Neurobiological markers for personality, inflammation, and stress

A naturalistic study in knee arthroplastic patients

Sara Bromander

Centre for Ethics, Law and Mental Health,
Institute of Neuroscience and Physiology,
Sahlgrenska Academy at University of Gothenburg

UNIVERSITY OF GOTHENBURG
Gothenburg 2013

Cover illustration: Mia Bromander

Neurobiological markers for personality, inflammation, and stress

Sara.bromander@rmv.se

http://hdl.handle.net/2077/32003
ISBN 978-91-628-8612-7

Printed in Stockholm, Sweden 2013
Universitetsservice US-AB

To see a world in a grain of sand

And a heaven in a wild flower

Hold infinity in the palm of your hand

And eternity in an hour

William Blake

A starlit or a moonlit dome disdains

All that man is;

All mere complexities,

The fury and the mire of human veins.

W.B Yeats

In memoriam

Anna Thunborg

Birga Bromander

Anne Rothlin

Magnus Bromander

ABSTRACT

Background: Psychiatry has strived to identify biomarkers elucidating the underlying biological mechanisms behind different disorders, to help in diagnostics and to assess treatment effects. In forensic psychiatric populations, findings have connected levels of cerebrospinal fluid (CSF) monoamine metabolites and blood-brain barrier (BBB) integrity with impulsivity and aggression. Other biomarkers, like insulin, inflammatory mediators, and different markers for neuronal and astroglial integrity have been studied in connection with cognition and psychiatric disorders but to a lesser degree in relationship to personality traits. **Aim:** The overall aims were to establish links between CSF markers for monoamine activity, BBB integrity, hormones, inflammation and neuronal and astroglial integrity, and aggressive and impulsive personality traits in a group of persons without psychiatric disorders, and to describe the distribution and dynamics of new biomarkers. **Methods:** Serum and CSF samples were collected before, three hours after, and on the morning following arthroplastic knee surgery in 35 patients who had completed two personality questionnaires, the Temperament and Character Inventory and the Karolinska Scales of Personality. **Results:** The CSF Homovanillic Acid/5-hydroxyindoleacetic Acid ratio correlated negatively with Cooperativeness. Beta-trace protein, as a marker of BBB dysfunction, correlated positively with Monotony Avoidance and Impulsiveness. Positive correlations were observed between CSF interleukin (IL)-10 and Verbal Aggression and between Self-Directedness, serum IL-10, and interferon-γ. CSF IL-10 correlated negatively with Inhibited Aggression, and CSF cortisol with Novelty Seeking. No correlations were detected between aggressive and impulsive personality traits and CSF levels of insulin, thyroid hormone, astroglial or neuronal integrity markers, or CSF/serum albumin ratio. Levels of CSF cytokines were markedly increased during and after the intervention compared to serum. Insulin levels in the brain seemed to be regulated differently from in the periphery, and modest increases in total-Tau were observed during surgery. **Conclusion:** Some aggressive and impulsive personality traits in non-disordered persons co-vary with various CSF biomarkers indicating lack of serotonergic control over dopamine signaling, decreased BBB integrity and inflammation.

Keywords: personality, cytokine, inflammation, insulin, impulsivity, aggression, blood-brain barrier

http://hdl.handle.net/2077/32003

ISBN: 978-91-628-8612-7

SAMMANFATTNING PÅ SVENSKA

Alltsedan psykiatrin började räknas som ett eget vetenskapligt fält har man strävat efter att identifiera tydliga och användbara biologiska markörer för olika beteenden och psykiatriska sjukdomar. Man har både velat hitta de biologiska mekanismerna bakom olika tillstånd och finna möjligheter för förbättrad diagnostik. Inom rättspsykiatrin har man särskilt riktat in sig på att identifiera biomarkörer för att förutse våldsbrott och även för att kunna förhindra återfall i våldsamt beteende. Genom åren har många markörer undersökts, från förekomst av en extra Y-kromosom till aktivitetsnivå i olika delar av hjärnan. I studier på rättspsykiatriska klienter har man under senare år funnit samband mellan impulsivt och aggressivt beteende och nivåer i ryggmärgsvätska av nedbrytningsprodukter av signalsubstanserna serotonin och dopamin, samt med tecken på blod-hjärnbarriärskada. För att ytterligare kunna belysa eventuella samband mellan personlighetsdrag, beteende och biomarkörer finns det dock ett behov av att studera dessa hos normala, icke våldsamma personer utan psykiatrisk problematik.

I denna avhandling undersöks eventuella samband mellan nivåer av nedbrytningsprodukter av signalsubstanser, markörer för blod-hjärnbarriärfunktion, hormoner, inflammatoriska ämnen (kortisol samt så kallade cytokiner), samt ämnen kända för att öka vid nervcellsskada och olika aggressiva och impulsiva personlighetsdrag hos en grupp personer utan känd psykiatrisk sjuklighet. Trettiofem personer som skulle genomgå en knäprotesoperation fick fylla i två personlighetsformulär före operationen, och prov på serum och ryggmärgsvätska togs sedan före, direkt efter och morgonen efter ingreppet. Vi fann flera samband mellan impulsiva och aggressiva personlighetsdrag och halten av olika cytokiner samt med kvoten mellan nedbrytningsprodukter av dopamin respektive serotonin i ryggmärgsvätska. Halten av ett ämne, beta-trace protein, som har visat sig ha samband med blod-hjärnbarriärfunktion, korrelerade också med impulsiva drag. Det visade sig också att nivåerna av flera cytokiner var förhöjda i ryggmärgsvätskan efter operation i högre grad än i perifert blod. En undergrupp av patienter visade sig också ha en mycket kraftigare stegring av halten av cytokiner än de övriga. Nivåerna av hormonet insulin i ryggmärgsvätska visade sig vara oberoende av nivåerna i perifert blod.

Viktigt är dock att notera att den aktuella studien inte har någon kontrollgrupp och alltså får ses som strikt deskriptiv. För att bekräfta eventuella orsakssamband skulle det krävas djurstudier och kvasi-experimentella kliniska studier med kontrollgrupp.

Resultaten i denna avhandling kan förhoppningsvis bidra till att öka förståelsen för hur personlighet och immunförsvar, hormonnivåer samt markörer för skada på det centrala nervsystemet hör ihop. Om än resultaten är på en mycket basal nivå, kan de stå till grund för vidare studier. Förhoppningsvis kan sådana leda till utvecklingen av nya behandlingsmöjligheter för till exempel aggressivt beteende respektive postoperativa komplikationer såsom konfusion och depression.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Nilsson T, Bromander S, Anckarsäter R, Kristiansson M, Forsman A, Blennow K, Zetterberg H, Anckarsäter H, Wass C. Neurochemical measures co-vary with personality traits: forensic psychiatric findings replicated in a general population sample.
Psychiatry Res. 2010; 178 (3):525-30.

II. Bromander S, Wass C, Anckarsäter R, Blennow K, Zetterberg H, Anckarsäter H, Nilsson T, Kristiansson M. Aggressive and impulsive personality traits and inflammatory markers in cerebrospinal fluid and serum: Are they interconnected?
(Manuscript).

III. Bromander S, Anckarsäter R, Ahrén B, Kristiansson M, Blennow K, Holmäng A, Zetterberg H, Anckarsäter H, Wass C. Cerebrospinal fluid insulin during non-neurological surgery.
J Neural Transm. 2010; 117:1167-70.

IV. Bromander S, Anckarsäter R, Kristiansson M, Blennow K, Zetterberg H, Anckarsäter H, Wass C. Changes in serum and cerebrospinal fluid cytokines in response to non-neurological surgery: an observational study.
J Neuroinflammation. 2012; 24:242.

V. Anckarsäter R, Bromander S, Anckarsäter H, Blennow K, Wass C, Zetterberg H. Non-neurological surgery and cerebrospinal fluid biomarkers for neuronal and astroglial integrity.
(Manuscript).

CONTENT

ABBREVIATIONS

5-HIAA	5-hydroxyindoleacetic Acid
5-HT	5-hydroxytryptamine (serotonin)
Aβ42	Amyloid beta, 42 amino acid form
AD/HD	Attention Deficit/Hyperactivity Disorder
BBB	Blood-brain Barrier
BMI	Body Mass Index
CNS	Central Nervous System
CRP	C-reactive Protein
CSF	Cerebrospinal Fluid
DA	Dopamine
GABA	Gamma-aminobutyric Acid
GFAp	Glial Fibrillary Acidic protein
HMPG	3-hydroxy-4-metoxyphelylglucol
HPLC	High-performance Liquid Chromotography
HVA	Homovanillic Acid
IFN-γ	Interferon-gamma
IL	Interleukin
KSP	Karolinska Scales of Personality
LPS	Lipopolysaccharide
MAO	Monoamine oxidase

NFL	Neurofilament Light
n.s.	Not significant
NMDA	N-methyl-D-aspartate
NO	Nitric Oxide
P-Tau	Phosphorylated Tau protein
SD	Standard Deviation
SSP	Swedish universities Scales of Personality
T3	Triiodothyronine
T4	Thyroxine
TCI	Temperament and Character Inventory
T-Tau	Total Tau protein
TNF	Tumor Necrosis Factor
TSH	Thyroid Stimulating Hormone

1 PREFACE

Since the first days of psychiatry as a scientific field, investigators have been eager to identify clear, useful biomarkers for different behaviours and psychiatric conditions. The aim of biomarker research has been to clarify the underlying biological mechanisms and to find diagnostic markers for developing stricter definitions for different disorders. In forensic psychiatry, the aim has more specifically been about identifying markers helpful in predicting future violent criminal acts and, more recently, in preventing relapse into violent behaviour. Many biomarkers have been examined through the years, from facial features to levels of activation in different brain areas. In forensic psychiatric populations, relationships between CSF levels of serotonin (5-HT) and dopamine (DA) metabolites, BBB integrity, and impulsive and aggressive behaviour and personality traits have been established. However, in order to fully elucidate possible connections between personality traits, behaviours and biomarkers, we need to study these relationships in normal, non-violent persons without psychiatric pathology. Investigating such connections might be valuable in clarifying the basis of different behaviours. Moreover, such knowledge could also be useful for identifying vulnerable persons in other contexts, like in assessing risk for developing psychiatric disorders, or in evaluating the risk of psychiatric complications in surgical patients before an operation.

The overall aim of this thesis was to investigate neurotransmitter metabolites, markers for BBB integrity, CSF hormones, inflammatory markers, and markers for neuronal and astroglial integrity in relation to aggressive and impulsive personality traits in a population of non-psychiatric patients. These biomarkers have previously been studied in forensic psychiatric groups, or in other ways attracted attention as possibly being connected with impulsivity and aggression, in human or animal studies. However, to get a fuller picture of their connection to aggression and impulsivity, they need to be examined in a non-disordered human population to see whether such connections can be generalized. Further exploring the neurochemical underpinnings of connected personality traits may contribute to a greater understanding of aggressive and impulsive behaviour in humans.

2 BACKGROUND

2.1 Biomarkers

A biomarker can be defined as an indicator of a biological state or trait. In psychiatry, where diagnosis is generally made during clinical examination, a search for clear, objective biomarkers for different disorders has gone on for decades. Biomarkers are measurable characteristics of an individual that may represent a normal variation; a risk factor for a disease; or an indicator of disease outcome, progression, or treatment response (1). Biomarkers might also be used to elucidate the difference between a "trait", meaning a relatively stable property of the assessed individual, like temperament, and a "state", meaning a more variable property like mood or affect. Biomarkers are usually taken to refer to explicitly "biological" markers identified via neuroimaging techniques, genetics, proteomics, peripheral and central neurochemistry, and cognitive measures.

The quest for clinically useful biomarkers in psychiatry has proved difficult. Several biomarkers have been investigated, for example for depression, chronic pain and Alzheimer's disease (2-4), but beside markers for neurological disorders such as dementias, these are mostly used in research. However, several biomarkers initially thought to be indicators for a specific disorder, like the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) for major depression, have instead been shown to be abnormal in persons displaying certain types of behaviours, such as violent suicide attempts or destructive aggressive acts (5, 6)

In this thesis, the focus will be limited to biochemical measures in serum and CSF for personality traits, especially those related to aggression and impulsivity, while important fields like genetics, neuroimaging, and other types of mental health problems, will be left out for reasons of brevity and clarity. We have used a non-disordered study group derived from orthopaedic patients to assess previously explored biomarkers from forensic psychiatry in a broader variation.

Biomarkers in forensic psychiatry

In forensic psychiatry, the main quest has been to elucidate the pathophysiology of, and markers for risk of, aggressive behaviour. Aggression and impulsivity are traits that are closely intertwined and of obvious importance for the development of violent behaviour (7). Identifying these traits in different individuals, through psychological testing or through measures of different biomarkers, might have important implications for assessment of risk and safety.

During the emergence of forensic psychiatry as a medical and scientific field, body measurements and facial features were considered promising biomarkers (8), although scientific validity was not particularly considered. In the mid-twentieth century, the extra Y chromosome of XYY syndrome was regarded as a promising biomarker, although later research could not confirm a general importance of sex chromosome aneuploidism for aggressive behaviour (9). Likewise, the relationship between serum levels of testosterone and aggressive behaviour has proved more inconsistent than initially anticipated, although later research has indicated relationships between the testosterone-cortisol ratio and aggression (10, 11). Moreover, low monoamine oxidase A (MAO-A) activity has been linked to aggressive behaviour, and genetic studies have shown important connections between different gene variants of this enzyme, environment, and aggression (12, 13). However, its predictive validity for aggression remains uncertain (14). Platelet monoamine Oxidase B (MAO-B) activity has also been investigated, but after initial enthusiasm, it has not been found to be clearly connected to aggressive and impulsive behaviour (13, 15). It is important to consider that a factor that might be of importance in explaining the behaviour of a single individual might have a weak correlation on a group level and thus be of very limited predictive value. Further biomarkers will be discussed in more detail below.

2.2 Personality

Personality may be described as a characteristic, enduring manner of feeling, thinking, behaving, and relating to others. It differs from other psychological constructs, such as cognition or mood, by being relatively stable over long periods of time, even a lifetime (16). The concept of personality is broader than the concept of behaviour; that is, any action or pattern of actions of an organism that changes its relationship to its environment. As behaviours are easier to observe and quantify, patterns of behaviour are central to the definition of personality. Examples of well validated and reliable personality

models are the Five Factor Model (17, 18), the Karolinska Scales of Personality (KSP) (19), revised as the Swedish universities Scales of Personality (SSP) (20), and the Temperament and Character Inventory (TCI) (21). Both genetic and biochemical biomarkers have been found to co-vary with several different personality traits, and psychiatric and somatic disorders (2, 22, 23).

Aggression

Aggression can be defined as a type of behaviour that either threatens, leads to, or causes harm to another organism. It is a complex phenomenon associated with psychosocial, neurobiological, and genetic factors. The prefrontal cortex, particularly the orbitofrontal and ventromedial regions, has been implied to play a crucial role in the regulation of aggression, controlling impulses from regions such as the temporal cortex, anterior cingulated cortex, periaqueductal grey, hippocampus, and amygdala (24). Impairment of several neurotransmitter systems has been implicated in aggression, including 5-HT, DA, and noradrenaline (NA). Dysregulation of many receptor systems and signalling pathways, such as γ-amino-butyric acid (GABA), N-methyl-D-aspartate (NMDA), nitric oxide (NO), MAO-A, and the inflammatory system, have also been implicated in aggression (25-29). When studying aggression, a single act of aggression must be differentiated from a more consistent pattern of aggressive behaviour. In animal studies, aggressive behaviour is often defined as either predatory or defensive (30). This might, in humans, correspond to the concepts of instrumental and reactive aggression (31). These two kinds of behaviour are distinguished by different patterns of neurobiological activation, with a higher degree of autonomous activation in reactive aggression (30).

Impulsivity

Impulsivity refers to acting without control or premeditation. This trait appears in every major conceptualization of personality, and encompasses a broad range of behaviours that reflect poor self-regulation, such as premature responding before considering consequences, inadequate planning, sensation-seeking, risk-taking, inability to inhibit responses, and preference for immediate rewards (32). Impulsivity is strongly linked to executive prefrontal dysfunction and aggression and when present, increases the risk of aggressive and violent behaviour (7, 33). Impulsivity is a key characteristic of several psychiatric disorders (34). 5-HT and DA, and also several other neurotransmitters like NA and glutamate, are strongly implicated in the regulation of impulsivity (35).

Protective factors

Personality factors regarded as protective against committing aggressive and impulsive acts can be deduced from studies comparing violent offenders to normal controls. As such, low scores in Self-directedness and Cooperativeness have been linked to aggressive behaviour, and to antisocial personality disorder, both in clinical groups (36) and in community-based samples (37). Low Cooperativeness has been shown to be strongly predictive of aggressive behaviour in a study of neuropsychiatric patients and violent offenders (38). Increased 5-HT transmission, GABA-signalling, neocortical inhibitory influence, and specific hypothalamic nuclei have been proposed to counteract aggressive impulses (39, 40). Self-directedness has been associated with low levels of C-reactive protein (CRP) (41), and with variations in the S-100b gene (42).

2.3 Current biomarker research regarding aggression and impulsivity

Biomarkers in the CSF

In biomarker research, CSF has the disadvantage of not being as readily accessible for sampling as serum. However, CSF is closer to and gives a more accurate picture of brain chemistry than measurements in peripheral blood. In clinical practice, measurement of different biomarkers in CSF is done routinely, aiding in the diagnosis of conditions like Alzheimer's disease (4). In research studies regarding aggression and impulsivity, markers like testosterone (43), GABA (44), monoamine metabolites (45), and thyroid hormone (46) are being evaluated in humans. When studying the roles of separate neurotransmitters, it is of utmost importance to remember that there are very close interactions between the different systems.

CSF

The CSF is a clear fluid produced by the choroid plexus in the ventricle system, as well as from the brain interstitial fluid (47, 48). It acts as a chemical buffer and cushion for the brain, providing a basic immunological protection, and serves a vital function in autoregulation of cerebral blood flow. Approximately 500 mL of CSF is produced every day, but the central nervous system (CNS) can only contain about 135-150 mL at a time. The caudal meningeal "sack" (from which lumbar punctures are drawn) has an estimated reabsorption of between 0.11-0.23 mL per minute. The CSF has an ionic composition similar to plasma, and normally should contain virtually no blood cells (47). It is sampled via lumbar puncture.

Monoamine transmitter systems

The monoamine transmitter systems (5-HT, DA, and NA) are of core importance for CNS function, as shown in their role in disorders such as depression and schizophrenia (49, 50). One way to approximate the activity in these systems is to measure the CSF concentrations of their main metabolites, 5-HIAA for 5-HT, homovanillic acid (HVA) for DA, and 3-hydroxy-4-metoxyphelylglucol (HMPG) for NA.

HMPG has shown very few correlations with aggressive and impulsive personality traits in earlier studies (51, 52).

Serotonin

Serotonin is synthesized from the amino acid tryptophan. 5-HT in the brain is crucial for a vast number of brain functions, such as control over mood and impulses, sleep, learning, appetite, and muscular tone (53). It is inactivated by degradation through MAO or by a specific reuptake transporter on the presynaptic neuron. The main 5-HT metabolite 5-HIAA can be measured in blood, urine and CSF samples (54).

Many studies suggest that 5-HT plays a significant inhibitory role with respect to aggressive behaviour. In humans, several studies have shown a decrease in aggressive and impulsive behaviour in persons treated with selective serotonin uptake inhibitors that increase postsynaptic serotonergic signals (30, 55-57). Other studies have shown an increased propensity for destructive acting out, such as violent suicide, arson, or killing a sexual partner, in subjects with decreased CSF 5-HIAA levels (5, 58, 59). Importantly, low 5-HIAA levels in CSF have been shown to correlate with impulsive, but not premeditated, aggression (60). Such findings suggest that the connections with the 5-HT system in the brain are stronger regarding impulsive aggressive behaviour than regarding premeditated, instrumental aggression. Also, polymorphisms in several genes encoding key enzymes and receptors in the 5-HT metabolism and neurotransmission systems have been shown to be associated with impulsivity (61). In all, however, studies are heterogeneous regarding the strength of the relationship between 5-HT and aggression (39), and the overall effect much smaller than initially thought.

Dopamine

Dopamine (DA) is synthesized from the amino acid L-tyrosine. It is inactivated by enzymatic breakdown by MAO or cathecol-O-methyltransferase (COMT). The main metabolite of DA, HVA, can be measured in CSF and urine (54).

The DA system in the brain seems to be involved in impulsivity and aggression, although the research regarding this system is not as extensive as for 5-HT. Drugs, like clozapine and olanzapine, blocking DA D2 receptors, have been repeatedly reported to decrease aggressive behaviour in different patient populations (62, 63). In patients with Parkinson's disease treated with DA replacement therapies, a number of impulse control impairments, including compulsive gambling and hypersexuality, have been identified (64). CSF HVA and 5-HIAA have been noted to co-vary in many studies (65), consistent with the notion that the monoamine systems interact (54). The ratio between 5-HT and DA metabolites is highly constant (54), and an increased HVA/5-HIAA ratio indicates an impaired 5-HT modulation of DA activity (65). A skewed ratio between CSF HVA and 5-HIAA has been found in psychotic patients (66), in suicide attempters (67, 68), and in groups of violent offenders undergoing forensic psychiatric examinations (69, 70).

The blood-brain barrier

The BBB is formed by specialized capillary endothelial cells, joined together by tight junctions, and having a close cellular connection with both astrocytes and specialized pericytes. These cells are closely applied to a continuous basement membrane. All these elements, together with neurons, form a functional neurovascular unit (71). The structure of the BBB, together with enzymes and transport systems, prevents the leakage of many substances and pathogens from blood to brain, but allows passage of necessary substances like nutrients (48). There are several types of selective, saturable transport mechanisms across the BBB: carrier-mediated transport (e.g. ions, glucose, amino acids), receptor-mediated (proteins like insulin and thyroxin) and adsorptive transcytosis (glycoproteins, viruses, albumin, and other plasma proteins), and diapedesis for immune cells (72, 73). Water, small gaseous molecules like O_2 and CO_2, and small lipophilic molecules like barbiturates and ethanol can diffuse freely across the BBB. The cells of the BBB can also produce and secrete neuroactive and immunoactive substances such as NO, prostaglandins, and cytokines (74). Perivascular macrophages, derived from blood-borne progenitors, reside by the vessel wall and protect the brain from infection (75, 76). Subsets of microglia resident in the brain have also been shown to regulate immune cell passage across the BBB (77).

BBB integrity

Signs of a disrupted or dysfunctional BBB have been observed in many pathologies of the CNS, from traumatic injury to psychiatric illness. Breakdown of the BBB can be helpful, allowing immune entry to clear away debris and repair injuries. It can also be damaging, causing oedema, neuronal injury, and degeneration (71). The integrity of the BBB has often been

assessed by measuring the ratio of the plasma protein albumin in CSF and serum (78). Increased ratios have been reported in patients with traumatic brain damage and tumours as well as psychotic illness (66), in suicide attempters (67), and in violent offenders (69, 79).

Beta-trace protein (βTP), identical with prostaglandin D synthase (80), is considered to be produced mainly in the leptomeninges (81) and then secreted into the CSF (80, 82-84). It is used in clinical practice to measure CSF leakage into peripheral fluids such as nasal secretions (85). Thus, serum βTP concentrations and CSF/serum albumin ratios represent two different aspects of BBB permeability (leakage in vs. leakage out of the CNS).

Insulin

Insulin is well known as the major, immediate regulator of peripheral blood glucose levels. Glucose transport across the BBB, and also brain glucose metabolism, are, however, regulated independently of insulin (86). Insulin is mainly synthesized in the periphery and crosses the BBB by saturable transport (87-89). Brain insulin has been found to play an important role in cognition and memory (possibly through regulating several receptors and transmitter systems, including NMDA (90) and DA (91, 92) receptors), and regulation of feeding behaviour (93, 94). Recently, insulin has been associated with Alzheimer's disease and depressive behaviour (95). CSF insulin has been found to be higher in patients who had made a violent suicide attempt than in those who had made a non-violent attempt (6), consistent with the hypothesis that habitually violent individuals have a dysregulated insulin secretion and glucose metabolism (96-98).

Thyroid hormones

The thyroid gland is a major regulator of metabolism in the whole body, producing thyroxin (T4) and the more active triiodothyronine (T3). Thyroid hormones have a profound influence on behaviour and mood, are essential for the development and maturation of the brain, and play an important role in the regulation of the monoamine systems (99). An association between increased serum thyroid activity (T3 or the T3/T4 ratio) and criminal recidivism, aggression, and psychopathic personality traits has been found in several studies (100-102). The ratio between T3 and T4 has been shown to be associated with ratings of psychopathy (100), and also with antisocial behaviour (103). In addition, a recent study in suicide attempters suggests that violent personality traits in men (high scores on aggressiveness and low scores on detachment on the KSP) are connected with decreased T3/T4 ratio (46).

Inflammation

Inflammation can be regarded as the reaction of the body to something perceived as dangerous- things as diverse as microorganisms, trauma, or psychological stress. The innate immune system is the first line of defence. It provides signals for the activation and regulation of the adaptive system, which mediates antigen-specific mechanisms. Inflammatory mediators have also been shown to play a role in processes such as normal embryonic development and learning (104, 105).

Accumulating evidence implicates a connection between the immune system, personality, and behaviour (106). Ever since the discovery that cytokine treatment of hepatitis and different forms of cancer can cause a form of depressive illness (107), the role of different inflammatory markers in psychiatric disorders like depression and schizophrenia has been subject to study (108-110).

Studies regarding personality traits and inflammation are scarce. However, increased production of proinflammatory cytokines, as induced by lipopolysaccharide (LPS) has been shown to be related to hostile and aggressive personality traits. Increased LPS-stimulated monocyte Tumor Necrosis Factor (TNF) expression has been shown to be associated with hostility and physical and verbal aggression in healthy men (111). LPS-stimulated expression of IL-1α, IL-1β, and IL-8 has also been shown to be associated with hostility in healthy women (112). Hostile behavioural tendencies have also been shown to be associated with IL-6 and CRP levels, independent of lifestyle factors like body mass index (BMI) and smoking (113). Impulsivity-related personality traits (high neuroticism and low conscientiousness) have been associated with higher levels of IL-6 in a population-based sample (114). Low CRP has been shown to be associated with Self-directedness, a trait regarded as protective against psychopathology (41).

Studies regarding central inflammatory reactions to peripheral trauma are scarce, but mainly show increases in both pro- and anti-inflammatory markers following surgery (115-119). Reactions in serum are, understandably, considerably more studied (120, 121). As it is becoming increasingly clear that inflammation plays an important role both in normal behaviour and post-operative cognitive and psychiatric complications (109, 122-125), the need for more knowledge about these reactions in a representative, normal patient material is evident.

Cortisol

Stress initiates several reactions in the body, including activation of the hypothalamus-pituitary-adrenal (HPA) axis. Cortisol regulates energy metabolism, and also activates immune system responses. It seems to enter the brain both through diffusion and through active transport, depending on the area involved. It has strong anti-inflammatory effects, although recent research shows some proinflammatory activities as well (126). The HPA axis and the inflammatory system are intertwined in a complex way. Greater anger in response to a stressor has been associated with higher cortisol, and greater fear with higher IL-6 (127). Two decades of research have implicated a relationship between cortisol and antisocial behaviour, especially in childhood (128). Cortisol and testosterone also seem to act together, with an imbalance in reciprocal inhibition resulting in a higher risk of aggression, possibly through action on the amygdala (11).

Markers for astroglial and neuronal integrity

Amyloid β (Aβ) is produced by proteolytic cleavage of amyloid precursor protein during normal cell metabolism. It is secreted into the CSF (129). The 42 amino acid form of this peptide (Aβ42) is the major component of senile plaque deposits. The most prevalent hypothesis for mechanisms of Aβ-mediated ''neurotoxicity'' is structural damage to the synapse (4). Alzheimer patients characteristically display low concentrations of Aβ42 and high total tau (T-tau) and phosphorylated tau (P-Tau) in their CSF (130). Tau proteins are involved in stabilizing microtubules in CNS neurons and can be regarded as markers of axonal damage (131). Patients with AD displaying agitated aggression during life have been shown to have increased phosphorylation of Tau in their frontal cortices (132). Neurofilaments are intermediate filaments found specifically in neurons. Neurofilament light (NFL) is the smallest sub-component of neurofilaments. Increased NFL levels in CSF are associated with brain damage, and are used as a biomarker for the integrity of large calibre myelinated axons. Glial fibrillary acidic protein (GFAp) is an intermediate filament protein that is used as a biomarker for astroglial cell integrity. Tau, as well as NFL, is known to increase in CNS damage (133, 134). Both GFAp and Aβ42 have been shown to be increased in CSF after open-heart surgery (135, 136).

Markers for BBB integrity, neurotransmitter metabolites, inflammatory markers, CSF hormones and markers for neuronal and astroglial integrity have all been shown to co-vary to different degrees with aggressive and impulsive personality traits. However, few studies have been done in non-psychiatric patients, showing whether these connections actually exist in the "normal" condition, or only when aggressive and impulsive behaviour is present.

3 AIMS

The overall aim of this thesis was to elucidate a number of biomarkers in relation to aggressive and impulsive personality traits in a non-psychiatric study group, using baseline serum and CSF samples collected before arthtroplastic surgery (A samples). In addition, the distributions of these CSF biomarkers and the effect of peripheral surgery (as an indicator of peripheral stress) on different CSF and serum biomarkers were also evaluated, with samples drawn three hours after (B samples) and on the morning (C samples) following the intervention. Previous published articles from this study have reported on changes in BBB permeability, CSF monoamine metabolites and thyroid hormones in response to peripheral surgery (137-139).

The specific aims were to:

I. identify possible co-variation between monoamine transmitter metabolites, BBB integrity, insulin, thyroid hormones, inflammatory markers, and markers for astroglial and neuronal integrity, and impulsive and aggressive personality traits;

II. assess changes in CSF and serum insulin during non-neurological surgery;

III. establish levels of inflammatory markers in CSF and serum and identify their changes during surgery and;

IV. investigate the levels of markers for astroglial and neuronal integrity before and after surgery.

4 SUBJECTS AND METHODS

4.1 Subjects

Patients scheduled for knee arthroplastic surgery at Kungälv Hospital were consecutively recruited from the anaesthesiological clinical practice doing preoperative assessments.

Thirty-five patients (20 men, 15 women, aged 51-82 years, median age 73) gave written and oral consent to participate in the study. One patient had bilateral surgery in the same session, while the others were unilateral interventions. The ambition was to select patients with as few complicating medical disorders as possible; however, given the age range of patients in need of knee arthroplasty, it was not possible to assemble a study group with overall "healthy" patients. Exclusion criteria included the systemic use of corticosteroids, anti-Parkinson medication, antipsychotics, antidepressants and anticoagulant treatment. Of these 35 patients, CSF and serum samples could be drawn from 34, who were to form the actual study group, with some further attrition due to technical problems (as detailed below for each paper).

Three of these patients were found to have creatinine concentration > 100 μmol/L, but otherwise routine preoperative blood tests were within normal reference values. Seven patients had diabetes mellitus (none treated with insulin) and 21 had hypertension. They had the following antihypertensive treatments: beta blockers (11 cases), angiotensin II-antagonists (2 cases), diuretics (2 cases), calcium channel blockers (2 cases), and ACE inhibitor (one case). Three patients had both diabetes and hypertension. Three patients used codeine and tramadol on an as-needed basis. All medications had been used for a long and stable period. One subject was originally thought to have taken the serotonin reuptake inhibitor citalopram; however, on thorough examination of their medical files, it became clear that they had discontinued this medication several months before the intervention. Any non-steroidal anti-inflammatory drugs were discontinued a week before the intervention. No abrupt washout of any drugs was performed for study purposes. On the day of surgery, no per-oral medication besides beta blockers was given.

All subjects had been fasting for at least 6 hours before surgery, and all glucose infusions administered during and after surgery were carefully registered. After surgery, patients were fasting until the second CSF sampling, as the spinal blockade was still active. They were free to eat in the evening after the interventions.

Twenty-six subjects had completed one or both of the personality questionnaires prior to arriving at the hospital for surgery. Of these, 26 had filled in the Temperament and Character Inventory and 23 had filled in the Karolinska Scales of Personality.

Detailed descriptions of which subjects were included in which papers are given below.

In **Paper I**, the study group at first consisted of the 26 subjects who had filled in one or both of the personality questionnaires before arriving at the hospital. In this paper, the individual believed to be on long-term medication with 10 mg of citalopram was excluded. Four subjects were excluded, as they had pathological CSF/serum albumin ratios (exceeding the normal reference value of 11.8) at the first sampling (A), and were therefore not considered as neurologically healthy. The final group analysed consisted of 21 subjects.

In **Paper II**, all 26 subjects who had filled in one or both of the personality questionnaires were included, including the four subjects with pathological CSF/serum albumin ratios, as we wanted a full picture of the correlations between cytokine levels in the CSF and personality traits, including any cytokine increases due to a "leaky" BBB. The number of individuals included in the correlations varied from 20–25, depending on which cytokine was studied, as it was not technically possible to measure all CSF cytokines in all individuals at all samplings. In this paper, cytokines with a mean concentration of 0.61 (lowest level of detection for the analysis kit) and SD of 0.00 pg/mL (i.e. IL-4, 5, 12 and 13) at A were excluded from further analysis.

In **Paper III**, one subject was included only in the analyses of the A and B (three hours after surgery) concentrations, because no C sample (the morning after) could be obtained. One subject was excluded due to deviant insulin concentrations along with a pathological CSF/serum albumin ratio at baseline, and one because of no back-flow in the spinal catheter at either the B or C samples. There were technical problems with the laboratory analyses of insulin in serum in one subject at the B and C samples, in another at A and B, and in six subjects at the CSF analyses of the C samples. After exclusion of these patients, serum samples from 30 patients and CSF samples from 24 patients were included in the final analyses.

In **Paper IV**, analysis of baseline CSF/serum albumin ratios identified four subjects with abnormal ratios (i.e. > 11.8) (54). Since one of the aims of the present study was to investigate relationships between variations in BBB

permeability and central inflammatory reactions, the initial analyses included all subjects, regardless of CSF/serum albumin ratios, in order to capture the full range of these relationships. Analysis of serum cytokines was possible in 34 individuals, and of CSF cytokines in 25–26 individuals.

In **paper V**, four subjects having pathological CSF/serum albumin ratios at A, and one with signs of bleeding at B, were excluded from further analyses (as in the previous report on protein chemistry from our research group (137)). Technical problems resulted in some further missing values, resulting in a final study group of 29 patients with the exact number varying from 26 to 29 at each sampling.

4.2 Procedures

CSF sampling

A lumbar puncture was performed with an 18-gauge Portex epidural needle in the L3-L4 interspace. About 2 mL of CSF was discarded at first, and then 12 mL of CSF was sampled and gently mixed before administration of any intrathecal drugs. At the same time, 15 mL of blood for serum analyses, 20 mL of EDTA blood for plasma analyses, and 20 mL of blood for possible later genetic analyses were sampled. These baseline samples are referred to as the "A samples". A catheter was inserted after the initial sampling, and "B-samples" were collected three hours after completion of the intervention. "C-samples" were drawn in the morning after the intervention by the same routine. CSF and blood samples were centrifuged at 2000g for 10 minutes to eliminate cells and other insoluble material, and pipetted in new tubes for transport to the neurochemistry laboratory. Aliquots were stored at -80° C until biochemical analyses.

Medications

All patients first had subcutaneous local anaesthesia with 10 mL 0.5% mepivacaine as part of the anaesthesiological procedure. After the A-samples were drawn, an initial spinal anaesthesia with 3 mL of bupivacaine 5mg /mL was administered. Propofol was administered during surgery as a continuous infusion to all patients, using the bispectral index (BIS) to titrate the dosage for an optimal sedation of BIS 70, yielding a total dosage of propofol ranging from 102 mg to 1423 mg, with a mean of 392 mg (standard deviation [SD] 228). All patients were given 1 g of tranexamic acid and 1 g of paracetamol before or during the intervention.

Between the A and B samplings, the following drugs were administered: phenylephrine (20 cases), ephedrine (4 cases), atropine (8 cases), metoprolol (1 case), droperidol (1 case), morphine and petidine chloride (1 case), ketobemidone (1 case), fentanyl (1 case), diazepam (1 case), and ondansetron (1 case).

Between the B and C samplings, ketobemidone (10 cases), morphine (7 cases), fentanyl (2 cases), ondansetron (4 cases), atropine (4 cases), dixyrazine (6 cases), ephedrine (1 case), phenylephrine (4 cases), diazepam (3 cases), betamethasone (1 case), droperidol (4 cases), and zolpidem or zopiclone in normal sleeping dosages (14 cases) were administered.

Bupivacaine was administered as a 5 mg/mL intrathecal solution in sodium chloride between the A and B samplings with a mean dosage of 3.7 mL (range 3–7.4 mL, SD 0.84), and between B and C with a mean dosage of 6.19 mL (range 2.80–11.40 mL, SD 2.46). Three patients had a local instillation of 30 mg ketorolac and 200 mg ropivacaine in the knee before termination of the operation, as part of another study protocol. No patients needed blood transfusions, and pain breakthrough was the only complication noted during the study. All but four patients were given glucose (5% or 10%) infusions, starting after the surgery and ending on the following morning.

All administered drugs and infusions were carefully monitored and introduced in the database used for the scientific analyses, in order to check for possible effects of drugs or infusions on the concentration or changes of the biomarkers investigated.

4.3 Measures

Neurochemical analyses

Monoamine metabolites were analysed by high-performance liquid chromatography (HPLC) with electrochemical detection as described by Blennow et al. [54].

Albumin analysis was made by nephelometry on the Immage instrument (Beckman Coulter, Brea, CA, USA). βTP protein was analysed by nephelometry on the BNProSpec instrument (Dade Behring, Deerfield, IL, USA) using the NLatex βTP kit.

Insulin was analysed using a double antibody radioimmunoassay (Linco Research, St Charles, MO, USA). Thyroid hormones were determined by biochip array technique on the Evidence Investigator (Randox, Crumlin, UK) using the total thyroid array for TSH, total T3, and total T4 [130].

The Human TH1/TH2 10-Plex Assay Ultra-Sensitive Kit (Meso Scale Discovery, Gaithersburg, MD, USA) was used for cytokine analyses. The kit used included analysis of IL-1β, IL-2, IL-4, IL-5, IL-10, IL-12, IL-13, IFN-γ, and TNF. The lowest level of detection for the kit was 0.61 pg/mL. For the cytokines having potentially lower concentrations, the level was thus set to 0.61 pg/mL in the computations. CSF cortisol was measured by radioimmunoassay using the Spectria Cortisol (125I) kit (Orion Diagnostica, Sollentuna, Sweden).

T-tau, P-tau, Aβ42, NFL, and GFAp were analysed by enzyme-linked immunosorbent assays.

All measurements were performed in batch mode by board-certified laboratory technicians, and intra-assay coefficients of variation were below 10% for all analyses.

Personality assessments

Personality questionnaires were distributed to the subjects during the anaesthesiological pre-operative consultation, and the patients were asked to fill them out at home prior to the operation. The completed forms were collected at the time of admission to the hospital.

Temperament and Character Inventory

The TCI is a 238-item true or false self-report questionnaire assessing four temperament dimensions, *i.e.* Novelty Seeking (impulsive vs. reflective), Harm Avoidance (anxious vs. calm), Reward Dependence (approval seeking vs. independent), and Persistence (steadfast vs. fickle), and three character dimensions, *i.e.* Self-directedness (resourceful vs. helpless), Cooperativeness (emphatic vs. hostile), and Self-transcendence (self-forgetful vs. acquisitive). It was developed to describe both normal and abnormal variation in personality. It is based on Cloninger's psychobiological model of personality (21) and has been translated into Swedish from the original American version (140).

Twenty-six subjects had filled in the TCI.

The Karolinska Scales of Personality

The KSP is a 135-item self-report questionnaire containing 15 personality scales, *i.e.* Somatic Anxiety, Psychic Anxiety, Muscular Tension, Social Desirability, Impulsiveness, Monotony Avoidance, Detachment, Psychasthenia, Socialization, Indirect Aggression, Verbal Aggression, Irritability, Suspicion, Guilt and Inhibition of Aggression, measuring stable temperament traits constituting vulnerability factors for different kinds of psychopathology (19). It has been widely used in studies involving biological correlates of personality traits (141, 142). A revised version (the Swedish Universities Scales of Personality, SSP) has been published since the study was designed (20).

Twenty-three subjects had filled in the KSP.

As the aim of this study was to investigate impulsive and aggressive personality traits, the following scales were chosen in paper I: to reflect impulsivity: Novelty Seeking (TCI), Monotony Avoidance (KSP), Impulsiveness (KSP); for aggression and aggression-related personality traits: Indirect Aggression (KSP), Verbal Aggression (KSP), Irritability (KSP), and for self-regulation, behaviour control and empathy: Inhibited Aggression (KSP), Detachment (KSP), Reward Dependence (KSP), Socialisation (KSP), Self-Directedness (TCI) and Cooperativeness (TCI), as described in Paper I, based on i.a. (37, 38). In paper II, we chose to concentrate on those scales more directly connected to impulsivity and aggression as stated above, and, in addition, we chose to look at Self-Directedness, as this trait has been shown to correlate with inflammatory markers in normal populations in other studies (41, 42).

No systematic differences in sex, age, or general medical status were noted between responders and non-responders. The main reason for non-response was stated to be lack of time to fill out the personality questionnaires before surgery.

The personality data were transformed into t-scores (with a mean of 50 and a SD of ± 10) derived from Swedish normal population groups, corrected for age and sex (143), and then compared to laboratory results. To assess the representativeness of the sample, personality traits were tested against the normal mean value of 50 by one-sample *t*-tests, and with the exception of the following KSP scales; Psychic Anxiety (< 50, $p = .001$), Indirect Aggression (< 50, $p = .001$), and Suspicion (> 50, $p = .006$) no significant differences from the expected mean were found. Strong tendencies for higher scores in women were found for the KSP traits Psychic Anxiety and Inhibited

Aggression, but these did not reach statistical significance according to the pre-set significance level ($p \leq .01$). No significant correlations were found between age and any of the KSP or the TCI dimensions.

4.4 Statistical and analytical methods

All statistical analyses were performed using the Statistical Package for Social Sciences Program (SPSS), version 17.0 (Papers I and IV), 18.0 (Paper III) and 20.0 (Papers II and V).

Statistical analyses

In **Papers I and II**, one-sample *t*-test was used to test the study group against the normal population (t-score of 50). This is a means of testing whether there were any statistically significant differences between the mean of the study group and the normal population. Spearman correlations were used to analyse associations between variables. To control for the risk of type I errors, significance level was set to $p < .01$.

In **Paper III**, repeated measures ANOVA and Bonferroni corrections were used to assess changes in insulin levels between samplings A, B and C. Repeated measures ANOVA is a test used to measure changes in a parameter between three or more time points. CSF and serum insulin concentrations were the dependent variable, while assessment points (A, B, and C) were the independent variable.

Pearson correlations were used for analyses of relations between different markers, and between CSF and serum concentrations and ratios, where a high positive correlation would indicate that subjects with high CSF concentrations also had high serum concentrations and vice versa with negative correlations.

In **Paper IV**, we used nonparametric methods, as the distribution of cytokine levels was skewed due to the lower detection level of the analysis kit (0.61 pmol/mL). Friedman's repeated measures analysis of variance by ranks, followed by Wilcoxon signed rank test for post hoc comparisons between pairs, were used to compare the cytokine concentrations at A, B, and C. Spearman correlations were computed to assess the relationship between peripheral and central levels of cytokines, cortisol, and CSF/serum albumin ratios (in absolute levels), and to investigate the relationship between peripheral and central fluctuations in cytokines (relative change in cytokine levels was computed as concentration at C divided by concentration at B, B divided by A, and C divided by A). Mann-Whitney U-test was used for

analysis of differences in CSF/serum albumin ratios and cytokine levels between subgroups. All tests were two-tailed, and significance levels were set to 0.05.

In **paper V**, mean CSF concentrations of T-Tau, P-Tau, Aβ42, NFL, and GFAp were calculated at sampling points A, B, and C, and compared first by a repeated measurement ANOVA. In case of an overall significant effect, the pair-wise comparison was done by repeated paired *t*-tests. Tests were two-tailed and the significance level was set to 0.05. Correlations with possible confounding factors and with changes in CSF/serum albumin ratios were calculated by Pearson's correlations.

Corrections for multiple comparisons

The level of significance is customarily set at 5% (meaning that the probability of making a type I error, or alpha, is set at 0.05). The more analyses we make in a study, the more likely we are to interpret random differences as true. In CSF studies, due to their invasive nature, it is difficult to gather large enough samples to reach adequate power to perform Bonferroni corrections without running a high risk of type II errors. This specifically poses a problem for correlational analysis between biomarkers and personality, where the number of comparisons are high. In paper I and II the level of significance was set to 1% in view of the large number of correlations analysed. Another statistical challenge regarding both the studied biomarkers and the personality factors, is that they are probably intracorrelated and not biologically independent. This might make Bonferroni correction, or similar measures, too strict.

5 ETHICAL CONSIDERATIONS

Written and oral information about the study was given to all potential subjects, as well as opportunity for questions and discussions. All subjects gave consent. The subjects earned no material compensation for their participation in the study. As they were undergoing spinal anaesthesia and blood draws as part of the normal anaesthesiological procedure for a knee arthroplasty, the only extra task for the subjects was filling out the personality questionnaires before arriving at the hospital. Copies of medical files and study data were kept in a fireproof, locked archive, and data kept in computer files were coded. The project was approved by the Research Ethics Committee at the University of Gothenburg.

Important to consider in all biomarker research, especially involving "sensitive" areas like aggression and impulsivity, is the possible risk of stigmatization in those who have been "labelled" as having increased levels of biomarkers associated with negative traits. A particular risk in biomarker research in forensic psychiatry is the propensity for oversimplification, which might lead to headlines like "simple blood test discloses risk of violent crime", and use of biomarkers that might be interesting on a group level in basic research to make important decisions in court about the fate of an individual, when in reality, the questions of free will, personal responsibility and their limitations are immensely complicated (144, 145). This presents a great pedagogical challenge, both for media and the scientific community.

In this particular study, however, the markers studied and the correlations found are on such a basic level that the danger of "labelling" somebody would be negligible. We are striving to elucidate basic mechanisms behind different behaviours, although impulsivity and aggression are generally considered quite negative traits. No inferences on individual forensic patients are possible based on the data collected and analysed here.

6 RESULTS

6.1 Aim I: Biomarkers for impulsive and aggressive personality traits

Monoaminergic metabolites

A negative correlation was detected between the HVA/5-HIAA ratio and Cooperativeness (*rho* = -.57, *p* = .008).

BBB integrity

Serum βTP correlated positively with Monotony Avoidance (*rho* = .62, *p* = .005) and Impulsiveness (*rho* = .64, *p* = .003). The correlations were stronger among women both for Monotony Avoidance (*rho* = .97, *p* = .001) and Impulsiveness (*rho* = .90, *p* = .002). No significant correlations were found between any of the personality traits and serum or CSF albumin levels, or CSF/serum albumin ratios.

Thyroid hormones

No significant correlations were found between total and free fractions of thyroid hormone in serum or CSF and any of the targeted personality traits.

Inflammatory markers and cortisol

IL-10 correlated positively with Verbal Aggression (*rho* = .60, *p* = .005), and showed a negative correlation with Inhibited Aggression (*rho* = -.57, *p* = .008). CSF cortisol showed a negative correlation with Novelty Seeking (*rho* = -.60, *p* = .006). Negative correlations were found between serum IL-10 and IFN-γ and Self-directedness (*rho* = -.51, *p* = .01 and *rho* = -.51, *p* = .009, respectively).

Insulin

No significant correlations were seen between insulin in serum or CSF or with the serum/CSF insulin ratio and any of the targeted personality traits.

Markers for neuronal and astroglial integrity

No significant correlations were seen between levels of the different markers for neuronal and astroglial integrity and any of the targeted personality traits.

Possible confounders

All analyses were redone after excluding the three subjects treated with lipophilic beta blockers (as these were considered to possibly influence levels

of monoamine metabolites). This did not change the direction or magnitude of the main findings; baseline serum βTP correlated positively with Monotony Avoidance (*rho* = .59, *p* = .017) and with Impulsivity (*rho* = .61, *p* = .013), and baseline CSF HVA/5-HIAA ratios correlated positively with Irritability (*rho* = .66, *p* = .005) and with Cooperativeness (*rho* = -.58, *p* = .012).

Overall serum βTP concentrations correlated with CSF/serum albumin ratios (*rho* = .51, *p* = .018). Serum βTP did not correlate with preoperative serum creatinine concentrations, even if renal pathology may be a confounder in research using this marker, which is considered as an indicator of glomerular filtration rate (146).

6.2 Aim II: Changes in insulin levels during peripheral surgery

Changes in serum and CSF insulin levels

Serum insulin levels did not markedly change from baseline to three hours following surgery. They did, however, increase dramatically in the morning after the intervention. Of note are the large standard deviations at all assessment points, which may have resulted in the lack of statistically significant fluctuations in serum insulin from A to B despite their appearing to decrease overall. In CSF, insulin levels decreased significantly from baseline to three hours after surgery; however, no significant change was observed between baseline and the morning after surgery. The effect sizes for the insulin changes in CSF ranged from small to large.

Table 1. CSF and serum mean concentrations and SD and CSF/serum ratios of insulin at the A, B, and C samplings with p-values from one-way ANOVAs with Bonferroni corrected p-values and Cohen´s d.

	A	B	C	ANOVA statistics	Bonferroni corrected p-values	Cohen´s *d*
Serum insulin (pmol/L) n=30	73.67 ± 39.19	65.40 ± 42.03	234.00 ± 192.47	F(2, 58) = 27.26 $p < .001$	AB n.s AC $p < .001$ BC $p < .001$	AB: 0.20 AC: 1.16 BC: 1.21
CSF insulin (pmol/L) n=24	5.78 ± 0.59	5.15 ± 0.71	5.49 ± 0.95	F(2, 46) = 5.46 $p < .01$	AB $p < .01$ AC n.s. BC n.s.	AB: 0.97
CSF/serum insulin n=23	0.013 ± 0.01	0.018 ± 0.014	0.0086 ± 0.0098	F(2, 44) = 24.45 $p < .01$	AB n.s. AC $p < .01$ BC $p < .01$	AC: 0.36 BC: 0.78

Serum and CSF insulin levels

Comparing overall CSF and serum insulin levels at both A, B, and C, showed a weak but statistically significant correlation between serum and CSF insulin concentrations (*rho* = .34, $p = .001$). When these correlations were assessed for each sampling separately, the serum and CSF insulin concentrations did not correlate either at A or B, while there was a significant positive correlation at C (*rho* = .58, $p = .002$).

Insulin and BBB integrity

Performing the analysis without the subjects who had CSF/serum albumin ratios above the clinical reference value at baseline only marginally reduced the mean CSF/serum insulin ratios in the remaining group and did not influence the results. No correlations were seen between serum or CSF insulin, CSF/serum insulin ratios and CSF/serum albumin ratio or βTP at any of the samplings.

Possible confounders

No difference was seen between genders in serum or CSF insulin concentrations or in the CSF/serum insulin ratios at A or B. Women had higher mean serum insulin ($t = 2.155$, $df = 29$, $p = .040$) and lower CSF/serum insulin ratios ($t = -2.59$, $df = 23$, $p = .016$). No significant relations were seen between age and serum or CSF insulin, at any of the samplings.

When subjects with diabetes mellitus were omitted, significant positive correlations emerged between serum and CSF insulin at A (*rho* = .38, $p < .05$) and C (*rho* = .58, $p < .01$), but not at B. Serum insulin concentrations were significantly correlated with BMI at A (*rho* = .54, $p < .01$), but not after surgery. There were no correlations between BMI and CSF insulin concentrations at any point.

6.3 Aim III: Changes in inflammatory makers during peripheral surgery

Changes in serum and CSF cytokines

In serum, IL-8 and IL-10 changed significantly from baseline to B and C (both $p < .001$). Serum TNF decreased significantly from A to C .In CSF, IL-2, IL-5, IL-8, IL-10, IL-13, and TNF showed significant change during the intervention (all $p < .001$).

Table 2. Serum and CSF mean (± SD) concentrations (pg/mL), of the cytokines showing significant change between the A, B, and C samplings with p-values from Friedman's repeated measures analysis of variance by ranks followed by Wilcoxon signed rank test for post hoc comparisons between pairs, α-level set to 0.05.

Serum	IL-8	IL-10	TNF
A	23.98 ± 116.12 N = 34	3.53 ± 4.09 N = 34	8.62 ± 3.05 N = 34
B	26.86 ± 123.50 N = 34	6.00 ± 7.43 N = 34	9.35 ± 3.71 N = 34
C	30.19 ± 126.82 N = 34	9.62 ± 7.28 N = 34	7.91 ± 2.25 N = 34
Friedman's statistics	$\chi 2$ (2) = 43.304 $p < .001$	$\chi 2$ (2) = 49.259 $p < .001$	$\chi 2$ (2) = 9.450 $p = .009$
Wilcoxon signed rank test *P*-values	AB $p < .001$ AC $p < .001$ BC n.s.	AB $p < .001$ AC $p < .001$ BC $p < .001$	AB n.s. AC $p = .03$ BC n.s.

CSF	IL-2	IL-5	IL-8	IL-10	IL-13	TNF
A	0.63 ± 0.54 N = 25	0.61 ± 0.00 N = 25	30.40 ± 8.80 N = 25	0.76 ± 0.22 N = 25	0.61 ± 0.00 N = 25	0.67 ± 0.14 N = 25
B	4.74 ± 10.77 N = 25	1.31 ± 1.87 N = 25	880.68 ± 1975.95 N = 25	6.91 ± 14.69 N = 25	3.02 ± 5.64 N = 25	2.76 ± 5.07 N = 25
C	3.38 ± 6.33 N = 25	0.99 ± 0.79 N = 25	543.20 ± 964.20 N = 25	5.15 ± 9.23 N = 25	3.26 ± 4.36 N = 25	1.77 ± 2.32 N = 25
Friedman's Statistics	$\chi 2$ (2) = 19.316 $p < .001$	$\chi 2$ (2) = 10.042 $p = .007$	$\chi 2$ (2) = 38.00 $p < .001$	$\chi 2$ (2) = 27.758 $p < .001$	$\chi 2$ (2) = 23.343 $p < .001$	$\chi 2$ (2) = 17.956 $p < .001$
Wilcoxon signed rank test	AB $p = .001$	AB n.s.	AB $p < .001$	AB $p < .001$	AB $p < .001$	AB $p < .001$
P-values	AC $p < .001$	AC $p = .002$	AC $p < .001$	AC $p < .001$	AC $p = .001$	AC $p < .001$
	BC n.s.	BC n.s.	BC n.s.	BC n.s.	BC n.s.	BC n.s.

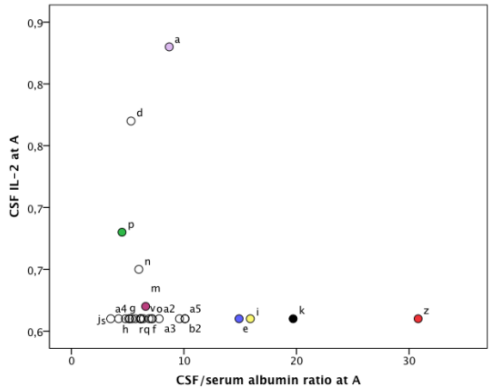
The increases in CSF cytokines were of a larger magnitude than those seen in plasma; IL-2, IL-8, IL-10, and IL-13 all increased to about 500% or more of their initial concentrations, while TNF more than doubled. Relative changes in these CSF cytokines showed highly significant intercorrelations, indicating that subjects with high increases in one cytokine also had increases in other cytokines. CSF concentrations of IL-2, IL-10, IL-12, and IL-13 displayed large interindividual variations following surgery (see Figure 1), while the changes in Il-8 and TNF were more consistent.

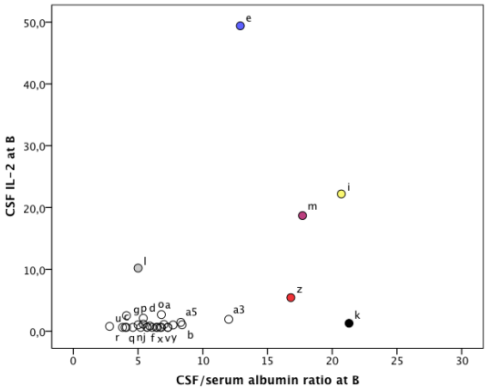
Serum and CSF cytokine levels

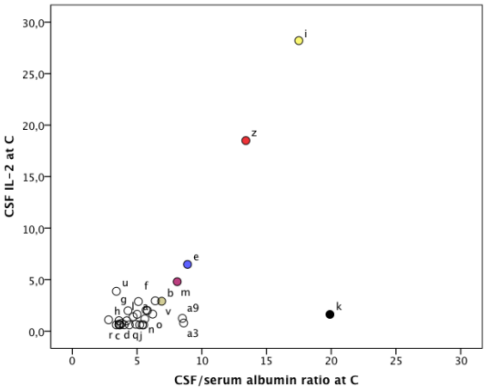
No significant correlations between serum and CSF concentrations (absolute values) of any of the cytokines at any sampling were detected.

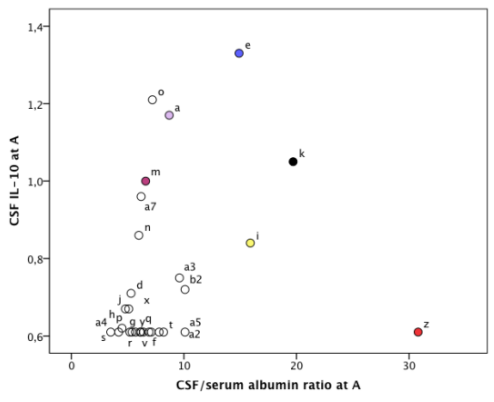
High responders

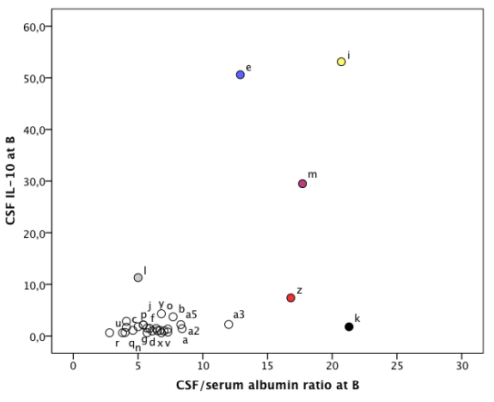
When plotting the data, a subgroup consisting of 10 individuals (defined as having remarkably large increases in IL-2, IL-10, IL-12 and IL-13) was shown to have markedly larger CSF cytokine increases. These individuals did not differ from the other subjects in any preoperative parameters. Moreover, this subgroup had significantly higher CSF/serum albumin ratios than the rest of the study group at B (U = 38.00, $p = .003$) and C (U = 45.00, $p = .008$); see Figure 1.

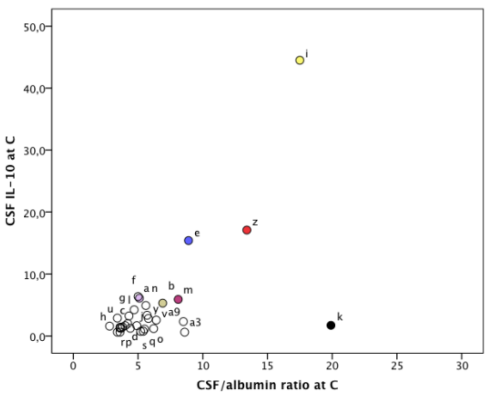

CSF IL-2 at A
CSF/serum albumin ratio at A


CSF IL-2 at B
CSF/serum albumin ratio at B


CSF IL-2 at C
CSF/serum albumin ratio at C


CSF IL-10 at A
CSF/serum albumin ratio at A


CSF IL-10 at B
CSF/serum albumin ratio at B


CSF IL-10 at C
CSF/albumin ratio at C

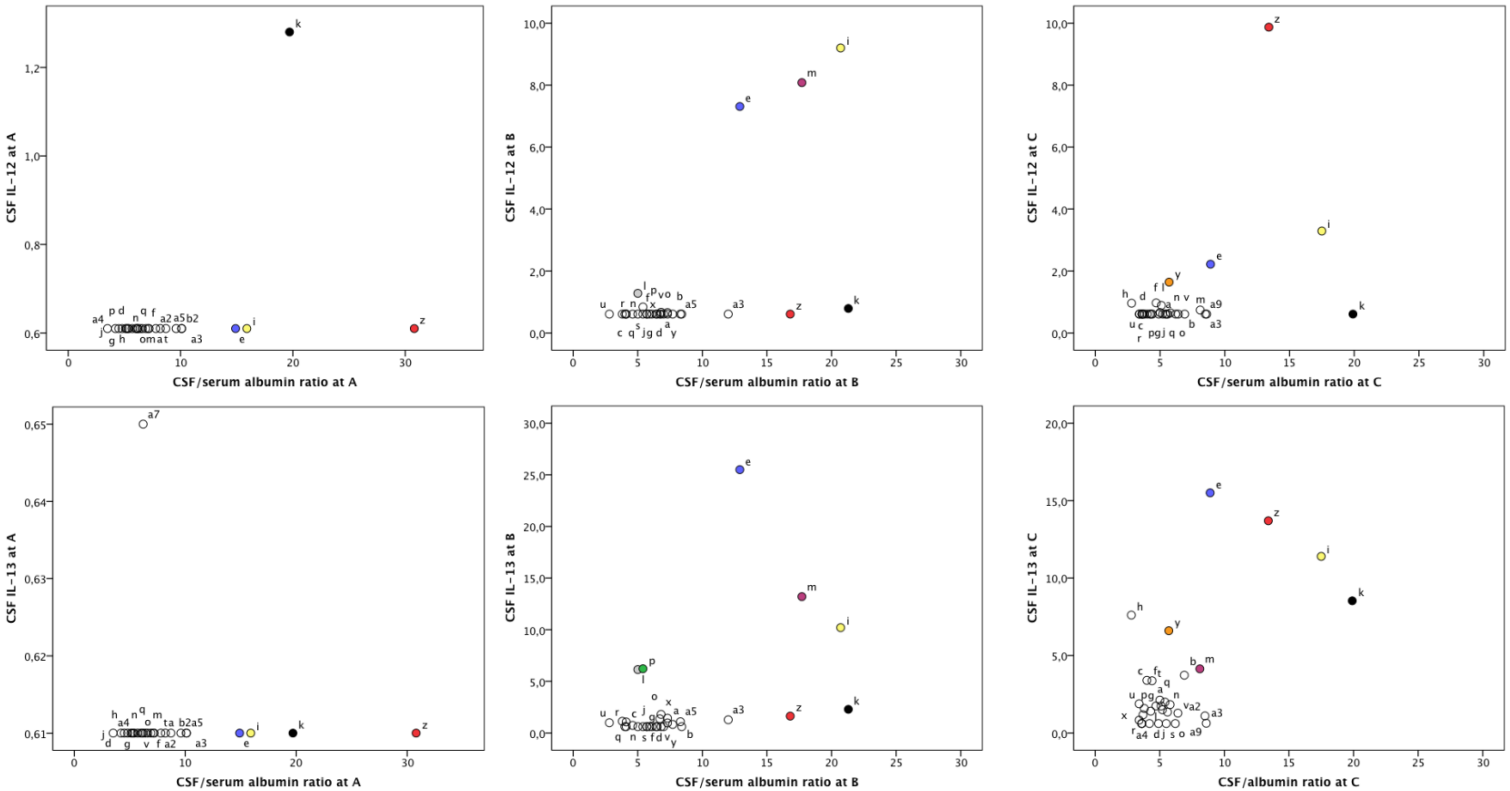


Figure 1. Cytokine levels of CSF IL-2, IL-10, IL-12 and IL-13 (pg/mL) plotted against CSF/serum albumin ratios at the three different assessment points; A, B, and C in the full sample (individuals re-coded as letters) with high responders colour coded.

Five of these subjects had abnormal CSF/serum albumin ratios (> 11.8 as the upper range of the established reference interval for the method (78)) at one or several of the assessment points. The remaining high responders had relatively stable CSF/serum albumin ratios within the normal range. The group of high responders had significantly higher CSF concentrations of almost all cytokines assessed (IL-2, -4, -5, -8, -10, -12, -13, TNF, and IFN-γ) at B and C (*p's* ranged from .042 to < .001), and had significantly higher serum concentrations of IL-4 ($p = .029$) at A and IL-8 at A ($p = .027$) and B ($p = .16$).

CSF cortisol

For cortisol, a significant increase was seen between A, B, and C (χ^2 (2) = 28.08, $p < .001$), and pairwise post-hoc comparisons revealed the following changes from A (20.61 ± 6.73 nmol/L) to C (51.69 ± 22.24 nmol/L; $p < .001$), and from B (22.05 ± 14.41 nmol/L) to C ($p < .001$) in the whole sample. Exclusion of those subjects who had abnormal CSF/serum albumin ratios did not markedly affect these results (χ^2 (2) = 21.09, $p < .001$).

No correlations between CSF/serum albumin ratios and CSF cortisol concentrations were detected at any of the assessment points. CSF cortisol showed a significant correlation with CSF IL-10 and IL-8 (*rho* = .42, $p = .025$ and *rho* = .39, $p = .043$, respectively) at B. Relative change in CSF cortisol from B to C correlated significantly with relative changes in CSF IL-2 (*rho* = .40, $p = .039$). No other correlations between cortisol and the different cytokines were identified at any of the sampling points.

Possible confounders

Confounding by age, gender, and BMI were assessed by computing correlation analyses with serum and CSF cytokine concentrations and CSF cortisol concentrations at A, B, and C. Age showed a positive correlation with serum IL-10 at B (*rho* = .54, $p = .001$) and BMI was negatively correlated with the CSF concentration of IL-8 at baseline (*rho* = -.41, $p = .034$). Correlations were found between age and cortisol concentrations at A (*rho* = .49, $p = .010$) and C (*rho* = .66, $p < .001$), such that older age was associated with higher levels of CSF cortisol. No other correlations were found.

6.4 Aim IV: Markers for astroglial and neuronal integrity in response to peripheral surgery

CSF markers for neuronal and astroglial integrity

For concentrations of the different markers, see Table 3. A significant increase in CSF T-tau concentrations during surgery (A to B, not significant, B to C, $p = .039$, A to C, $p = .026$) was noted. No significant changes in any of the other four biomarkers were seen.

Table 3. CSF protein concentrations (mean ± SD (number of samples)) at A, B, and C.

	A-samples	B-samples	C-samples	*ANOVA*
T-tau (ng/L)	368 ± 152 (n = 28)	375 ± 148 (n = 27)	404 ± 151 (n = 28)	*F = 3.86*
		A-B: n.s.	B-C: p = .039	*p = .028*
			A-C: p = .026	*(n = 26)*
P-tau (ng/L)	48 ± 22 (n = 29)	47 ± 22 (n = 29)	51 ± 20 (n = 29)	*n.s (n = 29)*
NFL (ng/L)	366 ± 493 (n = 28)	354 ± 428 (n = 27)	360 ± 449 (n = 28)	*n.s. (n = 26)*
A β 42 (ng/L)	708 ± 184 (n = 28)	710 ± 182 (n = 27)	697 ± 207 (n = 28)	*n.s. (n = 26)*
GFAp (ng/L)	813 ± 416 (n = 28)	827 ± 427 (n = 27)	1164 ± 1535 (n = 28)	*n.s. (n=26)*

Relationship between neuronal and astroglial integrity and BBB integrity

A significant correlation was found both between CSF T-tau (B: *rho* = .46, *p* = .016 and C: *rho* = .49, *p* = .009) and P-tau (B: *rho* = .49, *p* = .023, and C: *rho* = .47, *p* = .012) and albumin CSF/serum ratio at B and C. In addition, T-tau correlated with βTP at A (*rho* = .47, *p* = .01) but not at B or C.

Possible confounders

To investigate the relationship between T-tau and possible confounders such as age, gender, diabetes, and amount of propofol administered, correlational analysis was carried out. No correlations with any of these factors were detected. A positive correlation was seen between level of CSF T-tau and total dose of bupivacaine administered (*rho* = .41, *p* = .034 and *rho* = .43, *p* = .022, at A and B, respectively).

7 MAIN FINDINGS

Personality traits and biomarkers

- In this non-psychiatric sample, CSF HVA/5-HIAA ratios correlated negatively with Cooperativeness.
- CSF IL-10 was correlated with Verbal Aggression (positively) and Inhibited Aggression (negatively), and CSF cortisol co-varied with Novelty Seeking. Negative correlations were found between serum IL-10, IFN-γ and Self-directedness.
- Serum βTP, as a marker of BBB dysfunction, correlated positively with Monotony Avoidance and Impulsivity.
- No significant correlations were seen between aggressive and impulsive personality traits and CSF levels of insulin, thyroid hormones, markers for astroglial or neuronal integrity, or CSF/serum albumin ratios.

Biomarkers during surgery

- Insulin levels in the brain seemed to be regulated differently from the periphery during knee arthroplastic surgery.
- Levels of CSF cytokines were markedly increased during and after peripheral surgery, as compared with peripheral cytokine levels.
- A subgroup reacted with large increases in CSF cytokines during knee surgery. This group had higher CSF/serum albumin ratios than the rest of the subjects.
- T-tau increased significantly during surgery.

8 DISCUSSION

The main findings of this thesis pertain to two major areas: First, co-variation between aggressive and impulsive personality traits, CSF and serum biomarkers were identified in this non-psychiatric population. Second, levels of peripheral and central insulin, markers of inflammation, and neuronal and astroglial integrity displayed showed different patterns of change in response to peripheral surgery. This might be of use in future research, both regarding psychiatric and behavioural biomarkers and in studies on psychiatric and somatic complications to surgery.

8.1 Personality traits and biomarkers

Specifically, we found correlations between impulsive and aggressive personality traits and HVA/5-HIAA ratio, IL-10, cortisol, and βTP, thus confirming some previous findings from studies on forensic psychiatric patients (69, 70). In contrast to our study group, forensic patients often display frequent heightened or outright pathological impulsive and/or aggressive behaviours, which are likely due to the underlying neuropathophysiology. In our normal population group, we cannot be expected to find connections between biomarkers and personality traits or behaviours as strong as those found in more extreme populations. However, the fact that some co-variations were also confirmed in our study makes a stronger case for these biomarkers actually reflecting specific pathophysiological mechanisms in the regulation of impulsivity and aggression. Previous studies have also established a connection between aggression, impulsivity, and the immune system, with different patterns of immune activation in instrumental and reactive aggression (29). However, the connection between IL-10 levels and personality traits has not been described previously.

Monoamine systems

In the present study, we found that increased CSF HVA/5-HIAA ratio was inversely correlated with Cooperativeness (a character dimension measuring degree of empathy versus hostility). Increased ratios between the CSF concentrations of HVA over 5-HIAA have been found to correlate with psychopathic personality dimensions, mainly relating to behaviour, in two forensic psychiatric study groups (69, 70), and with measures of impulsive aggression in normal controls as well as in personality-disordered subjects (45). Our results thus point towards confirming a relationship between aggressive and impulsive personality traits and increased HVA/5-HIAA ratio, even in non-psychiatric patients.

BBB integrity

In this study, no correlations were seen between CSF/ serum albumin ratio and any of the personality traits studied. However, levels of βTP in serum, which can be interpreted as signs of a damaged BBB, correlated with impulsive personality traits. Even though we found no correlations between CSF/serum albumin ratios and personality traits, these findings might be seen as pointing in the same direction as earlier findings, showing a connection between BBB dysfunction and impulsive behaviour. Increased CSF/serum albumin ratios have previously been found in forensic psychiatric groups of violent offenders (52, 69) in suicide attempters (67), and in individuals with psychosis (66). It should, however, be noted that βTP is a protein with many functions, and has been associated with risk for several cardiovascular disorders (147). It has also been shown to play a proinflammatory role in the immune response (148). Thus, it might be possible that the connections seen here between levels of βTP and impulsive personality traits are related to inflammation and not specifically to BBB dysfunction.

Insulin

No significant correlations were seen between aggressive or impulsive personality traits and CSF levels of insulin. Insulin has been found to play a number of different and important roles in the CNS, for example in cognition and regulation of feeding behaviour (93-95). Higher levels of CSF insulin have been shown in persons making a violent suicide attempt, than in persons making a less violent one (6). However, this connection between insulin, impulsivity and aggressive behaviour was not replicated regarding personality traits in our study in psychiatrically healthy persons. Apart from this study, research regarding behaviour, personality traits, and levels of CSF insulin seems to be scarce, as most studies concentrate on diabetes and dementia.

Thyroid hormone

An association between increased serum thyroid activity and criminal recidivism, aggression, and psychopathic personality traits has been found in several studies (101, 102). In the present study, we were unable to replicate any findings regarding impulsive and aggressive personality traits and T3 or T4. This might be due to specific mechanisms in incarcerated individuals, such as the most violent being less prone to depressive reactions.

Inflammation

Correlations between aggressive and impulsive personality traits and levels of CSF inflammatory markers were observed, the strongest one between IL-10 and Verbal Aggression. IL-10 functions mostly as an anti-inflammatory cytokine. In animal studies, it has been found to inhibit the depression-like behavioural effects of proinflammatory cytokines like IL-1, IFN-γ, and TNF (149, 150). No correlation between hostility and stimulated Th2 cytokine production (including serum IL-10) was seen in a study of a healthy sample of 193 men and women (151). As shown both in the present study and earlier research (152), serum levels of IL-10 do not necessarily correspond with CSF levels. Thus, the relevance of the correlations between serum IL-10 and behaviour remains uncertain. In all, IL-10 does not seem to have been extensively studied in connection with personality traits. However, it is gaining increasing interest in studies regarding depression, looking at its role linking disruption of the HPA axis with decreased cytokine production (152). Low Self-directedness (a character dimension reflecting resourcefulness versus helplessness) has been shown to be associated with inflammatory activation in earlier studies (41, 42), and our results might further elucidate that connection.

Biomarkers for axonal and neuroglial integrity

We did not find any significant correlations between biomarkers for axonal and neuroglial integrity and any of the studied personality traits. Tau levels have been associated with aggressive behaviour in Alzheimer patients (132), but we found no connections with aggressive personality traits in this group without dementia.

8.2 Change in biomarkers during surgical stress

In earlier papers from this study, we have reported on changes in BBB permeability, CSF monoamine metabolites and thyroid hormones in response to peripheral surgery (137-139). In summary, CSF concentrations of both HVA and 5-HIAA increased substantially after surgery, but returned to their initial levels overnight, thyroid hormone levels seemed to be separately regulated in CSF and serum, and CSF/serum albumin ratio decreased significantly during the intervention. Besides looking at the connections between personality and different biomarkers described above, we wanted to further investigate the reactions of the CNS to a surgical intervention.

Insulin

Insulin concentrations in CSF were approximately 10% of those in serum at sampling A and B, and concentrations in CSF showed no significant change even as serum concentrations of insulin rose to 200% at C. The present study is one of the first to describe insulin changes in the CNS as compared to those in serum during a stressful event, such as orthopaedic surgery, in humans. The results, with small fluctuations in CSF insulin compared to very large changes in serum insulin, unrelated to the CSF/serum albumin ratio, indicate that the BBB is actively protecting the brain from large variations in this hormone. The weak correlations found between serum and CSF insulin are consistent with results seen in other studies (153). Our findings are in line with the data showing that insulin transport across the BBB is highly regulated (87-89), and that the brain is protected from peripheral fluctuations in insulin. Since insulin is increasingly recognized as an important player in cognition (90), (although its possible role in postoperative delirium remains unclear (154)), studying the way it is affected by different forms of stress is important.

Cytokines

Cytokine levels were found to increase substantially more in CSF than in serum, in response to peripheral surgery. This might reflect a quicker turnover of inflammatory markers peripherally, or, more probably, is a sign of the fact that inflammatory reactions in the brain are regulated separately from those in the periphery (155). The length of the observed reaction in the CSF, which lasted at least until the morning following surgery, may be a reflection of the relatively high age of the study population, as older individuals have been shown to have a more prolonged immune reaction (156).

The fact that ten of the individuals had a much higher increase in central cytokines might indicate that some persons are prone to a stronger central inflammatory reaction, which might be a sign of greater vulnerability to complications such as postoperative delirium, cognitive decline, and depression. Increased levels of CSF IL-8 in hip fracture patients have been found to have a relationship with the development of delirium (125). On the contrary, previously published data from the present study show decreased CSF albumin concentrations and thus lower CSF/serum albumin ratios, indicating decreased permeability of the BBB for macromolecules during surgery (137). Interestingly, the same individuals who had a larger CSF cytokine increase also had CSF/serum albumin ratios in the top part of the variance indicating a "leakier" BBB which might indicate that the reduced permeability seen in most patients is really part of the normal reaction to stress (an "oyster effect" protecting the CNS). None of the subjects in this study were reported to have any postoperative complications, although no formal neuropsychiatric testing or follow-up was done. However, it might be possible that the increased CSF/serum albumin ratios and stronger immune activation are both signs of an increased vulnerability to post-surgical complications.

Cortisol

Cortisol levels increased significantly during surgery. However, it cannot be excluded that the increase seen reflects the normal diurnal variation rather than a direct effect of the surgery, although cortisol diurnal variations may be influenced by stress (157, 158). As there was no non-surgical control group, the reason for the changes seen cannot be determined with certainty. Serum levels of cortisol are known to reflect a pulsatile secretion and were therefore considered to be too unreliable to merit inclusion in the study.

Biomarkers for axonal and neuroglial integrity

Some signs implicated impaired cortical axonal integrity, as shown as a significant increase in biomarkers for Alzheimer's pathology (T-tau). However, it should be noted that tau is also released from neurons in the absence of damage, possibly reflecting physiological neuronal plasticity and/or function, which in turn could be influenced by stress, anaesthesia, and/or surgical trauma (159), something which limits the interpretation of these results. In a recent report, Tang and co-workers (2011) showed that T-tau and P-tau increased progressively in CSF after surgery for idiopathic nasal CSF leak correction (119). However, the study sample was small (n = 11) and the surgical procedures must have involved manipulation of the meninges and thus, to some extent, the neural tissue. Our aim was to replicate this study in a population undergoing peripheral surgery. We were able to

partly replicate the findings by Tang and co-workers (2011), and our findings might be reflecting more of a real CNS reaction to peripheral trauma. Accumulating evidence suggests that surgery and anaesthesia may have harmful effects on the brain, inducing neurodegeneration and Alzheimer-like changes (160). Geriatric patients are particularly vulnerable. The risk of postoperative delirium increases with age, and cognitive decline is reported to occur in about a quarter of cases, following surgery in elderly persons (160-162).

8.3 Limitations

There are important limitations. This exploratory, naturalistic study reflects a true clinical sample (although subjects with psychiatric or neurological illness were excluded). Thus, the data represent a real clinical variation. However, the nature of the study limits any interpretation of causality of the findings. One important limiting factor is that the study did not include a non-surgical, age- and sex-matched control group, and thus confounding across the majority or all subjects from, for example, the surgical procedure, potential chronic pain and anesthesia on biomarkers, cannot be discounted. Another limitation is the sample size.

Statistical limitations

The study sample was small, leading to difficulties in obtaining sufficient power for multiple comparisons. We addressed this problem by setting the significance level lower ($p < .01$) when computing correlations regarding personality traits. Neither personality and character dimensions nor the different markers can be said to be independent of each other, and thus Bonferroni correction, or similar, would be too conservative, yielding a high risk of false negatives. In paper I, a total of 24 comparisons were made, resulting in a risk of 0.24 false positive outcomes. In paper II, a total of 117 comparisons were made between twelve personality scales and seven biomarkers, resulting in a risk of 1.17 false positive findings. The correlations between biomarkers and personality traits were strong with *rho's* > .50 and significant at the $p < .01$ level. However, due to the larger number of comparisons between personality traits and biomarkers, these findings would not survive Bonferroni corrections. In addition, the correlations described in papers I and II should merely be regarded descriptive as binominal correlations indicate a systematic dependence between two variables without disclosing anything about their causal relationship.

Variation in insulin and inflammatory markers during surgery was marked and survived Bonferroni correction. This was in part due to a low number of comparisons to be corrected for. (three). Of note is that the concentrations of IL- 4, 5, and 13 were at the lower detection level of the analysis kit and may not reflect the true concentration. Consequently, this may have influenced the variation and statistical analysis. In addition, fluctuations in T-tau were moderate and did not survive Bonferroni correction, although pairwise comparisons between A and C, and B and C identified significant differences at the $p < .05$ level.

Possible confounders

The subjects were elderly and heterogeneous regarding long-term use of medications and concomitant medical disorders. It should be noted that levels of several inflammatory markers increase with age (163, 164). Nineteen of the patients were overweight, with a BMI over 25, a condition characterized by increased inflammatory activation (165). Seven of the patients had diabetes mellitus, also known to affect levels of different inflammatory markers (166). We cannot rule out that propofol, bupivacaine or other medications might have had effects on neuroendocrine or inflammatory responses (167-172).

Procedures

We were unable to have continuous CSF samplings. Thus, we chose to collect samples three hours after termination of surgery to be able to pinpoint chemical reactions during the operation (considering absorption time for CSF (173)) and in the morning after surgery to assess the continued neurobiological activity during the night. It was not regarded as feasible to continue sampling for a longer period, as the patients no longer had any need for intrathecal catheters. We also cannot rule out an effect of the catheter or lumbar puncture; this, however, would be a problem in all studies of CSF, and it would be unlikely given the magnitude of changes. Yeager et al. (1999) found that when comparing a group of patients who had spinal anaesthesia to those who had general anaesthesia, inflammation, as measured in CSF levels of IL-6, was more marked in the spinal anaesthesia group (116). Studies of animals have shown that prolonged catheterization may lead to changes in the spinal cord cytokine environment (174). As the catheter was made from an inert material, and all lumbar punctures were performed by an experienced anaesthesiologist, we hypothesize that the inflammatory changes caused by this procedure would be minimal, although we cannot rule them out. Also, the stress of lumbar puncture has been shown to influence levels of monoamine metabolites, though not the HVA/5-HIAA ratio (175). Another limitation common to all research on CSF is that it is not possible to fully

evaluate the relationship between the concentration at the site of synthesis in the brain and the lumbar area where the samples are drawn (176). For serum samples, one limitation is that no baseline samples were drawn before the start of the anaesthesiological procedure.

Pain

The main reason to perform knee arthroplasty is pain, something that affected all of the patients to a varying degree. This pain in itself may have influenced the degree of inflammatory activation in the subjects (3). Measures of subjective pain were not assessed in the present investigation.

Inflammatory markers

IL-6 is a proinflammatory cytokine, which has been extensively studied as a marker for inflammatory activation in recent years, especially in connection with psychiatric disorders and cognition; for example, IL-6 has been shown to be significantly higher in CSF from suicide attempters than from healthy controls, and also to be elevated in Alzheimer's disease (177, 178). Due to limitations of the analysis kit, which was chosen for its suitability for CSF analysis, this cytokine was not included in our study.

Another important limitation is that several of the cytokines had median concentrations at the lower detection levels of the assays used, indicating that the laboratory assays truncated the true variation considerably. It can therefore not be ruled out that these cytokines may have fluctuated in response to the surgical intervention, as their true concentration levels may not have been detected in this study.

Insulin

With regard to the post operative insulin changes, it cannot be ruled out that these were caused by the catabolic insulin resistance resulting from a long fasting period (179). Even though patients had intravenous glucose infusions, food intake may have stimulated insulin secretion by neural reflexes in addition to the regulation in response to serum glucose levels. We found no correlations between insulin concentrations and glucose administered in this study.

βTP

The measurement of βTP holds some promise for future neuropsychiatric research, as it is an interesting potential marker for BBB leakage into blood; in that case, kidney function should be carefully examined before any conclusions are drawn, as βTP is known to be elevated in kidney failure (146). One advantage of assessing BBB integrity by the measurement of

serum βTP is that there is no need to draw CSF. More likely, however, is that further studies regarding the connections between βTP, cardiovascular risk, inflammation, sleep and personality factors might yield interesting results.

9 CONCLUSIONS, FUTURE PERSPECTIVES, AND CLINICAL IMPLICATIONS

In summary, correlations between CSF HVA/5-HIAA ratio, CSF IL-10 and cortisol, serum IL-10 and IFN-γ, and aggressive and impulsive personality traits were found in this non-psychiatric study group. However, we were unable to replicate some of the other findings in violent offenders of correlations between impulsive and aggressive behaviour, CSF levels of 5-HT and DA metabolites, and damaged BBB integrity, as indicated through increased CSF/serum albumin ratio (58-60, 70). This should come as no surprise, as it is becoming increasingly clear that biomarkers like the CSF HVA/5-HIAA ratio may be extremely aberrant in some individuals displaying very unusual and violent behaviour, while having limited predictive value on a group level. Nevertheless, the fact that some of the previously established relationships were replicated in this non-psychiatric sample, suggests that there may be common neurochemical pathways that modulate aggression and impulsivity (or protective factors). While different biomarkers might be more indicative of recent behaviour than of a certain trait underlying propensity to that behaviour, these findings do indicate that there may be a certain degree of overlap between disordered and non-disordered populations.

In addition, we found aggressive and impulsive personality traits to be connected with inflammatory activation in the brain and in the periphery. The correlation seen between CSF IL-10 and Verbal Aggression is strong, and has not been described before. Correlations between inflammatory activation and personality have, however, been reported in other healthy populations (41). It seems increasingly likely that such a connection exists both in healthy individuals and persons displaying aggressive and impulsive behaviour, and this would be worthy of further study. One possibility may be to further investigate different gene variants coding for inflammatory markers in such populations. Another approach in the future might be to do a placebo-controlled, double-blinded, treatment trial, investigating the effects of anti-inflammatory treatments on impulsivity and aggression in patient populations.

The results of this study also indicate that, during non-neurological surgery, the BBB protects the brain from large hormonal variations in the periphery. Results from the present study also suggest that the immune reactions in the

brain during and after the intervention are regulated separately from those in the periphery. Changes in inflammatory markers in the brain were found to be of a larger magnitude than those in serum. A subgroup with a larger CSF cytokine increase also had heightened BBB macromolecular permeability. Inflammatory activation in the CNS has been linked to postoperative cognitive decline, delirium, and depression (125, 160, 161). Our results might aid in future studies identifying individuals at risk for such complications. In accordance with Tang et al (119), we also found a modest, but significant increase in a biomarker for Alzheimer's pathology following surgery. The relevance of such changes for post-operative neurological status is important to pursue, especially as the population keeps getting older and surgeries of this kind will become even more frequent. Along the same lines, it would be interesting to look at long-term effects of peripheral surgery on neurological and psychiatric functioning. In all, these results further show that reactions to surgical stress are regulated differently in the brain than in the periphery.

It has become increasingly clear that personality traits play a role in vulnerability to psychological trauma, and also to cardiovascular disease, in a way that is possibly connected with inflammation (180, 181). Our findings may help in planning future research elucidating these connections.

The present study has no control group and should be regarded as descriptive. Specific relationships between the different biomarkers and personality dimensions described herein would need to be formally tested in case-controlled and experimental clinical studies.

Clinical implications

The possible connections between a high CSF cytokine response and a disrupted BBB are worthy of further exploration. Individuals with a heightened vulnerability to trauma, and an increased risk of post-surgical complications, might be identified before surgery. Our results may aid in finding methods of identification, and also in the development of possible preventive treatments.

ACKNOWLEDGEMENT

I wish to express thanks to the following:

My family.

My main supervisor Caroline Wass, whose excellent guidance has been invaluable.

My co-supervisors Marianne Kristiansson, Henrik Anckarsäter, and Henrik Zetterberg, for their patient, quick, and generous help and guidance.

Rolf Anckarsäter, who together with Henrik Anckarsäter planned the original study and then collected all the samples.

Thomas Nilsson, who was exceedingly helpful in interpreting the results of the personality testing.

My other co-authors, Kaj Blennow, Bo Ahrén, Anders Forsman, and Agneta Holmäng, for their generous assistance.

Åse Holl and Stefan Axelsson, for swift, competent, and invaluable practical help.

Monika Montell, and the late Agneta Brimse for excellent secretarial help.

Jan Kowalski, for excellent statistical help.

My colleagues and friends, in research, at work and everywhere, for outstanding support.

The National Board of Forensic Medicine (Rättsmedicinalverket) has been very generous regarding both funding and practical support. Data collection and analyses were funded by grants from Gothenburg Medical Society, the Health & Medical Care Committee of the Regional Executive Board, Region Västra Götaland to Dr Rolf Anckarsäter, to Prof Kaj Blennow, ALF-agreement ALFGBG-139671 and to Prof Henrik Zetterberg, ALF-agreement ALFGBG-144341. The PhD program, including participation in group activities and education, was funded through grants from the Swedish Research Council (Henrik Anckarsäter, VR521-2010-2689). Gothenburg Medical Society, the Greta Johansson memorial fund, the Wilhelm and Martina Lundgren Scientific fund and the Bror Gadelius Foundation also

have given generous grants, both for time to write up the dissertation and for travel to international conferences to present my work. I also want to thank the European Association for the Study of Diabetes and the Swedish Medical Society for travel grants.

REFERENCES

1. Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharmacol Ther* 2001;69(3):89-95.
2. Schmidt HD, Shelton RC, Duman RS. Functional biomarkers of depression: diagnosis, treatment, and pathophysiology. *Neuropsychopharmacology* 2011;36(12):2375-94.
3. Slade GD, Conrad MS, Diatchenko L, Rashid NU, Zhong S, Smith S, Rhodes J, Medvedev A, Makarov S, Maixner W, Nackley A. Cytokine biomarkers and chronic pain: association of genes, transcription, and circulating proteins with temporomandibular disorders and widespread palpation tenderness. *Pain* 2011;152(12):2802-12.
4. Blennow K, Hampel H, Weiner M, Zetterberg H. Cerebrospinal fluid and plasma biomarkers in Alzheimer disease. *Nat Rev Neurol* 2010;6(3):131-44.
5. Asberg M, Traskman L, Thoren P. 5-HIAA in the cerebrospinal fluid. A biochemical suicide predictor? *Arch Gen Psychiatry* 1976;33(10):1193-7.
6. Westling S, Ahren B, Traskman-Bendz L, Westrin A. High CSF-insulin in violent suicide attempters. *Psychiatry Res* 2004;129(3):249-55.
7. Mazzarello P. Cesare Lombroso: an anthropologist between evolution and degeneration. *Funct Neurol* 2011;26(2):97-101.
8. Hook EB. Behavioral implications of the human XYY genotype. *Science* 1973;179(4069):139-50.
9. Terburg D, Morgan B, van Honk J. The testosterone-cortisol ratio: A hormonal marker for proneness to social aggression. *Int J Law Psychiatry* 2009;32(4):216-23.
10. Montoya ER, Terburg D, Bos PA, van Honk J. Testosterone, cortisol, and serotonin as key regulators of social aggression: A review and theoretical perspective. *Motiv Emot* 2012;36(1):65-73.
11. Brunner HG, Nelen M, Breakefield XO, Ropers HH, van Oost BA. Abnormal behavior associated with a point mutation in the structural gene for monoamine oxidase A. *Science* 1993;262(5133):578-80.
12. Verhoeven FE, Booij L, Kruijt AW, Cerit H, Antypa N, Does W. The effects of MAOA genotype, childhood trauma, and sex on trait and state-dependent aggression. *Brain Behav* 2012;2(6):806-13.
13. Gustavson C, Wass C, Mansson JE, Blennow K, Forsman A, Anckarsater H, Nilsson T.. Platelet monoamine oxidase B activity did not predict destructive personality traits or violent recidivism: a prospective study in male forensic psychiatric examinees. *Neuropsychobiology* 2010;61(2):87-96.

14. Cloninger CR. A unified biosocial theory of personality and its role in the development of anxiety states. *Psychiatr Dev* 1986;4(3):167-226.
15. Zammit S, Jones G, Jones SJ, Norton N, Sanders RD, Milham C, McCarthy GM, Jones LA, Cardno AG, Gray M, Murphy KC, O'Donovan MC, Owen MJ.. Polymorphisms in the MAOA, MAOB, and COMT genes and aggressive behavior in schizophrenia. *Am J Med Genet B. Neuropsychiatr Genet* 2004;128B(1):19-20.
16. McCrae RR, John OP. An introduction to the five-factor model and its applications. *J Pers* 1992;60(2):175-215.
17. Rantanen J, Metsapelto RL, Feldt T, Pulkkinen L, Kokko K. Long-term stability in the Big Five personality traits in adulthood. *Scand J Psychol* 2007;48(6):511-8.
18. Asberg M, Montgomery SA, Perris C, Schalling D, Sedvall G. A comprehensive psychopathological rating scale. *Acta Psychiatr Scand Suppl* 1978(271):5-27.
19. Gustavsson JP, Bergman H, Edman G, Ekselius L, von Knorring L, Linder J. Swedish universities Scales of Personality (SSP): construction, internal consistency and normative data. *Acta Psychiatr Scand* 2000;102(3):217-25.
20. Cloninger CR, Svrakic DM, Przybeck TR. A psychobiological model of temperament and character. *Arch Gen Psychiatry* 1993;50(12):975-90.
21. Sutin AR, Costa PT, Jr., Uda M, Ferrucci L, Schlessinger D, Terracciano A. Personality and metabolic syndrome. *Age (Dordr)* 2010;32(4):513-9.
22. Service SK, Verweij KJ, Lahti J, Congdon E, Ekelund J, Hintsanen M, Raikkonen K, Lehtimaki T, Kahonen M, Widen E, Taanila A, Veijola J, Heath AC, Madden PAF, Montgomery GW, Sabatti C, Jarvelin, M-R, Palotie A, Raitakari O, Viikari J, Martin, NG, Eriksson, JG, Keltikangas-Jarvinen L, Wray NR, Freimer NB. A genome-wide meta-analysis of association studies of Cloninger's Temperament Scales. *Transl Psychiatry* 2012;2:e116.
23. de Almeida RM, Ferrari PF, Parmigiani S, Miczek KA. Escalated aggressive behavior: dopamine, serotonin and GABA. *Eur J Pharmacol* 2005;526(1-3):51-64.
24. Patrick CJ. Psychophysiological correlates of aggression and violence: an integrative review. *Philos Trans R Soc Lond B Biol Sci* 2008;363(1503):2543-55.
25. Buckholtz JW, Meyer-Lindenberg A. MAOA and the neurogenetic architecture of human aggression. *Trends Neurosci* 2008;31(3):120-9.
26. Nelson RJ, Trainor BC, Chiavegatto S, Demas GE. Pleiotropic contributions of nitric oxide to aggressive behavior. *Neurosci Biobehav Rev* 2006;30(3):346-55.

27. Comai S, Tau M, Gobbi G. The psychopharmacology of aggressive behavior: a translational approach: part 1: neurobiology. *J Clin Psychopharmacol* 2012;32(1):83-94.
28. Zalcman SS, Siegel A. The neurobiology of aggression and rage: role of cytokines. *Brain Behav Immun* 2006;20(6):507-14.
29. Siegel A, Bhatt S, Bhatt R, Zalcman SS. The neurobiological bases for development of pharmacological treatments of aggressive disorders. *Curr Neuropharmacol* 2007;5(2):135-47.
30. Meloy JR. Empirical basis and forensic application of affective and predatory violence. *Aust N Z J Psychiatry* 2006;40(6-7):539-47.
31. Gvion Y, Apter A. Aggression, impulsivity, and suicide behavior: a review of the literature. *Arch Suicide Res* 2011;15(2):93-112.
32. Garcia-Forero C, Gallardo-Pujol D, Maydeu-Olivares A, Andres-Pueyo A. Disentangling impulsiveness, aggressiveness and impulsive aggression: an empirical approach using self-report measures. *Psychiatry Res* 2009;168(1):40-9.
33. Derefinko K, DeWall CN, Metze AV, Walsh EC, Lynam DR. Do different facets of impulsivity predict different types of aggression? *Aggress Behav* 2011;37(3):223-33.
34. Moeller FG, Barratt ES, Dougherty DM, Schmitz JM, Swann AC. Psychiatric aspects of impulsivity. *Am J Psychiatry* 2001;158(11):1783-93.
35. Pattij T, Vanderschuren LJ. The neuropharmacology of impulsive behaviour. *Trends Pharmacol Sci* 2008;29(4):192-9.
36. Fassino S, Daga GA, Piero A, Leombruni P, Rovera GG. Anger and personality in eating disorders. *J Psychosom Res* 2001;51(6):757-64.
37. Yoo HJ, Kim M, Ha JH, Chung A, Sim ME, Kim SJ,Lyoo, IK. Biogenetic temperament and character and attention deficit hyperactivity disorder in Korean children. *Psychopathology* 2006;39(1):25-31.
38. Hofvander B, Stahlberg O, Nyden A, Wentz E, degl'Innocenti A, Billstedt E, Forsman A, Gillberg C, Nilsson T, Rastam M, Anckarsater H. Life History of Aggression scores are predicted by childhood hyperactivity, conduct disorder, adult substance abuse, and low cooperativeness in adult psychiatric patients. *Psychiatry Res* 2011;185(1-2):280-5.
39. Duke AA, Begue L, Bell R, Eisenlohr-Moul T. Revisiting the Serotonin-Aggression Relation in Humans: A Meta-Analysis. *Psychological bulletin* 2013.
40. Haller J. The neurobiology of abnormal manifestations of aggression-A review of hypothalamic mechanisms in cats, rodents, and humans. *Brain research bulletin* 2012.
41. Henningsson S, Baghaei F, Rosmond R, Holm G, Landen M, Anckarsater H, Ekman A. Association between serum levels of C-

reactive protein and personality traits in women. *Behav Brain Funct* 2008;4:16.
42. Suchankova P, Baghaei F, Rosmond R, Holm G, Anckarsater H, Ekman A. Genetic variability within the S100B gene influences the personality trait self-directedness. *Psychoneuroendocrinology* 2011;36(6):919-923.
43. Coccaro EF, Beresford B, Minar P, Kaskow J, Geracioti T. CSF testosterone: relationship to aggression, impulsivity, and venturesomeness in adult males with personality disorder. *J Psychiatr Res 2007*;41(6):488-92.
44. Lee R, Petty F, Coccaro EF. Cerebrospinal fluid GABA concentration: relationship with impulsivity and history of suicidal behavior, but not aggression, in human subjects. *J Psychiatr Res* 2009;43(4):353-9.
45. Coccaro EF, Lee R. Cerebrospinal fluid 5-hydroxyindolacetic acid and homovanillic acid: reciprocal relationships with impulsive aggression in human subjects. *J Neural Transm* 2010;117(2):241-8.
46. Sinai C, Hirvikoski T, Vansvik ED, Nordstrom AL, Linder J, Nordstrom P, Jokinen J. Thyroid hormones and personality traits in attempted suicide. *Psychoneuroendocrinology* 2009;34(10):1526-32.
47. Segal MB. The choroid plexuses and the barriers between the blood and the cerebrospinal fluid. *Cell Mol Neurobiol* 2000;20(2):183-96.
48. Banks WA. Physiology and pathology of the blood-brain barrier: implications for microbial pathogenesis, drug delivery and neurodegenerative disorders. *J Neurovirol* 1999;5(6):538-55.
49. Alamo C, Lopez-Munoz F. New antidepressant drugs: beyond monoaminergic mechanisms. *Curr Pharm Des* 2009;15(14):1559-62.
50. Conn PJ, Tamminga C, Schoepp DD, Lindsley C. Schizophrenia: moving beyond monoamine antagonists. *Mol Interv* 2008;8(2):99-107.
51. Nordin C, Sjodin I. CSF monoamine patterns in pathological gamblers and healthy controls. *J Psychiatr Res* 2006;40(5):454-9.
52. Soderstrom H, Blennow K, Manhem A, Forsman A. CSF studies in violent offenders. I. 5-HIAA as a negative and HVA as a positive predictor of psychopathy. *J Neural Transm* 2001;108(7):869-78.
53. Lesch KP, Araragi N, Waider J, van den Hove D, Gutknecht L. Targeting brain serotonin synthesis: insights into neurodevelopmental disorders with long-term outcomes related to negative emotionality, aggression and antisocial behaviour. *Philos Trans R Soc Lond B Biol Sci* 2012;367(1601):2426-43.
54. Geracioti TD, Jr., Keck PE, Jr., Ekhator NN, West SA, Baker DG, Hill KK, Bruce AB, Wortman MD. Continuous covariability of dopamine and serotonin metabolites in human cerebrospinal fluid. *Biol Psychiatry* 1998;44(3):228-33.
55. Coccaro EF, Kavoussi RJ, Hauger RL. Serotonin function and antiaggressive response to fluoxetine: a pilot study. *Biol Psychiatry* 1997;42(7):546-52.

56. Coccaro EF, Kavoussi RJ. Fluoxetine and impulsive aggressive behavior in personality-disordered subjects. *Arch Gen Psychiatry* 1997;54(12):1081-8.
57. Salzman C, Wolfson AN, Schatzberg A, Looper J, Henke R, Albanese M, Schwartz J, Miyawaki E. Effect of fluoxetine on anger in symptomatic volunteers with borderline personality disorder. *J Clin Psychopharmacol* 1995;15(1):23-9.
58. Lidberg L, Asberg M, Sundqvist-Stensman UB. 5-Hydroxyindoleacetic acid levels in attempted suicides who have killed their children. *Lancet* 1984;2(8408):928.
59. Virkkunen M, Goldman D, Linnoila M. Serotonin in alcoholic violent offenders. *Ciba Found Symp* 1996;194:168-77; discussion 177-82.
60. Linnoila M, Virkkunen M, Scheinin M, Nuutila A, Rimon R, Goodwin FK. Low cerebrospinal fluid 5-hydroxyindoleacetic acid concentration differentiates impulsive from nonimpulsive violent behavior. *Life Sci* 1983;33(26):2609-14.
61. Stoltenberg SF, Christ CC, Highland KB. Serotonin system gene polymorphisms are associated with impulsivity in a context dependent manner. *Prog Neuropsychopharmacol Biol Psychiatry* 2012;39(1):182-91.
62. Volavka J, Czobor P, Nolan K, Sheitman B, Lindenmayer JP, Citrome L, et al. Overt aggression and psychotic symptoms in patients with schizophrenia treated with clozapine, olanzapine, risperidone, or haloperidol. *J Clin Psychopharmacol* 2004;24(2):225-8.
63. Volavka J, Citrome L, Huertas D. Update on the biological treatment of aggression. *Actas Esp Psiquiatr* 2006;34(2):123-35.
64. O'Sullivan SS, Evans AH, Lees AJ. Dopamine dysregulation syndrome: an overview of its epidemiology, mechanisms and management. *CNS Drugs* 2009;23(2):157-70.
65. Agren H, Mefford IN, Rudorfer MV, Linnoila M, Potter WZ. Interacting neurotransmitter systems. A non-experimental approach to the 5HIAA-HVA correlation in human CSF. *J Psychiatr Res* 1986;20(3):175-93.
66. Bauer K, Kornhuber J. Blood-cerebrospinal fluid barrier in schizophrenic patients. *Eur Arch Psychiatry Neurol Sci* 1987;236(5):257-9.
67. Bayard-Burfield L, Alling C, Blennow K, Jonsson S, Traskman-Bendz L. Impairment of the blood-CSF barrier in suicide attempters. *Eur Neuropsychopharmacol* 1996;6(3):195-9.
68. Engstrom G, Alling C, Blennow K, Regnell G, Traskman-Bendz L. Reduced cerebrospinal HVA concentrations and HVA/5-HIAA ratios in suicide attempters. Monoamine metabolites in 120 suicide attempters and 47 controls. *Eur Neuropsychopharmacol* 1999;9(5):399-405.

69. Anckarsater H, Forsman A, Blennow K. Increased CSF/serum albumin ratio: a recurrent finding in violent offenders. *Acta Neurol Scand* 2005;112(1):48-50.
70. Soderstrom H, Blennow K, Sjodin AK, Forsman A. New evidence for an association between the CSF HVA:5-HIAA ratio and psychopathic traits. *J Neurol Neurosurg Psychiatry* 2003;74(7):918-21.
71. Abbott NJ, Friedman A. Overview and introduction: The blood-brain barrier in health and disease. *Epilepsia* 2012;53 Suppl 6:1-6.
72. Abbott NJ. Inflammatory mediators and modulation of blood-brain barrier permeability. *Cell Mol Neurobiol* 2000;20(2):131-47.
73. Brasnjevic I, Steinbusch HW, Schmitz C, Martinez-Martinez P. European NanoBioPharmaceutics Research I. Delivery of peptide and protein drugs over the blood-brain barrier. *Prog Neurobiol* 2009;87(4):212-51.
74. Quan N, Banks WA. Brain-immune communication pathways. *Brain Behav Immun* 2007;21(6):727-35.
75. Williams K, Alvarez X, Lackner AA. Central nervous system perivascular cells are immunoregulatory cells that connect the CNS with the peripheral immune system. *Glia* 2001;36(2):156-64.
76. Polfliet MM, Zwijnenburg PJ, van Furth AM, van der Poll T, Dopp EA, Renardel de Lavalette C, van Kesteren-Hendrikx EM, van Rooijen N, Dijkstra CD, van den Berg TK. Meningeal and perivascular macrophages of the central nervous system play a protective role during bacterial meningitis. *J Immunol* 2001;167(8):4644-50.
77. Hudson LC, Bragg DC, Tompkins MB, Meeker RB. Astrocytes and microglia differentially regulate trafficking of lymphocyte subsets across brain endothelial cells. *Brain Res* 2005;1058(1-2):148-60.
78. Blennow K, Fredman P, Wallin A, Gottfries CG, Karlsson I, Langstrom G, Skoog I, Svennerholm L, Wikkelso C. Protein analysis in cerebrospinal fluid. II. Reference values derived from healthy individuals 18-88 years of age. *Eur Neurol* 1993;33(2):129-33.
79. Soderstrom H, Blennow K, Manhem A, Forsman A. CSF studies in violent offenders. II. Blood-brain barrier dysfunction without concurrent inflammation or structure degeneration. *J Neural Transm* 2001;108(7):879-86.
80. Urade Y, Hayaishi O. Prostaglandin D synthase: structure and function. *Vitam Horm* 2000;58:89-120.
81. Beuckmann CT, Lazarus M, Gerashchenko D, Mizoguchi A, Nomura S, Mohri I, Uesugi A, Kaneko T, Mizuno N, Hayaishi O, Urade Y. Cellular localization of lipocalin-type prostaglandin D synthase (beta-trace) in the central nervous system of the adult rat. *J Comp Neurol* 2000;428(1):62-78.
82. Hoffmann A, Nimtz M, Conradt HS. Molecular characterization of beta-trace protein in human serum and urine: a potential diagnostic marker for renal diseases. *Glycobiology* 1997;7(4):499-506.

83. Schell C, Frungieri MB, Albrecht M, Gonzalez-Calvar SI, Kohn FM, Calandra RS, Mayerhofer A.. A prostaglandin D2 system in the human testis. *Fertil Steril* 2007;88(1):233-6.
84. White DM, Takeda T, DeGroot LJ, Stefansson K, Arnason BG. Beta-trace gene expression is regulated by a core promoter and a distal thyroid hormone response element. *J Biol Chem* 1997;272(22):14387-93.
85. Arrer E, Meco C, Oberascher G, Piotrowski W, Albegger K, Patsch W. Beta-Trace protein as a marker for cerebrospinal fluid rhinorrhea. *Clin Chem* 2002;48(6 Pt 1):939-41.
86. McEwen BS, Reagan LP. Glucose transporter expression in the central nervous system: relationship to synaptic function. *Eur J Pharmacol* 2004;490(1-3):13-24.
87. Banks WA, Jaspan JB, Kastin AJ. Effect of diabetes mellitus on the permeability of the blood-brain barrier to insulin. *Peptides* 1997;18(10):1577-84.
88. Banks WA, Jaspan JB, Kastin AJ. Selective, physiological transport of insulin across the blood-brain barrier: novel demonstration by species-specific radioimmunoassays. *Peptides* 1997;18(8):1257-62.
89. Banks WA, Jaspan JB, Huang W, Kastin AJ. Transport of insulin across the blood-brain barrier: saturability at euglycemic doses of insulin. *Peptides* 1997;18(9):1423-9.
90. Zhao WQ, Chen H, Quon MJ, Alkon DL. Insulin and the insulin receptor in experimental models of learning and memory. *Eur J Pharmacol* 2004;490(1-3):71-81.
91. Lozovsky DB, Kopin IJ, Saller CF. Modulation of dopamine receptor supersensitivity by chronic insulin: implication in schizophrenia. *Brain Res* 1985;343(1):190-3.
92. Ghasemi R, Haeri A, Dargahi L, Mohamed Z, Ahmadiani A. Insulin in the Brain: Sources, Localization and Functions. *Mol Neurobiol* 2012.
93. Figlewicz DP, Sipols AJ, Seeley RJ, Chavez M, Woods SC, Porte D, Jr. Intraventricular insulin enhances the meal-suppressive efficacy of intraventricular cholecystokinin octapeptide in the baboon. *Behav Neurosci* 1995;109(3):567-9.
94. Bruning JC, Gautam D, Burks DJ, Gillette J, Schubert M, Orban PC, Klein R, Krone W, Müller-Wieland D, Kahn CR. Role of brain insulin receptor in control of body weight and reproduction. *Science* 2000;289(5487):2122-5.
95. Grillo CA, Piroli GG, Kaigler KF, Wilson SP, Wilson MA, Reagan LP. Downregulation of hypothalamic insulin receptor expression elicits depressive-like behaviors in rats. *Behav Brain Res* 2011;222(1):230-5.
96. Virkkunen M. Reactive hypoglycemic tendency among habitually violent offenders. A further study by means of the glucose tolerance test. *Neuropsychobiology* 1982;8(1):35-40.

97. Virkkunen M. Reactive hypoglycemic tendency among habitually violent offenders. *Nutr Rev* 1986;44 Suppl:94-103.
98. Virkkunen M, Rissanen A, Franssila-Kallunki A, Tiihonen J. Low non-oxidative glucose metabolism and violent offending: an 8-year prospective follow-up study. *Psychiatry Res* 2009;168(1):26-31.
99. Bauer M, Heinz A, Whybrow PC. Thyroid hormones, serotonin and mood: of synergy and significance in the adult brain. *Mol Psychiatry* 2002;7(2):140-56.
100. Soderstrom H, Forsman A. Elevated triiodothyronine in psychopathy - possible physiological mechanisms. *J Neural Transm* 2004;111(6):739-44.
101. Yhede R, Blennow K, Forsman A, Soderstrom H. The activity in the CNS catecholaminergic systems covaries with thyroid hormone metabolism in humans. *J Neural Transm* 2003;110(12):1369-73.
102. Strawn JR, Ekhator NN, D'Souza BB, Geracioti TD, Jr. Pituitary-thyroid state correlates with central dopaminergic and serotonergic activity in healthy humans. *Neuropsychobiology* 2004;49(2):84-7.
103. Alm PO, af Klinteberg B, Humble K, Leppert J, Sorensen S, Tegelman R, Thorell LH, Lidberg L.. Criminality and psychopathy as related to thyroid activity in former juvenile delinquents. *Acta Psychiatr Scand* 1996;94(2):112-7.
104. Goshen I, Kreisel T, Ounallah-Saad H, Renbaum P, Zalzstein Y, Ben-Hur T, Levy-Lahad E, Yirmiya R. A dual role for interleukin-1 in hippocampal-dependent memory processes. *Psychoneuroendocrinology* 2007;32(8-10):1106-15.
105. Raman D, Sobolik-Delmaire T, Richmond A. Chemokines in health and disease. *Exp Cell Res* 2011;317(5):575-89.
106. Ranjit N, Diez-Roux AV, Shea S, Cushman M, Seeman T, Jackson SA, Ni H.. Psychosocial factors and inflammation in the multi-ethnic study of atherosclerosis. *Arch Intern Med* 2007;167(2):174-81.
107. Kraus MR, Schafer A, Faller H, Csef H, Scheurlen M. Psychiatric symptoms in patients with chronic hepatitis C receiving interferon alfa-2b therapy. *J Clin Psychiatry* 2003;64(6):708-14.
108. Elomaa AP, Niskanen L, Herzig KH, Viinamaki H, Hintikka J, Koivumaa-Honkanen H, Honkalampi K, Valkonen-Korhonen M, Harvima IT, Lehto SM.. Elevated levels of serum IL-5 are associated with an increased likelihood of major depressive disorder. *BMC Psychiatry* 2012;12:2.
109. Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat Rev Neurosci* 2008;9(1):46-56.
110. Monji A, Kato T, Kanba S. Cytokines and schizophrenia: Microglia hypothesis of schizophrenia. *Psychiatry Clin Neurosci* 2009;63(3):257-65.

111. Suarez EC, Lewis JG, Kuhn C. The relation of aggression, hostility, and anger to lipopolysaccharide-stimulated tumor necrosis factor (TNF)-alpha by blood monocytes from normal men. *Brain Behav Immun* 2002;16(6):675-84.
112. Suarez EC, Lewis JG, Krishnan RR, Young KH. Enhanced expression of cytokines and chemokines by blood monocytes to in vitro lipopolysaccharide stimulation are associated with hostility and severity of depressive symptoms in healthy women. *Psychoneuroendocrinology* 2004;29(9):1119-28.
113. Marsland AL, Prather AA, Petersen KL, Cohen S, Manuck SB. Antagonistic characteristics are positively associated with inflammatory markers independently of trait negative emotionality. *Brain Behav Immun* 2008;22(5):753-61.
114. Sutin AR, Terracciano A, Deiana B, Naitza S, Ferrucci L, Uda M, Schlessinger D, Costa PT Jr.. High neuroticism and low conscientiousness are associated with interleukin-6. *Psychol Med* 2010;40(9):1485-93.
115. Woiciechowsky C, Asadullah K, Nestler D, Glockner F, Robinson PN, Volk HD, et al. Different release of cytokines into the cerebrospinal fluid following surgery for intra- and extra-axial brain tumours. *Acta Neurochir (Wien)* 1997;139(7):619-24.
116. Yeager MP, Lunt P, Arruda J, Whalen K, Rose R, DeLeo JA. Cerebrospinal fluid cytokine levels after surgery with spinal or general anesthesia. *Reg Anesth Pain Med* 1999;24(6):557-62.
117. Reis HJ, Teixeira AL, Kalman J, Bogats G, Babik B, Janka Z, Schlessinger D, Costa PT Jr.Different inflammatory biomarker patterns in the cerebrospinal fluid following heart surgery and major non-cardiac operations. *Curr Drug Metab* 2007;8(6):639-42.
118. Pearson A, de Vries A, Middleton SD, Gillies F, White TO, Armstrong IR, Andrew R, Seckl JR, MacLullich AM. Cerebrospinal fluid cortisol levels are higher in patients with delirium versus controls. *BMC Res Notes* 2010;3:33.
119. Tang JX, Baranov D, Hammond M, Shaw LM, Eckenhoff MF, Eckenhoff RG. Human Alzheimer and inflammation biomarkers after anesthesia and surgery. *Anesthesiology* 2011;115(4):727-32.
120. Kohl BA, Deutschman CS. The inflammatory response to surgery and trauma. Curr Opin Crit Care 2006;12(4):325-32.
121. Reikeras O. Immune depression in musculoskeletal trauma. *Inflamm Res* 2010;59(6):409-14.
122. Kalman J, Juhasz A, Bogats G, Babik B, Rimanoczy A, Janka Z, Penke B, Palotás A.. Elevated levels of inflammatory biomarkers in the cerebrospinal fluid after coronary artery bypass surgery are predictors of cognitive decline. *Neurochem Int* 2006;48(3):177-80.
123. Suchankova P, Henningsson S, Baghaei F, Rosmond R, Holm G, Ekman A. Genetic variability within the innate immune system

influences personality traits in women. *Genes Brain Behav* 2009;8(2):212-7.
124. Cibelli M, Fidalgo AR, Terrando N, Ma D, Monaco C, Feldmann M, Takata M, Lever IJ, Nanchahal J, Fanselow MS, Maze M. Role of interleukin-1beta in postoperative cognitive dysfunction. *Ann Neurol* 2010;68(3):360-8.
125. MacLullich AM, Edelshain BT, Hall RJ, de Vries A, Howie SE, Pearson A, Middleton SD, Gillies F, Armstrong IR, White TO, Cunningham C, de Rooij SE, van Munster BC. Cerebrospinal fluid interleukin-8 levels are higher in people with hip fracture with perioperative delirium than in controls. *J Am Geriatr Soc* 2011;59(6):1151-3.
126. Lannan EA, Galliher-Beckley AJ, Scoltock AB, Cidlowski JA. Proinflammatory actions of glucocorticoids: glucocorticoids and TNFalpha coregulate gene expression in vitro and in vivo. *Endocrinology* 2012;153(8):3701-12.
127. Moons WG, Eisenberger NI, Taylor SE. Anger and fear responses to stress have different biological profiles. *Brain Behav Immun* 2010;24(2):215-9.
128. Alink LR, van Ijzendoorn MH, Bakermans-Kranenburg MJ, Mesman J, Juffer F, Koot HM. Cortisol and externalizing behavior in children and adolescents: mixed meta-analytic evidence for the inverse relation of basal cortisol and cortisol reactivity with externalizing behavior. *Dev Psychobiol* 2008;50(5):427-50.
129. Seubert P, Vigo-Pelfrey C, Esch F, Lee M, Dovey H, Davis D,, Sinha S, Schlossmacher M, Whaley J, Swindlehurst C. Isolation and quantification of soluble Alzheimer's beta-peptide from biological fluids. *Nature* 1992;359(6393):325-7.
130. Wiltfang J, Lewczuk P, Riederer P, Grunblatt E, Hock C, Scheltens P, Hampel H, Vanderstichele H, Iqbal K, Galasko D, Lannfelt L, Otto M, Esselmann H, Henkel AW, Kornhuber J, Blennow K. Consensus paper of the WFSBP Task Force on Biological Markers of Dementia: the role of CSF and blood analysis in the early and differential diagnosis of dementia. *World J Biol Psychiatry* 2005;6(2):69-84.
131. Forlenza OV, Diniz BS, Gattaz WF. Diagnosis and biomarkers of predementia in Alzheimer's disease. *BMC Med* 2010;8:89.
132. Guadagna S, Esiri MM, Williams RJ, Francis PT. Tau phosphorylation in human brain: relationship to behavioral disturbance in dementia. *Neurobiol Aging* 2012;33(12):2798-806.
133. Pouw MH, Hosman AJ, van Middendorp JJ, Verbeek MM, Vos PE, van de Meent H. Biomarkers in spinal cord injury. *Spinal Cord* 2009;47(7):519-25.
134. Zetterberg H, Hietala MA, Jonsson M, Andreasen N, Styrud E, Karlsson I, Edman A, Popa C, Rasulzada A, Wahlund LO, Mehta PD,

Rosengren L, Blennow K, Wallin A.. Neurochemical aftermath of amateur boxing. *Arch Neurol* 2006;63(9):1277-80.
135. Reinsfelt B, Westerlind A, Blennow K, Zetterberg H, Ricksten SE. Open-heart surgery increases cerebrospinal fluid levels of Alzheimer-associated amyloid beta. *Acta Anaesthesiol Scand* 2012.
136. Reinsfelt B, Ricksten SE, Zetterberg H, Blennow K, Freden-Lindqvist J, Westerlind A. Cerebrospinal fluid markers of brain injury, inflammation, and blood-brain barrier dysfunction in cardiac surgery. *Ann Thorac Surg* 2012;94(2):549-55.
137. Anckarsater R, Vasic N, Kristiansson M, Zetterberg H, Blennow K, Anckarsäter H. Cerebrospinal fluid protein reactions during non-neurological surgery. Acta Neurol Scand 2007;115(4):254-9.
138. Anckarsater R, Zetterberg H, Blennow K, Anckarsater H. Association between thyroid hormone levels and monoaminergic neurotransmission during surgery. *Psychoneuroendocrinology* 2007;32(8-10):1138-43.
139. Anckarsater R, Zetterberg H, Mansson JE, Blennow K, Anckarsater H. Non-neurological surgery results in a neurochemical stress response. *J Neural Transm* 2008;115(3):397-9.
140. Brandstrom S, Schlette P, Przybeck TR, Lundberg M, Forsgren T, Sigvardsson S, Nylander PO, Nilsson LG, Cloninger RC, Adolfsson R. Swedish normative data on personality using the Temperament and Character Inventory. *Compr Psychiatry* 1998;39(3):122-8.
141. Laakso A, Wallius E, Kajander J, Bergman J, Eskola O, Solin O, Ilonen T, Salokangas RK, Syvälahti E, Hietala J. Personality traits and striatal dopamine synthesis capacity in healthy subjects. *Am J Psychiatry* 2003;160(5):904-10.
142. Gustavsson JP, Pedersen NL, Asberg M, Schalling D. Origins of individual differences in anxiety proneness: a twin/adoption study of the anxiety-related scales from the Karolinska Scales of Personality (KSP). *Acta Psychiatr Scand* 1996;93(6):460-9.
143. Schalling D, Edman G. The Karolinska Scales of Personality (KSP) manual: An inventory for assessing temperament dimensions associated with vulnerability for psychosocial deviance. Stockholm, Sweden: Department of Psychiatry, Karolinska Institutet; 1993.
144. Siegel A, Douard J. Who's flying the plane: serotonin levels, aggression and free will. *Int J Law Psychiatry* 2011;34(1):20-9.
145. Miller G. Science and the courts. In mock case, biological evidence reduces sentences. *Science* 2012;337(6096):788.
146. Ferguson MA, Waikar SS. Established and emerging markers of kidney function. *Clin Chem* 2012;58(4):680-9.
147. Orenes-Pinero E, Manzano-Fernandez S, Lopez-Cuenca A, Marin F, Valdes M, Januzzi JL. Beta-Trace Protein: From GFR Marker to Cardiovascular Risk Predictor. *Clin J Am Soc Nephrol* 2013.

148. Joo M, Sadikot RT. PGD synthase and PGD2 in immune resposne. *Mediators Inflamm* 2012
149. Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. *Annu Rev Immunol* 2001;19:683-765.
150. Mesquita AR, Correia-Neves M, Roque S, Castro AG, Vieira P, Pedrosa J, Palha JA, Sousa N. IL-10 modulates depressive-like behavior. *J Psychiatr Res* 2008;43(2):89-97.
151. Janicki-Deverts D, Cohen S, Doyle WJ. Cynical hostility and stimulated Th1 and Th2 cytokine production. *Brain Behav Immun* 2010;24(1):58-63.
152. Roque S, Correia-Neves M, Mesquita AR, Palha JA, Sousa N. Interleukin-10: a key cytokine in depression? *Cardiovasc Psychiatry Neurol* 2009;2009:187894.
153. Woods SC, Porte D, Jr. Relationship between plasma and cerebrospinal fluid insulin levels of dogs. *Am J Physiol* 1977;233(4):E331-4.
154. Bisschop PH, de Rooij SE, Zwinderman AH, van Oosten HE, van Munster BC. Cortisol, insulin, and glucose and the risk of delirium in older adults with hip fracture. *J Am Geriatr Soc* 2011;59(9):1692-6.
155. Irwin MR, Cole SW. Reciprocal regulation of the neural and innate immune systems. *Nat Rev Immunol* 2011;11(9):625-32.
156. Shaw AC, Joshi S, Greenwood H, Panda A, Lord JM. Aging of the innate immune system. *Curr Opin Immunol* 2010;22(4):507-13.
157. Buckingham JC. Glucocorticoids: exemplars of multi-tasking. *Br J Pharmacol* 2006;147 Suppl 1:S258-68.
158. Wong ML, Kling MA, Munson PJ, Listwak S, Licinio J, Prolo P, Karp B, McCutcheon IE, Geracioti TD Jr, DeBellis MD, Rice KC, Goldstein DS, Veldhuis JD, Chrousos GP, Oldfield EH, McCann SM, Gold PW.Pronounced and sustained central hypernoradrenergic function in major depression with melancholic features: relation to hypercortisolism and corticotropin-releasing hormone. *Proc Natl Acad Sci U S A* 2000;97(1):325-30.
159. Yamada K, Cirrito JR, Stewart FR, Jiang H, Finn MB, Holmes BB, Binder LI, Mandelkow EM, Diamond MI, Lee VM, Holtzman DM. In vivo microdialysis reveals age-dependent decrease of brain interstitial fluid tau levels in P301S human tau transgenic mice. *J Neurosci* 2011;31(37):13110-7.
160. Terrando N, Eriksson LI, Ryu JK, Yang T, Monaco C, Feldmann M, Jonsson Fagerlund M, Charo IF, Akassoglou K, Maze M. Resolving postoperative neuroinflammation and cognitive decline. *Ann Neurol* 2011;70(6):986-95.
161. Moller JT, Cluitmans P, Rasmussen LS, Houx P, Rasmussen H, Canet J, Rabbitt P, Jolles J, Larsen K, Hanning CD, Langeron O, Johnson T, Lauven PM, Kristensen PA, Biedler A, van Beem H, Fraidakis O,

Silverstein JH, Beneken JE, Gravenstein JS. Long-term postoperative cognitive dysfunction in the elderly ISPOCD1 study. ISPOCD investigators. International Study of Post-Operative Cognitive Dysfunction. *Lancet* 1998;351(9106):857-61.
162. O'Mahony R, Murthy L, Akunne A, Young J. Guideline Development G. Synopsis of the National Institute for Health and Clinical Excellence guideline for prevention of delirium. *Ann Intern Med* 2011;154(11):746-51.
163. Gomez CR, Boehmer ED, Kovacs EJ. The aging innate immune system. *Curr Opin Immunol* 2005;17(5):457-62.
164. Krabbe KS, Pedersen M, Bruunsgaard H. Inflammatory mediators in the elderly. *Exp Gerontol* 2004;39(5):687-99.
165. Fresno M, Alvarez R, Cuesta N. Toll-like receptors, inflammation, metabolism and obesity. *Arch Physiol Biochem* 2011;117(3):151-64.
166. Nikolajczyk BS, Jagannathan-Bogdan M, Shin H, Gyurko R. State of the union between metabolism and the immune system in type 2 diabetes. *Genes Immun* 2011;12(4):239-50.
167. Ansley DM, Lee J, Godin DV, Garnett ME, Qayumi AK. Propofol enhances red cell antioxidant capacity in swine and humans. *Can J Anaesth* 1998;45(3):233-9.
168. Takaono M, Yogosawa T, Okawa-Takatsuji M, Aotsuka S. Effects of intravenous anesthetics on interleukin (IL)-6 and IL-10 production by lipopolysaccharide-stimulated mononuclear cells from healthy volunteers. *Acta Anaesthesiol Scand* 2002;46(2):176-9.
169. Semba K, Adachi N, Arai T. Facilitation of serotonergic activity and amnesia in rats caused by intravenous anesthetics. *Anesthesiology* 2005;102(3):616-23.
170. Lu Y, Li L, Zhao X, Huang W, Wen W. Beta blocker metoprolol protects against contractile dysfunction in rats after coronary microembolization by regulating expression of myocardial inflammatory cytokines. *Life Sci* 2011;88(23-24):1009-15.
171. Takenami T, Yagishita S, Nara Y, Tsai YH, Hiruma H, Kawakami T, Hoka S. Spinal procaine is less neurotoxic than mepivacaine, prilocaine and bupivacaine in rats. *Reg Anesth Pain Med* 2009;34(3):189-95.
172. Wolfe JW, Butterworth JF. Local anesthetic systemic toxicity: update on mechanisms and treatment. *Curr Opin Anaesthesiol* 2011;24(5):561-6.
173. Edsbagge M, Tisell M, Jacobsson L, Wikkelso C. Spinal CSF absorption in healthy individuals. *Am J Physiol Regul Integr Comp Physiol* 2004;287(6):R1450-5.
174. DeLeo JA, Colburn RW, Rickman AJ, Yeager MP. Intrathecal catheterization alone induces neuroimmune activation in the rat. *Eur J Pain* 1997;1(2):115-22.

175. Hill KK, West SA, Ekhator NN, Bruce AB, Wortman MD, Baker DG, Geracioti TD Jr. The effect of lumbar puncture stress on dopamine and serotonin metabolites in human cerebrospinal fluid. *Neurosci Lett* 1999;276(1):25-8.
176. Reiber H. Dynamics of brain-derived proteins in cerebrospinal fluid. *Clin Chim Acta* 2001;310(2):173-86.
177. Conrad R, Schilling G, Bausch C, Nadstawek J, Wartenberg HC, Wegener I, Geiser F, Imbierowicz K, Liedtke R. Temperament and character personality profiles and personality disorders in chronic pain patients. *Pain* 2007;133(1-3):197-209.
178. Lindqvist D, Janelidze S, Hagell P, Erhardt S, Samuelsson M, Minthon L, Hansson O, Björkqvist M, Träskman-Bendz L, Brundin L. Interleukin-6 is elevated in the cerebrospinal fluid of suicide attempters and related to symptom severity. *Biol Psychiatry* 2009;66(3):287-92.
179. Spooren A, Kolmus K, Laureys G, Clinckers R, De Keyser J, Haegeman G, Gerlo S. Interleukin-6, a mental cytokine. *Brain Res Rev* 2011;67(1-2):157-83.
180. Mommersteeg PM, Pouwer F. Personality as a risk factor for the metabolic syndrome: a systematic review. *J Psychosom Res* 2012;73(5):326-33.
181. Elovainio M, Merjonen P, Pulkki-Raback L, Kivimaki M, Jokela M, Mattson N, Koskinen T, Viikari JS, Raitakari OT, Keltikangas-Jarvinen L. Hostility, metabolic syndrome, inflammation and cardiac control in young adults: The Young Finns Study. *Biological psychology* 2011;87(2):234-40.