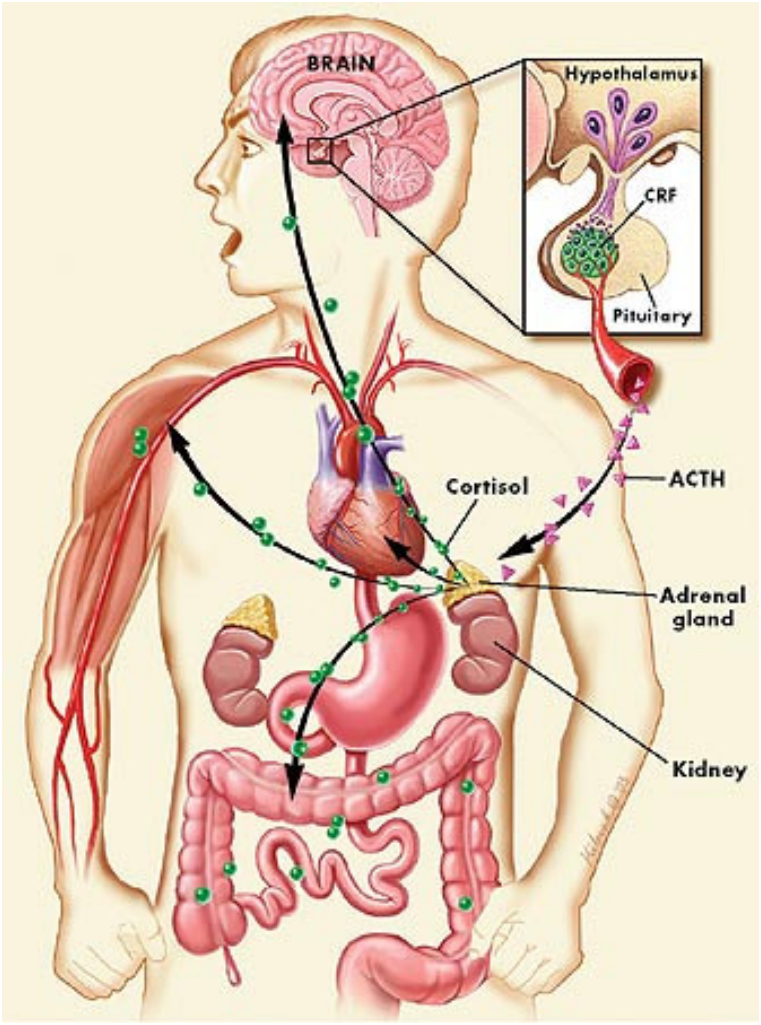
Integrating the Homeostatic Imbalances

Genetics and Physiology of Stress and the Emotions



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For my Dad, who showed me how to read slowly

Contents

1 Introduction and Concept 11

1.1 A Vulnerability that is more than skin deep……... 11
1.1.1 Fusion of Mind and Body…… 14
1.2. But where lies the roots to feelings…… 16
1.2.1 Stress and the Emotions…….. 17
1.2.2 Embodied Feelings…….. 19
1.3 The Question at Hand…….. 20
1.3.1 Empirical Approach…… 22
1.4 Bibliography…… 25

2 Common Cingulate response to Emotions 30

2.1 Abstract…….. **31**
2.2 Introduction…… **31**
2.3 Materials and Methods…….. **33**
2.3.1 Subjects and Procedure…….. 33
2.3.2 Experimental Design…….. 33
2.3.3 Image Analysis…… 34
2.3.4 Data Analysis……... 34
2.4 Results…….. **35**
2.4.1 Behavioural Data…… 35
2.4.2 Table 1…….. 35
2 4.3 Common Activations…… 36
2.4.4 Table 2…….. 37
2.4.5 Table 3…… 37
2.5 Discussion…… 38
2.6 Acknowledgements…… 39
2.7 Bibliography…… 40

3 Empathy in the Insula 43

3.1 Abstract …….. **44**
3.2 Introduction…….. **44**
3.3 Materials and Methods…….. **46**
3.3.1 Subjects and Procedures…….. 46

3.3.2 Visual Runs…… 46
3.3.3 Gustatory Runs…… 47
3.3.4 Self Report Measures…… 48
3.3.5 fMRI Acquisition…… 48
3.3.6 General fMRI data Analysis…… 49
3.3.7 Functional gustatory IFO definition…… 49
3.3.8 Correlation Analysis…… 49
3.4 Results…… **50**
3.4.1 Ratings…… 50
3.4.2 Neuroimaging Results……51
3.5 Discussion……**52**
3.6 Acknowledgements…… 55
3.7 Bibliography…… 56

4 COMT and Diagnostic Status Modulates Stress response 60

4.1 Abstract…… **61**
4.2 Introduction…… **61**
4.3 Materials and Methods…… **63**
4.3.1 Subjects…… 63
4.3.2 Laboratory Stress Test…… 64
4.3.3 Endocrine Assessment…… 65
4.3.4 Genotyping…… 65
4.3.5 Statistical Analysis……65
4.3.6 Table 1…… 67
4.4 Results……**67**
4.4.1 Demographics……..67
4.4.2 Endocrine and Subjective Stress response……67
4.4.3 Table 2……..71
4.4.4 Discussion……**71**
4.5 Table 3…… 72
4.6 Acknowledgements…… 74
4.7 Bibliography…… 75

5 Convergent MAOA, COMT and 5-HTT modulation of Stress 80

5.1 Abstract…… 81
5.2 Introduction…… 81
5.3 Materials and Methods…… 83

5.3.1 Participants……..……..……..……. 83
5.3.2 Laboratory Tasks……..……..……..……. 83
5.3.3 Endocrine Assessment……..……..……..... 84
5.3.4 Genotyping……..……..……..... 84
5.3.4.1 COMT Genotyping……..……..……..... 84
5.3.4.2 MAOA Genotyping……..……..……..... 84
5.3.4.3 5-HTT genotyping……..……..……..... 84
5.3.5 Statistical Analysis……..……..……..... 85
5.4 Results……..……..……..... 85
5.5 Discussion……..……..……..... 88
5.6 Acknowledgements……..……..……..... 91
5.7 Bibliography……..……..……..... 92

6 Summary and Conclusions 94
6.1.1 Summary……..……..……..... 94
6.2 Is Stress an Emotion……..……..……..... 97
6.3 Conclusions and Future Directions……..……..……..... 99
6.4 Nederlandse Samenvatting……..……..……..101
6.5 Bibliography……..……..……..……110
6.6 Related Bibliography……..……..……..... 114
6.7 Dankwoord..... 115

Chapter 1

1. Introduction and concept

> Men ought to know that from the brain, and from the brain only, arise our pleasures, joys, laughter and jests, as well as our sorrows, pains, griefs and tears. Through it, in particular, we think, see, hear, and distinguish the ugly from the beautiful, the bad from the good, the pleasant from the unpleasant. . . . It is the same thing which makes us mad or delirious, inspires us with dread and fear, whether by night or by day, brings sleeplessness, inopportune mistakes, aimless anxieties, absent-mindedness, and acts that are contrary to habit…..
>
> Attributed to Hippocrates; Fifth Century, B.C.

1.1. A Vulnerability That is More than Skin Deep

The Metmetian archipelago was founded in the year 2293 AD by a group of people who could not cope with the fast pace of the world at that time. For generations, there has been a string of severe psychiatric and physiological conditions haunting their blood lines and although the various nations they live in fairly succeeded in adopting strategies to cure these highly vulnerable people of their ills, the frequency of relapse and the status of constantly being at risk became virtually unbearable. The idea of moving away to start a settlement of the vulnerable was born during a group meeting of an advocate group for individuals suffering from psychiatric and other related disorders.

One very emotionally labile lady called Jexina, who was later recognized as the first Metmetian, had been suffering from emotional disorders all her adult life. She expressed her sorrow during one of the meetings and pondered as to how her life would have been better if she would be living on an island that is free from all the hassles of modern life. Every one in the gathering instantly recognized her thoughts and consensus was easily attained, among all those present, that this might be the only way for them to survive the world as it is. This led to the idea of moving to an uninhabited island where the vulnerable ones that could not withstand stressful life events, could start an alternative community based on protecting the people from environmental pathogens that led to series of disorders. These include both psychiatric and physiological conditions such as major depression, anxiety disorders, psychotic symptoms, gastrointestinal disorders, cardiovascular disorders, and a series of pain related disorders that had haunted these individuals for generations.

When the disease burden of the world became unbearable, suicide rampant among the youth, and there was no turning back to a slower pace of life, a slow but

steady exodus to a southern island called Hetzygon took place and the Metmetian archipelago was born.

The Metmetians lend their name from an inherited genetic trait. They carry more than seventy percent of the low activity variants of the major neuromodulatory enzymes. This allelic variation in their genes results in a reduced activity of enzymes such as catechol-O-methyltransferase (*COMT*) and brain derived neurotropic factor (*BDNF*). *COMT* plays a role in the degradation of catecholamines such as dopamine (DA) and norepinephrine (NA) with the *met* allelic variation of the functional polymorphism of the *COMT* genotype leading to a three to fourfold reduction in this degradation process. In the case of *BDNF*, this neurotropic factor is implicated in intracellular trafficking and activity dependent *BDNF* release, with the *met* allelic variation leading to a marked reduction of this process. Additionally, low activity allelic variations for the glucocorticoid, glutamtergic, gamma-aminobutyric acid (*GABA*), and monoaminergic neuromodulators, which were later called *met* alleles as in *COMT* and *BDNF*, were frequent in Metmetians. This genetic condition that is passed onto future Metmetian generations is the result of a rare mutation that led to a general inability of the body to produce enzymes responsible for the breakup of a broad class of the above named neuromodulators in the central and peripheral nervous system classified as G^3M^2B. These substances are neurotransmitters, hormones, biogenic amines, or neurotropic factors that make up the G^3M^2B subclass of neuromodulators. They are very functional in the maintenance of homeostasis and facilitate the central and peripheral response to cognitive, emotional and physiological processing necessary for the organism's adaptation to the ever changing environment.

A rare combination of these low catabolic activity allelic variations of the major neuromodulators led to the genetic trait that later became to be known as the Metmetian genetic condition. As a consequence of this reduced catabolic activity in their neuromodulatory system, Metmetians have an increased availability of catecholamines, glucocorticoids, *BDNF* and other stress-related markers in their brain/body which makes them especially vulnerable for stress related disorders.

The fact that the mammalian physiology has evolved such that adaptive mechanisms can in themselves become maladaptive and lead to disorders of severe etiologies in the face of persistent adverse circumstances, is rather unfortunate for the Metmetians. Given their genetic vulnerability to a variety of diseases triggered by environmental pathogens such as stressful experiences, their idea was that moving to an island where life could be adjusted such that even the most vulnerable individuals could prosper without a significant risk for developing stress-related diseases.

At the peak of Metmetian harmony, when fatalities or severe health conditions as a result of stressful life experience were reduced to zero, disaster struck hard at the heart of their social fabrics. Without warning of any kind, an extremely fast rolling object that was later identified as a huge boulder, the size of a foot ball field, broke off the southern cliff of mount Hetza and rolled down towards the sea, destroying everything in its path, including the sports complex with a huge boom. Pandemonium broke out, the survivors were in panic and people started running towards the open shores with hundreds venturing into the violet sea, desperate for a safer abode. The destruction was huge; the loss devastating; the whole sports complex and most of the surrounding buildings and virtually anything in the path of the giant rock was crushed. Bodies could be seen jumbled up amongst the debris. Remarkably however, a lot of the dead bodies were of those who died not directly due to the explosion but rather, as a result of a mysterious after effect they must have suffered. Bodies were scattered along the pavements that marked the flight path of survivors of the impact.

The damage was so immense that no emergency service no matter how organized and well equipped, could have handled the situation and taken care of the needs of the victims adequately. Most of all, the Metmetians were ill prepared for such a calamity. Their society was organized such that all difficulties which potentially could be experienced as stressful were avoided. One could imagine that the number of deaths that was directly caused by the disaster on the Metmetian Island of Hetzygon may just be the tip of the iceberg. One month after the disaster, a string of individuals began to develop sleeping disorders, and in a short time, half the youth were either suffering from major depression or other related disorders, or were affected by some form of somatic disorder like chronic pain or cardiovascular diseases. It became common place to see one person suddenly bursting out loud in cries which is most certainly echoed by others, for Metmetians are very well known for their highly empathic nature.

People began to report a multitude of complaints some of which were so complex that the physicians together with Metmetian thinkers and researchers were left dumfounded as to how to deal with the situation. The prevalence of emotional disorders like generalized anxiety, major depression (MD), post traumatic stress disorder (PTSD), and psychotic symptoms rose from near zero marks to about forty eight percent of individuals in the general population. Suicide, that was never heard of for years, became the number one cause of death and about seventy percent of the youth were addicted to at least one form of psychoactive drug or alcohol. Thus, the long term consequences of such a disaster became indeed apparent in Hetzygon.

The highly efficient dietary system that succeeded in maintaining health and prevents disease was not effective any more. The successful philosophy of prevention became rather useless when psychiatric disorders with somatic symptoms reached pandemic proportions. The strain of events that followed the disaster impacted the Metmetian society enormously. Even the preservation of law and order that every one took for granted, crumbled away when some highly emotional youngsters started indulging themselves in behaviors that were far from being acceptable.

This unfortunate turn of events was later commented on by one of the Metmetian physiologists as a reminder of the outdated hypothesis in psychoneuroendocrinology, also known as the *stress diathesis theory*. As he put it in an emergency conference designed to find solutions to the crisis: our present crisis is a resonance of the *stress diathesis theory*. According to this theory, appropriate responsiveness to daily life stressors is crucial for adequate functioning, while inappropriate responsiveness impair growth and lead to a number of physiological and psychiatric disorders (Plotsky 1998; McEwen 1998; Sapolsky 2000; Chamandari et al. 2005; Moffitt et al. 2005).

The history of the Metmetians was strongly influenced by their existing genetic risk. This was the strongest motivating factor behind the creation of a near stress free archipelago. However, unpredictable disasters such as these leading to the prevalence of emotional disorders at pandemic proportions underscore the complex nature of gene-environment interaction in the maintenance of health. Thus the Metmetian tragedy and the turn of events that led to it, epitomizes that some vulnerabilities are simply more than skin deep. Such vulnerabilities, that are strong determinants of a large proportion of the disease burden facing the world today, warrants careful and meticulous scientific investigations, as suggested in Figure 1.1 below.

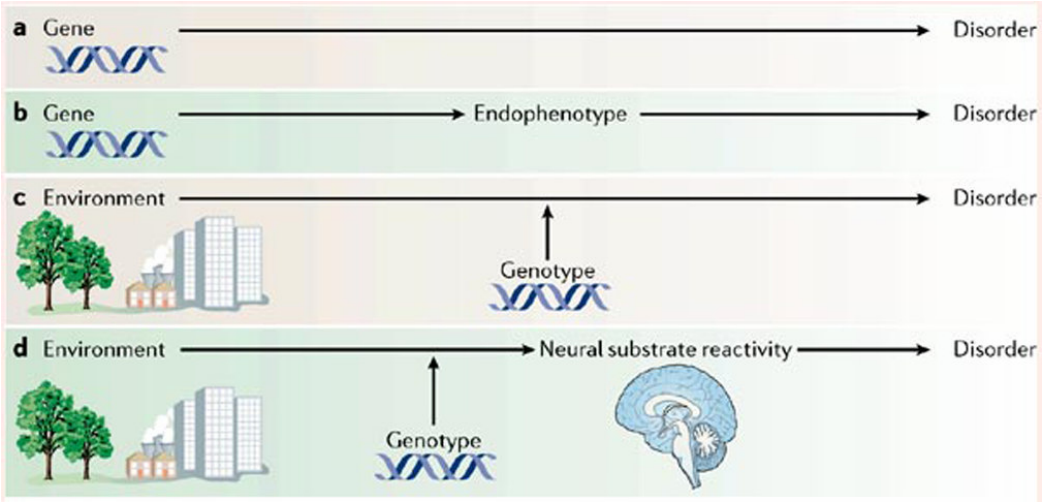

Figure 1.1. Adopted from Caspi and Moffitt 2006. Approaches to psychiatric genetic research. (a) The gene-to-disorder approach assumes a direct linear relationship between genes and disorder. (b) The endophenotypes approach replaces the disorder outcomes with intermediate phenotypes. (c) The gene-environment interaction approach assumes that genes moderate the effect of environmental pathogens on disorder. (d) Neuroscience complements the latter research by specifying the proximal role of nervous system reactivity in the gene-environment interaction.

1.1.1. Fusion of Mind and Body

Modern day reality however, is far from being Metmetian. While the Metmetians are unique in their enormous homogeneity relating to their genetic risk factors, present day humans tend to be diverse in their genetic susceptibility to stress related disorders. A bulk of the Metmetians suffered the unbearable consequence of the fateful disaster; however, it is likely that similar effects will be evident in not more than 20 percent of any typical human population in the year 2006, given a similar disaster. This implies that unlike the Metmetian population, there exists a normal distribution within a given human population as to how prone individuals are to the consequences of life stress. Regardless, the disease burden in industrial economies of present times is strongly impacted by a prevalence of stress related psychiatric disorders like MD, that are believed to have genetic origins (Caspi and Moffitt 2006; Meyer-Lindenbergh and Weinberger 2006).

The human body has evolved to react to the outside world through a cascade of mechanisms. Stressors that are of evolutionary importance, like predator encounters, leads to a cascade of bodily responses. These physiological mechanisms in conjunction with cognitive processes are necessary for adaptation and survival. However, given the individual differences in the bodily responses to environmental challenges, these biologically determined factors that to some extent mediates personality, may perhaps also modulate disease vulnerability to complex diseases like MD. Co-occurring psychiatric disorders could therefore be seen as an ‘extreme’ of interindividual difference in coping to stressful experience. Indeed, in some people, stress and extreme emotional encounters leads to coping and successful adaptation, while in other, similar experiences can lead to severe health consequences, and in worst cases death.

Present day reality that is continuously recruiting our abilities to adapt to either desirable or adverse changes in our environment. This process of environmental adaptation goes hand in hand with internal bodily changes in the form of autonomic arousal. Similar to avoiding burns or extremely heated environments, fighting off hard and fierce an attack of a disorientated rascal on the streets, running away from a

menacing predator, or avoiding psychologically stressful or emotional conditions, the human body undergoes physiological changes that are designed to maintain balance (homeostasis) during the experience of daily stressors like a job interview. Unlike evolutionarily valid stressors such as predator encounter, modern day stressors like job interviews are social rather than physical in nature. Similar to these very somatic examples like a predator encounter, humans undergo internal bodily changes consciously or unconsciously during exposure to socially emotional and stressful experiences. The physiological responses that accompany these experiences enable us to maintain the integrity of our body and thereby enhance survival in challenging situations. Such actions of either avoidance or approach in situations where the environmental events are favorable or threatening to our psychological and physiological well being, are designed to maintain the balance of our inner milieu as well as our needs and goals.

As Claude Bernard, the nineteenth century French physiologist, was intrigued by the seemingly trivial distinction between an animal's external environment and its internal environment, he was struck by the *non*trivial fact that while the external environment can fluctuate considerably, an animal's *internal* condition is kept relatively constant. For example, the human body temperature remains about 37º C, and a mere five degrees difference leads to death (Churchland 2002). Homeostasis—maintenance of biological values within narrowly defined range—is a buffer against environmental fluctuations. This way, organisms are in constant interaction with their environment and are homeodynamic rather than homeostatic (Rose 2001). As Patricia Churchland puts it; "the brain keeps track of levels of blood sugar, oxygen, and carbon dioxide, as well as blood pressure, heart rate, and body temperature, in order to detect perturbations to the internal milieu that are detrimental to the animal's health." Deviation from the normal set points cause an orchestrated set of neuronal responses that ultimately cause the animal to seek either food, water, warmth, a hiding place, or the like, thereby restoring deviant values to their normal values.

By homeostasis, we are referring to the term of "being in balance" and whatever knocks homeostasis out of balance causes stress and is eventually emotionally appraised as negative or positive (Sapolsky 2000). Homeostatic functions—and, in particular, the ability to switch between the different internal configurations for *flight* and *fight* from that needed for *rest* and *digest*—require coordinated control of heart, lungs, viscera, liver, and adrenal medulla in a set of interconnected structures, with the brain stem being anatomically important in coordinating this process (Damasio 1999).

The maintenance of an internal milieu requires the setting of a dynamic range enabling the brain to know when an organism is being threatened. Thermal pain therefore, should be coordinated with withdrawal, not approach, cold-temperature with shelter seeking, not with sleeping and very hostile environmental conditions with fear and flight and not with approach-behavior (Churchland 2002). These above mentioned behavioral repertoires are directly under the influence of brain mechanisms that enables our ability to assign value to surrounding events and directs our choices.

By the same token, emotions can therefore be seen as the brain's way of making us do and pay attention to certain things. Like in the face of a severely threatening situation, say an approaching predator, our bodies are programmed such that flight away from the predator will be the most logical option. Thus, sensory feelings of emotions evolved in response to those environmental events that have consistently presented opportunities or threats to biological survival in ancestral environments. That is, emotions are assignments of value that direct us one way rather than another,

and they seem to have a role in every aspect of self-representation, and certainly in body representation (Churchland 2002). A typical example is the choice of running away from any bodily source of harm instead of approaching. In physiological terms, brief periods of oxygen deprivation give rise to overwhelming feelings of needing air; extreme hunger and thirst can make us feel so desperate as to banish all thoughts of anything but water and food. Satisfaction is felt after feeding, sex, and successful predator avoidance. More generally, self-representation is underpinned by powerful feelings (MacLean 1949; Damasio 1999).

1.2. But Where Lies The Roots to Feelings?

> "PLEASURE, ELATION, EUPHORIA,
> ecstasy, sadness, despondency, depression,
> fear, anxiety, anger, hostility, and calm—these
> and other emotions color our lives. They
> contribute to the richness of our experiences
> and imbue our actions with passion and character."
>
> Iverson, Kupferman and Kandel 2000.

States of bodily excitement distinguish "passion" from "cold reason" (Critchley 2005). Everyone is familiar with the phenomenon of shivering with fear, flushing with anger or embarrassment, and quickening of heart during anxiety or love. These bodily states represent the peripheral components of subjective emotional experience, with a hierarchy of homeostatic mechanisms being responsible for regulating functions within and across bodily systems (Critchley 2005). As humans, we perceive feelings from our bodies that relate to our state of well-being, our energy and stress levels, our mood and disposition (Craig 2002). At the organ level, coordinated control is mediated primarily by neural and humoral pathways, with the adaptive control of behaviour within the environment topping the hierarchy. In this sense, internal bodily changes could be seen as evolutionary adaptations that influence behavior, signaling physical needs such as hunger, but also preparatory bodily responses such as states of readiness evoked by threat, all shaped by experience and stored in memory to serve future behavior (Darwin, 1898).

The autonomic nervous system represents the principal regulatory route of internal bodily functions. Sympathetic and parasympathetic autonomic axes provide neural input into every major bodily system (Brading, 1990). Their interaction enables continuous control of "vegetative" processes and the dynamic modification of bodily states in response to environmental challenges. By dilating vessels of the musculature as a result of concomitant reduction of blood supply to the gut, sympathetic activities facilitate motor behavior and thereby increase cardiac output. These physiological activities facilitates the *fight* and *flight* behavior in times of danger. In contrast, parasympathetic activity promotes recuperative functions effecting heart rate reduction, lowering of blood pressure, and slowing of gut motility, facilitating *rest* and *digest* related states. Bodily states of arousal associated with survival (e.g., fight and flight responses) are typically characterized by increased sympathetic activity and, usually, decreased parasympathetic activity (Cannon, 1929; Porges, 1995; Morrison, 2001). Thus subsets of autonomic arousal responses that affect visible visceral regions have developed into potent social cues that can betray an individual's motivational state (Darwin, 1898; Ekman et al. 1983).

1.2.1 Stress and the Emotions

In conjunction with the feelings of emotions, exposure to hostile conditions initiates responses organized to enhance the probability of survival. Such adaptations, known as the stress response, consist in a variety of changes in behavior, autonomic and neuroendocrine function, leading to release of different hormones. The activation of the hypothalamic-pituitary adrenocortical (HPA) axis, in concert with epinephrine and norepinephrine from the sympathetic nervous system and adrenal medulla, plays a pivotal role in the stress response. Stress initiates a cascade of events in the brain and peripheral systems that enable organisms to cope with and adapt to new and challenging situations. For this reason, the physiological and behavioral responses to stress are generally considered to be adaptive reactions. However, when stress is maintained for long periods of time, most physiological systems are negatively affected because of the prolonged exposure of target cells to physiological stress mediators (McEwen 2002).

Physical stressors include temperature, pain, itch, muscular and visceral sensations. Sir Charles Sherington conceptualized a sense of 'the material me' by considering that all of these feelings are related and form a foundation of the sense of one's physical self (Sherington 1900). Sherington later codified the senses into teloreceptive (vision and hearing), exteroceptive (touch), chemoreceptive (smell and taste), proprioceptive (limb position) and interoceptive (visceral) modalities, and categorized temperature and pain as aspects of touch (Sherington 1948). Recent findings on the functional anatomy of lamina 1 spinothalamocortical system indicate that interoception should be redefined as sense of the physiological condition of the entire body and not just the viscera (Craig 2002; Craig et al.. 2000). This system is believed to be a homeostatic afferent pathway that conveys signals from small-diameter primary afferents that represent the physiological status of all tissues of the body (Craig 2002). However, it is only now that the fundamental recognition of pain, temperature and other bodily sensations as interoceptive, rather than exteroceptive is beginning to emerge (Craig 2002; Critchley 2005).

A key feature that is common to pain, temperature and other bodily feelings like sensual touch is their inherent association with emotion (Craig 2002). These feelings have not only sensory, but also affective and motivational aspects. Physiological stressors like pain, temperature and touch, all generate inseparable affect (pleasantness or unpleasantness) representative of the physiological condition of the body that are directly related to homeostatic needs and associated with behavioral motivations that are crucial for the maintenance of body integrity, with their neural representations reflecting this homeostatic primacy (Craig 2002). Interestingly, these seemingly different sensations are represented to some extent by a common brain network, suggesting that even if there may exist different subcomponents of homeostasis, certain brain regions such as the insula and cingulate cortices, may be involved in the regulation of mechanisms relevant for general homeostatic maintenance (Craig 2002).

Recent neuroimaging studies have greatly enriched our understanding of the neuroanatomical substrates underlying perception, cognition and emotion (Wang et al. 2005). Data on the processing of different emotions suggest a common neural network involving the prefrontal cortex, anterior cingulate, amygdala, insula, basal ganglia (Davidson and Irwin 1999; Dolan 2002). The neural correlates of vigilance and sustained attention have been largely localized to the right prefrontal and parietal lobes and the thalamus (Sarter et al. 2001). The right prefrontal cortex may play a key

role in the brain's response to stress, because this brain area is a primary part of both the emotion and vigilance network (Wang et al. 2005). Moreover, animal and human studies have demonstrated descending influences from prefrontal and limbic cortices (cingulate, medial temporal. and insula) and amygdala on autonomic control mediated by hypothalamic and brainstem centers (Pool and Ransohoff, 1949; Kaada, 1951; Gelsema et al. 1989; Neafsey, 1990; Fish et al. 1993; Oppenheimer et al. 1992; Mangina and Buezeron-Mangina, 1996; Asahina et al. 2003; Critchley 2005). Most importantly, neurons that are either the target or the releasing site of an array of stress mediators (neurotransmitters and hormones such as corticotrophin releasing hormone 'CRH') have been identified in the amygdala and cingulate areas (Charney 2004; Carrasco et al. 2003; Craig 2002).

Where autonomic arousal occurs in anticipation of behavioral responses, feedback of bodily changes reinforces stimulus processing to influence behavioral judgments, implicitly or explicitly (Damasio et al. 1991; Damasio, 1994; Bechara et al. 1997). Central representation of these internal motivational signals is hypothesized as the origin of emotional feeling states (Lange, 1885; James 1894; Damasio 1994, 1999). Furthermore, William James argued that the feelings from our bodies are the basis of self awareness and emotion (James 1890). As James wrote in the *Principles of Psychology*; "our natural way of thinking about these coarser emotions is that the mental perception of some fact excites the mental affection called the emotion, and that this latter state of mind gives rise to the bodily expression." According to his theory he noted that *the bodily changes follow directly the perception of the exciting fact, and that our feeling of the same changes as they occur* IS *the emotion* (James 1890). James went on to urge the vital point of his theory: *If we fancy some strong emotion, and then try to abstract from our consciousness of it all the feelings of its bodily symptoms, we find we have nothing left behind*, no 'mind-stuff' out of which the emotion can be constituted, and that a cold and neutral state of intellectual perception is all that remains (James 1890).

Notably, the absences of this direct interoceptive representation in sub-primates imply that they cannot experience feelings from the body in the same way that humans do (Craig 2002). In humans, lesions of the dorsal insula interrupt these feelings (Schmahmann & Leifer 1992; Greenspan & Winfield 1992), disrupts homeostatic processing (Craig 2002) and cause permanent loss of discriminative thermal sensation. Several studies in the domain of pain, interoceptive awareness of the body, but also studies employing positively valenced stimuli like pleasant music, sensual touch and sexually arousing stimuli, have often implicated the anterior insula/frontal opercular cortex (Craig 2002) (see Figure 1.2). Such functions enable structures like the insula to code the physiological correlates of the body's needs (Cabenac 1972; Mower 1976). As Craig puts it; compare the pleasant feeling of cool water when your body is overheated with gnawing discomfort generated by the very same cool stimulus when you are 'chilled to the bone'. Thermoregulation is therefore a primal evolutionary requirement for all animals, particularly homeothermic mammals, and the affective aspects of such feelings correspond to the motivations that are essential for behavioral thermoregulation and homeostasis—that is survival (Satinoff 1978; Blatteis 1998). These cortical regions constitute a primary interoceptive image of homeostatic afferents that codes distinct sensations including temperature, pain, itch, muscular and visceral sensations, sensual touch, but also experiences of stress and emotions that are related to feelings from the body (Craig 2002) (see Figure 1.2).

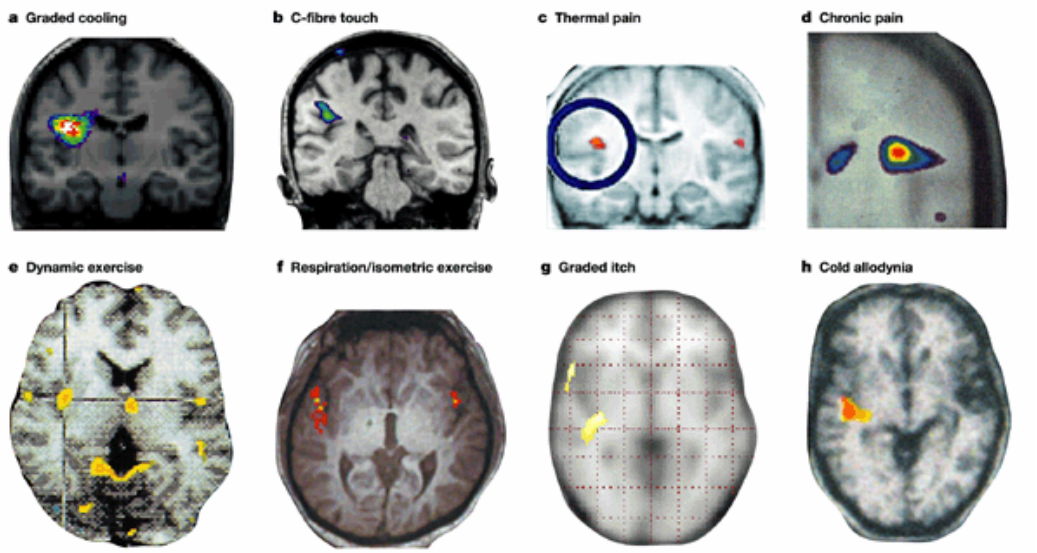

Figure 1.2. Adopted from Craig 2002. This figure shows that several experiences ranging from (a) graded cooling, (b) sensual touch, (c) heat pain, (d) chronic pain, (e and f) exercise, (g) itch, and (h) cold experience all recruit the anterior insula/frontal opercular cortex. As noted earlier in the text, a common feature of these stimuli is their strong relationship to bodily feeling states. A consistent activation of the insular cortex in all these feeling states regardless of modality and valence implicates this region to be involved in the regulation of bodily feeling states and thereby ascribes this region a pivotal role in the maintenance of homeostasis.

1.2.2. Embodied Feelings

A rather intriguing illustration of how emotional feelings are not so strongly distinguishable from bodily feelings is the following. An individual who went amok and attempted to carry out a mass murder in a market place on the edges of a populous city in sub-Saharan Africa was overpowered and tied down. He started a desperate lamentation in the Malinke language, screaming: **"**I have this strong feeling of failing myself and all my people and do not wish to live any longer because the *crawling of extreme sadness in my spine* became simply unbearable. Although the fear of killing myself has strongly *stiffened my marrows*, the idea keeps coming to my mind that if I kill a few people, this fear may wane and as a result, I may even feel a bit of *happiness warming up my glands* which may give me courage to shoot myself *cold*. Yesterday, I felt enough *strength in my fibers* and had decided to go some place and kill as many people as possible, a place where I have no *bodily affecting individuals*. After loading my assault rifle, I walked the whole night till I got here this morning. I chose the moment when I saw that enough people were around at a closer range and started shooting straight out. But then during the shooting, I saw the one *who usually stresses up my heart* in my line of firing and *my body died* in an instant, and that was when you guys took hold of me."

What the poor fellow was trying to convey was a highly tragic story full of bodily emotional experiences or anticipation of these experiences. Sadness crawls in his spine; fear stiffens his marrows; happiness warms his glands. But most profoundly, he calls his relatives and loved ones as those that affects his bodily responses; and indeed after seeing the lady who usually stresses up his heart in his line of firing (referring to someone he is desperately in love with), his body simply died of sympathy for that individual. This story demonstrates some intriguing qualities of the Malinke language. It shows that speakers of such a tongue do indeed inevitably see emotional feelings as abstract as being in love or sympathizing with someone as strongly embedded in bodily feelings. Even though these people are not advanced in the study of human physiology as far as the modern western scientific methods are

concerned, their language is such that they recognize the importance of bodily changes in the generation of emotions.

If we step from the southern lamentation and examine an even more elaborate manifestation of grief as described by a nineteen century Danish physiologist, C. Lange, here is a summarized version his embodied account of grief: "The chief feature in the physiognomy of grief is perhaps its paralyzing effect on the voluntary movements. This effect is by no means as extreme as that which fright produces, being seldom more than that degree of weakening which makes it cost an effort to perform actions usually done with ease. It is, in other words, a feeling of weariness; and (as in all weariness) movements are made slowly, heavily, without strength, unwillingly, and with exertion, and are limited to the fewest possible. By this, the grieving person gets his outward stamp: he walks slowly, unsteadily, dragging his feet and hanging arms. His voice is weak and without resonance, in consequence of the feeble activity of the muscles of expiration and of the larynx. The tonicity or 'latent innervations' of the muscles is strikingly diminished. The neck is bent, the head hangs ('bowed down' with grief), the relaxation of the cheek- and jaw-muscles makes the face look long and narrow, the jaw may even hang open. And what is not obvious to the eye is that the mouth grows dry, the tongue is sticky, and a bitter taste ensues which, it would appear, is only a consequence of the tongue's dryness. The expression 'bitter sorrow' may possibly arise from this. There is one most regular manifestations of grief, which apparently contradicts these other physiological phenomena, and that is weeping, with its profuse secretion of tears, its swollen reddened face, red eyes, and augmented secretion from the nasal mucous membrane."

If we try to forget the depressing physiology of grief and delve into the exciting state of fear as Darwin wrote of its bodily effects: "Fear is often preceded by astonishment, and is so far akin to it, that both lead to the senses of sight and hearing being instantly aroused. In both cases the eyes and mouth are widely opened, and the eyebrows raised. The frightened man at first stands like a statue motionless and breathless, or crouches down as if instinctively to escape observation. The heart beats quickly and violently, so that it palpitates or knocks against the ribs; but it is very doubtful whether it then works more efficiently than usual, so as to send a greater supply of blood to all parts of the body; for the skin instantly becomes pale, as during insipient faintness. That the skin is much affected under the sense of great fear, we see in the marvelous manner in which perspiration immediately exudes from it. The hairs also on the skin stands erect; and the superficial muscles shiver. In connection with the disturbed action of the heart, the breathing is hurried. One of the best-marked symptoms is the trembling of all the muscles of the body; and this is often first seen in the lips. From this cause, and from the dryness of the mouth, the voice becomes husky or indistinct, or may altogether fail."

1.3. The Question at Hand

By now the reader might be reminded of the existing relationship between emotional and stressful experiences and bodily feeling states. The main purpose of the present thesis is to investigate the underlying physiological correlates of emotional and psychological stress response. We will address the bodily responses (in terms of the functional neuroanatomical correlates of emotional experience on the one hand, and peripheral hormonal responses to the experience of psychological stress on the other) to emotional and stressful experiences and their role in the maintenance of individual well being.

It is very important to recognize that the sensory system that codes for emotional, physiological as well as psychologically stressful experiences recruits part

of an entire physiological network involved in the maintenance of homeostasis (Wang et al. 2005). In line with this view, it has been proposed in the somatic marker hypothesis that the subjective process of feeling emotions requires the participation of brain regions that are involved in the mapping and/or regulation of our continuously changing internal states—that is, in homeostasis (Damasio 1993). These feelings help to guide behavioral decisions that affect survival and quality of life by producing a 'perceptual landscape' that represents the emotional significance of a particular stimulus that is being experienced, or of a projected future action by means of a further 'as-if-body loop' mechanism (Damasio et al. 2000). Evidence indicating the existence of a relationship between brain stem activity (a structure strongly implicated in homeostasis regulation) and subjective feelings comes from human imaging studies showing an interaction of feelings and emotions with many aspects of subconscious homeostatic processes. (Sawchenko et al. 1996; Craig et al. 2000; Damasio et al. 2000; Craig 2002).

Such a conceptualization of considering both the emotions and psychological stress as involving similar homeostatic processes of the body provides an easy formulation for somatization under emotional stress (i.e., perceiving both the stress and emotional experience as rooted in related bodily responses). Thus, the *chronic* homeostatic imbalance one undergoes after a traumatic event, (e.g., such stressful experience like surviving a terrorist attack in which some closely-related individuals lost their lives), may be similar to the *acute* homeostatic imbalance that might result from a near death escape from a fierce predator or traffic accident. Similarly, these considerations imply that several unexplained somatic/pain syndromes such as fybromyalgia, chronic pain, diabetes as well as psychosomatic disorders, could be related to homeostatic dysfunctions, rather than to tissue damage (Craig 2002). Support for such a concept stems from the theory that stress promotes adaptation ("allostasis"), whereas a perturbed diurnal rhythm or a failed shutoff of mediators after stress ("allostatic state") may in time, lead to wear and tear on the body ("allostatic load") (McEwen 1998; 2003). Thus, neural changes mirror the pattern seen in the cardiovascular, metabolic, and immune systems, that is, short-term adaptation versus long-term damage.

Allostatic load can therefore lead to impaired immunity, atherosclerosis, obesity, bone demineralization, and atrophy of nerve cells in brain. Allostatic load is seen in major depressive illness and may also be expressed in other chronic anxiety disorders such as post traumatic stress disorder (McEwen 2003). Thus allostasis depends on personality type and the associated stress response (Korte et al. 2005). According to this view, the benefits of allostasis and the costs of allostatic load, leads to different trade-offs in health and disease, thereby reinforcing a Darwinian concept of stress. *But how does the bodies' quest for the maintenance of homeostasis in the face of environmental challenges (i.e. stress and emotions) affect the brain?*

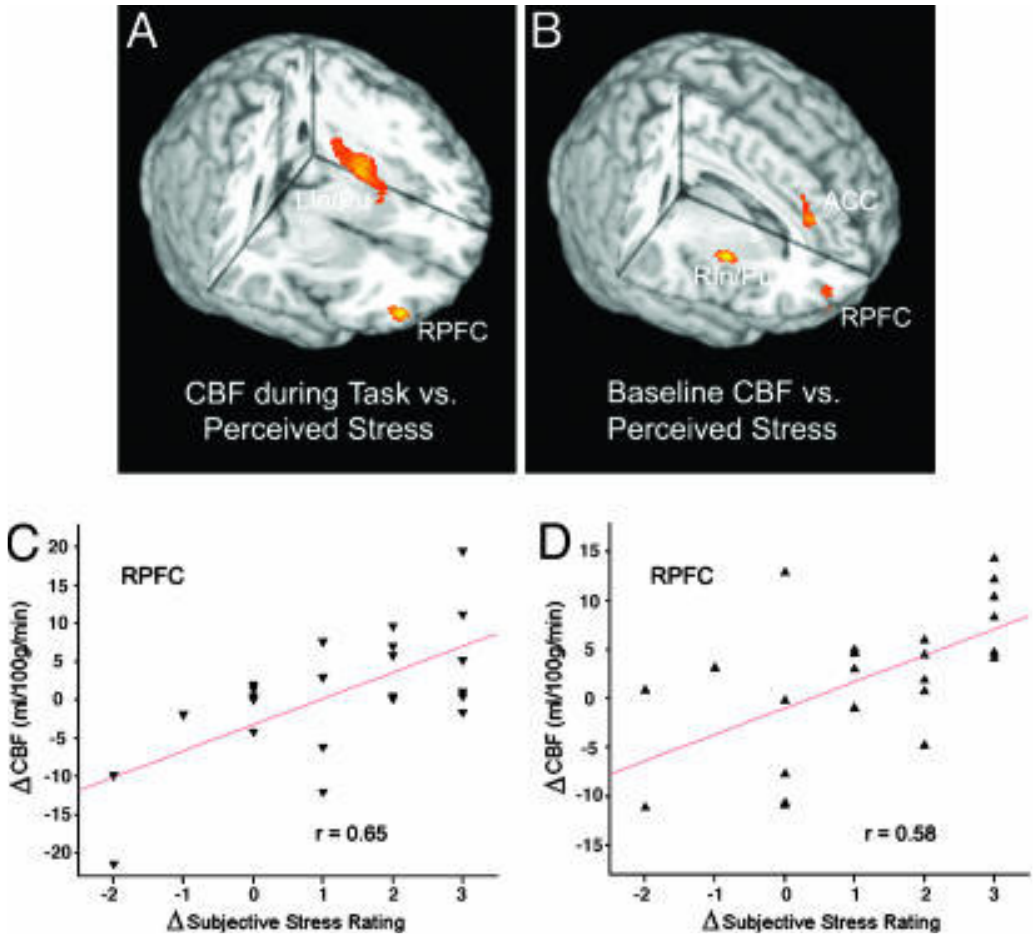

Figure 1.3. Adopted from Wang et al. 2005 PNAS. Three-dimensional rendering of the regression-analysis results, which use the CBF change during stress tasks (high-stress - low-stress task) (*A*) or the CBF change at baseline (baseline 2 - baseline 1) (*B*) as the dependent variable and the change in perceived stress from the low- to high-stress task as the predictor. Also shown are scatter plots of changes in CBF during stress tasks (*C*) and at baseline (*D*) as a function of changes in perceived stress between the two stress tasks. Each data point represents one subject. Mean CBF values are drawn from the ROI defined by the activation cluster. Right prefrontal cortex (RPFC) $x = 42$, $y = 54$, $z = -10$, 211 pixels, $Z = 3.59$ in *A*; $x = 32$, $y = 58$, $z = -2$, 118 pixels, $Z = 2.98$ in *B*. Anterior cingulate cortex (ACC) $x = 10$, $y = 38$, $z = 24$, 156 pixels, $Z = 3.22$; left insula/putamen $x = -32$, $y = -8$, $z = 4$, 811 pixels, $Z = 3.46$; right insula/putamen $x = 38$, $y = 2$, $z = 2$, 144 pixels, $Z = 3.73$.

1.3.1. Empirical Approach

In this thesis, individual differences in the perception and experience of social emotional interactions, psychologically stressful experiences, and genetic determinants of endocrine and subjective psychological stress response in low and high risk individuals will be examined. The cascade of peripheral endocrine response to psychological stress, coupled with the neural response to emotional experience could be seen as an organisms adaptation processes that have developed to serve short term purposes. In the long-term however, the result of this processes may be either adaptive or maladaptive depending on the nature of individual's genetic make-up and the severity of the environmental stressor (Plotsky et al., 1998). This is supported by the accumulating empirical evidence indicating that traumatic and stressful life-events are potential risk factors for the onset of certain psychiatric disorders (Brown and Harris 1978; Ormel et al. 2001).

In this vein, genetic determinants of the stress responses are believed to influence the degree of individual's vulnerability to stress-related emotional disorders (Caspi et al. 2003; Charney 2004). In *chapter 2* an experimental approach will be used to study the involvement of neural networks in the brain by inducing social emotions in healthy volunteers while undergoing functional magnetic resonance imaging (fMRI). The primary research question was to determine if the same brain areas that are implicated in the regulation of homeostasis as reported in studies examining the human stress response are recruited during the experience of these emotions. To these aims, we will induce the emotional states of happiness, sadness, and fear in individuals using film clips with emotional content as a sort of pilot study in which environmentally valid social emotional stimuli will be presented. The goal of this emotion-induction paradigm was to map the neural substrates commonly involved in the processing of all these emotions. Although not many studies have focused on the neural underpinnings of general emotional processing, identifying such areas may consolidate the idea of an existing biological substrate of homeostatic maintenance. This way, neural mechanisms underlying the arousal and bodily responses involved in emotional experiences, may not be specific for one emotion, but rather encompassing different emotions. Inducing emotions, however, is not an easy task in a laboratory environment. However, gustatory emotions like disgust and food related pleasure (hereafter called food-emotions, where with food, we mean both liquid and solid nutrients) are particularly well suited for scientific investigation in humans. This is possible because unlike other basic emotions, they can be reliably and repeatedly triggered in ethically sound ways by presenting participants with pleasant or unpleasant olfactory or gustatory stimuli.

In *chapter 3*, we capitalized on this possibility and investigated the neural basis of our understanding of other individual's basic emotions relevant for feeding behavior. Using event related fMRI, we investigated if subject's empathy scores are predictive of the intensity of activations in their insula and other regions that do not discriminate between perception and experience of both food related disgust and pleasure.

Disgusting food substances always induce aversive emotional responses that can go as far as causing people to become sick (nausea) and eventually vomit because such bodily feelings may prevent food poisoning and enhance survival. Delicious food substances on the other hand, do in fact induce a pleasant emotional response that signal appetitive reward and enhance survival. What is common to both food related disgust and pleasure however, is the ability of both experiences in affecting the internal bodily states and thereby homeostasis.

In *chapter 4*, we will investigate effects of individual differences in the functional polymorphic variations of *COMT* (a monoaminergic gene involved in the metabolism of catecholamines) on subjective and endocrine responses to acute psychosocial stress in a laboratory paradigm. Linking behavioral and neuroendocrine responses to validated experimental stress paradigms may enable us to examine the role of genetic polymorphisms in determining differences in individual susceptibility to neuropsychiatric disorders. Thus, individuals with varying degrees of susceptibility to major depression will be included to enable us to delineate the role of *COMT* allelic variation in the human stress response, as well as the relationship between this genetic variable and the individual's degree of susceptibility to major depression in the face of stress. Establishing such a relationship will increase our understanding of the role of genes in the maintenance of homeostasis and psychological well being.

Interactions between genetic makeup as determined by individual's *COMT*, monoamine oxidase-A (*MAOA*) and serotonin transporter (*5-HTT*) allelic variations in terms of their physiological and subjective response to acute psychosocial stress will be examined in Chapter 5. Additionally, a similar complex genetic involvement in homeostatic maintenance will be studied during an endocrine challenge (Combine Dexamethason/CRH challenge) in the same chapter. This will enable the examination to genetic effects of hormonal regulation, in the absence of psychological stress.

To the best of our knowledge, this is the first time that the relationship has been studied between genetic polymorphisms and the behavioral and neuroendocrine response to an experimental stressor in both healthy volunteers, first degree relatives and patients with major depression. A finding of gene-gene interaction in endocrine response to environmentally challenging experiences will enable us to demonstrate the complex modulatory roles of genes in individuals' continuous quest for homeostatic maintenance, failure of which might lead to disease in the long run.

The attempt to investigate environmental experiences be they, psychologically stressful, or emotional in nature, is a key goal of this thesis. Integrating findings of brain responses to emotional induction and peripheral endocrine responses to the experience of stress with earlier findings of neural mechanisms underlying stress and emotional experience may enable us to suggest the existence of shared networks involved in the processing of stress and the emotions. Here, we will focus on the idea that stress and the emotions are linked by homeostasis. Key to understanding the biological basis of vulnerability to stress related disorders like MD may therefore be the understanding of normal and abnormal physiological mechanisms relating to the maintenance of homeostasis, in the face of environmental challenges. We will examine the individual differences in the cascade of physiological responses by examining how individual differences modulate brain reactions to other people's emotions. Second, we will study the influence of genetic makeup in peripheral endocrine and behavioral response to stressors.

Social emotional behaviour, like psychosocial stress, can be adaptive (e.g. understanding another individual) or maladaptive (e.g. Social Phobia). The examination of interactions between individual differences in genetic make up in relation to physiological responses to psychosocial stress, in conjunction with the neural mechanisms regulating empathy, may help bridge the gap between self and other homeostasis. Studying the individual difference in terms of both empathic tendency as well as genetic differences in physiological response to psychosocial stress, may enable us to make a small contribution in the understanding of social emotional behaviour relevant for mental health.

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Chapter 2

Common Cingulate response to Happy, Sad and Fearful social interactions during fMRI

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A Modified version of this chapter is under consideration in Neuropsychologia

2.1. Abstract

Effectively inducing the emotions under scrutiny is essential in understanding the underlying neural mechanisms during neuroimaging sessions. Considerable evidence exists to support the idea that watching emotions (including facial expressions) unfold in others, can induce the same emotion in the observer. The generation of autonomic arousal lies central to these processes and may involve the somatosensory and cingulate cortices in a similar manner during the processing of different emotions. With this in mind, we examined the hypothesis that there exists a common neural circuitry involved in the processing of happiness, sadness and fear, in addition to the neural substrates specifically involved in the processing of each of these emotions. Dynamic emotional movie scripts capable of inducing happiness, sadness and fear were used instead of autobiographical memory recall during functional magnetic resonance imaging (fMRI).

We found common activations in all three emotional conditions in the bilateral anterior cingulate cortex (ACC), right posterior cingulate cortex (PCC), left visual areas, including the Cuneus, fusiform gyrus and middle occipital region, in addition to the emotion specific neural activations that are in line with earlier findings in neuroimaging studies of emotional processing. These results suggest the existence of common underlying neural substrate involved in the processing of more than one basic emotion. These structures may variably contribute to the perceptual. mnemonic, somatic and experiential aspects of emotions.

2.2. Introduction

In the last decade, the neuronal underpinning of emotions has been an important focus of research. Studies that have investigated the neural substrates of emotional feeling have consistently implicated the sub-cortical. limbic, paralimbic and frontal regions (George et al. 1995; Reiman et al. 1997; Beauregard et al. 1998; Damasio et al. 2000; Levesque et al. 2003; Calder et al. 2001; Phan et al. 2002; Adolphs 2003). Interestingly, many of the same structures have been reported to be involved in the perception of the emotional facial expressions of others (Reiman et al. 1997; Adolphs et al. 1996; Dolan et al. 1996; Morris et al. 1998; Lane et al. 1997). In the face of this similarity, it has been argued that perception of an emotion in a conspecific involves simulation of the emotional state within the relevant cortical circuitry of the observer (Damasio 1994; Gallese et al. 2004; Adolphs 2006). The critical mechanism linking other people's emotions to our own though remains a matter of debate. Some authors believe that somatosensory representations of the bodily state associated with the emotion is critical "see somatic marker hypothesis" (Damasio 1994), others believe that the neural activity that triggers the bodily responses to emotional experience is sufficient (Gallese et al. 2004). Yet common to all these accounts is the hypothesis that the perception of the emotions of others triggers a state in the observer that is similar to the endogenous experience of the emotion in the observer as caused for instance by the memory of an emotional event or the exposure to a primary emotional stimulus (e.g. a disgusting smell).

The main stream of research in affective neuroscience, encouraged by the findings of distinct substrates for fear (amygdala) and disgust (anterior insula) has focused on the identification of neural substrates *specific* for particular emotions

(Calder et al. 2001). To many of these papers, neural structures commonly activated in the perception and/or feeling of many emotions are considered of little or no interest. In comparison to reports that focused on the involvement of a particular area in a specific emotion, there are relatively few reports on the involvement of certain brain regions in the processing of more than one type of emotion, but see (Lane et al. 1997; Winston et al. 2003; Schienle et al. 2002; Liberzon et al. 2003; Habel et al. 2005).

While we watch an emotional movie, we perceive the emotions of the actors, and indeed often share these emotions, for instance weeping at the sight of a child mourning his father's death. In such cases perceiving the emotions of others and the feeling of our own emotions is even more intimately linked: the emotions of others are then powerful inducers of our own emotions. A similar situation is often encountered in social interactions: the sadness of our partner makes us sad. On the other hand, frequently perceived emotions in a social context could also induce a different emotion in the observer (e.g., anger evoked by perceiving an unfair treatment that led to someone's crying). Thus, by studying emotions in the laboratory using films or pictures, perceptual and appraisal processes on the input side, in concert with processes involved in the generation of the somatic, visceral. and experiential features of emotion, as well as processes involved in the regulation of different emotions, all recruit similar as well as distinct neural pathways at the same time (Davidson 2003). With a bulk of the literature focusing more on specific pathways involved in the processing of individual emotions (Phan et al. 2002), the question of which neural pathways may be involved in the processing of more than one basic emotion still remains unresolved.

To study the neural structures involved in the processing of happiness, sadness and fear, we carried out an fMRI study using dynamic movie clips of popular movies known to induce these emotions. We choose happiness, sadness and fear among the basic emotions in the present study, because even though anger, disgust and surprise are also termed as one of the so-called basic emotions, the three emotions chosen here were earlier shown to recruit similar somatic as well as cortical routes during emotional autobiographical memory recall (Damasio et al. 2000). Furthermore, while low mood/sadness is a core symptom of major depression, this symptom seems to go hand in hand with anhedonia (i.e. the inability to experience pleasure/happiness) (Beauregard et al. 1998). Most importantly, these sadness and lack of happiness related symptoms of major depression tend to coexist quite often with anxiety symptoms (George et al. 1996; Reiman et al. 1997; Beauregard et al. 1998; Levesque et al. 2003; Calder et al. 2001; Phan et al. 2002), underscoring the likely existence of an interlinked biological substrate relevant for processing of these discrete emotions in both health and during disorders of affect.

The aim of the present study is therefore not just to study the existence of areas specifically involved in one emotion (Phan et al. 2002), but rather, our aim is to further investigate the existence of areas commonly involved in the processing of all three emotions studied here. Emotional movie samples were used because by using dynamic movie clips with sound tracks, we choose to increase the ecological validity by focusing more generally on the involvement of certain neural areas in the processing of emotional stimuli during the observation of a more complex social interaction than say facial expressions. Thus, the present study capitalizes on the reported similarities to focus on the identification of areas involved in the processing of happiness, sadness and fear, along side the confirmation of areas that are more specifically involved in the processing of different emotions. In line with earlier

reports, we expect to find a dominance for the processing of happiness and reward related stimuli in striatal. ventral prefrontal and sub-genual cingulate areas (Damasio et al. 2000); dominance for sadness processing in prefrontal and cingulate regions (Damasio et al. 2000; Phan et al. 2002; Murphy et al. 2003); and a dominance for processing of fear related stimuli in the amygdala, anterior cingulate and frontal regions (Damasio et al. 2000; Calder et al. 2001; Phan et al. 2002; Adolphs et al. 2003; Murphy et al. 2003). To these aims, we exposed subjects to comparatively long movies excerpts (~2 minutes) in this study. These movie excerpts are pre-validated, shown earlier to successfully induce happiness/amusement, sadness and fear during fMRI (Beauregard et al. 1998; Gross et al. 1995; Phillipot et al. 1993). Most importantly, we expect these movies to activate some common structures across all emotional conditions in addition to emotion specific structures (Damasio et al. 2000; Calder et al. 2001; Phan et al. 2002; Adolphs 2003; Murphy et al. 2003; Critchley et al. 2003; Northoff and Bermpohl 2004).

2.3. Materials and Methods

2.3.1. ***Subjects and Procedures****:* Prior to the fMRI session, 16 (8 females, mean age = 22.31years, SD = 2.39) took part in the rating of the edited movie lips in terms of the subjective emotional experience they felt while watching the movies. During fMRI, 10 healthy individuals (5 females, mean age = 19.75, SD = 1.38)), all native Dutch speakers, right handed without a history of neurological/psychiatric disorder with normal/corrected to normal vision took part in this study in accordance with the local medical ethics committee guidelines. The data of 8 subjects (4 female) were used in the final analysis. Data of two subjects was excluded from final analysis due to technical problems. All subjects gave written informed consent and were naïve of the contents of the movies before the fMRI session. More subjects could not be added to the study because the scanner became unavailable to us, and adding subjects scanned on another machine is statistically problematic.

2.3.2. ***Experimental Design****:* The experiment consisted of three pairs of ~2 minutes emotional videos with sound, validated to induce happiness, sadness and fear (Beauregard et al. 1998; Phillipot et al. 1993; Gross and Levenson 1995), plus six neutral video clips (of which only two were used in the final analysis for all participants, while the remaining four were used as emotional wash-outs i.e., presented between two emotional movie clips when none of the two neutral clips are presented). A one minute resting condition (fixation cross in the middle of a black screen), was presented at the onset of the experiment and after every movie clip. The emotional movie excerpts were presented in pseudo-random order where the same emotion never followed itself to avoid habituation, while the two neutral and four washout movies were presented in a fully randomized order. We choose to use the two neutral clips from the same job interview that also compose the emotional wash-out movies to ascertain that the neutral clips were indeed free of any emotional interaction while still adequately depicting social interactions, even though this might be at the expense of the novelty of the neutral clips relative to the emotional ones. Although we did not include any explicit task during the vision condition, subjects were instructed to carefully attend to the contents of the movies for the purpose of answering some questions after the fMRI session. This measure was important to make sure the subjects stay fully awake and attend to the movies. During debriefing,

participants were asked to recall the most amusing, sad and frightening scenes, and whether they laughed, cried or felt physically tensed as a result of the fearful scenes during the observation of the movies. Additionally, participants were asked how they felt about the two individuals in the job interview (neutral/washout movies). All the eight participants included in the final analysis were able to recall all emotional and neutral scenes adequately.

A total of 12 movies were shown (six of which were emotional and six were neutral) only once. Four of these emotional movie clips with sound tracks were fragments of popular Hollywood movies validated to induce amusement/happiness (When Harry met Sally), Sadness (The champ and Kramer versus Kramer), fear (The shinning and Silence of the lambs) adopted from Gross and Levenson (Gross and Levenson 1995) and Phillipot's sample movies (Phillipot 1993). A pair of movie excerpts was chosen each to evoke happiness/amusement, sadness and fear. For the neutral condition, we selected 2 minute excerpts taken from a longer movie of two actors pretending to have a job interview. These excerpts were taken at moments, in which the actors displayed no particular emotions. These neutral movies were indeed considered to be significantly less emotional than the emotional movies (see below). We used movies instead of static pictures for their higher ecological validity (Beauregard et al. 1998; Adolphs 2003; Wicker et al. 2003).

2.3.3. ***Image Acquisition****:* Subjects were scanned on a 1.5T Siemens Vision scanner. An echo planar imaging (EPI) series of 836 volumes (TE=60ms, TR=3000, FA=90), with 36 slices (3.5 mm thick, 5% slice gap) each, was acquired parallel to the AC-PC line. The first two scans were discarded to allow entrance to the equilibrium state. In addition to the functional scans, a T1 weighted 3D flash (TE=5ms, TR=30ms, FA=30) series was acquired to provide a high-resolution (1x1x1 mm^3) anatomical image. During the 42 minutes and 18 seconds EPI scan, participants viewed movie excerpts with sound tracks, presented on a large screen (approx. 20 degrees of visual angle wide) and an MR-compatible headphone. The movie could be seen with the help of a mirror mounted on the head coil directly in front of their eyes, at an angle of 45 degrees. Movies were projected via an LCD projector onto the screen located at the foot end of the MRI bed.

2.3.4 ***Data analysis****:* Data were processed (realign, normalized and smoothed using Statistical Parametric Mapping (SPM99)). Each functional volume for each subject was realigned to the first in the sequence to remove motion artefacts and the average head movement as determined by translation during realignment for the x, y and z coordinates were 0.07mm, 1.58mm and -0.62mm respectively. Data were normalized [28] to the SPM99 EPI MNI template (Montreal Neurological Institute coordinate space). Images were resampled into 2-mm cubic voxel and smoothed spatially with an 8-mm full width half-maximum isotropic Gaussian kernel. Regarding the laterality, we note that in the SPM conventional Talairach coordinate system for images, right means the subjects anatomical right hemisphere and structures on that side have a positive MNI *x* coordinate. Further analysis was performed using (SPM2) after all images were replaced in neurological convention, Wellcome Department of Cognitive Neurology, London, UK; http://www.fil.ion.ucl.ac.uk.

Basis functions were calculated as box-car functions corresponding to the presentation time of each movie, convolved with the hemodynamic response function. Global volume effects were removed by normalizing the raw signal in each volume element to the global mean (SPM: global scaling). A high-pass filtering (cut-off

frequency = 512 s) was used to remove possible effects of low-frequency changes. Even though scanner drift was not explicitly modelled, this measure could be seen as a way of accounting for scanner drift. Effects at each and every voxel were estimated with the use of the general linear model. Random-effects analysis was carried out using single subject's contrasts. Additionally, a conjunction analysis was used to determine the common activations across the three emotional conditions (Nichols et al. 2005). Predicted peaks for the common activations were considered significant at $p < 0.01$, which is equivalent to $p<0.000001$ (Friston et al. 2005) (see table 1). Specific activation for individual emotional conditions was determined using a masking in SMP2 at 0.05 exclusively with the other two emotional conditions at p values of 0.001 with a threshold level of 5 voxels (see Table 3). This more stringent statistical threshold was adopted during the analysis of the specific activations for the purpose of demonstrating a more reliable selectivity for specific emotions. In addition to the masking procedure, analysis of the individual activations of each of the three emotional conditions relative to the neutral condition was carried out at a statistical threshold of $p < 0.005$ uncorrected for multiple comparisons with a threshold level of 10 voxels (see Table 4-6).

2.4. Results

2.4.1. Behavioural data

A likert scale ranging from 0-6 was used to obtain individual emotional experience scores immediately after every movie excerpt, with a score of 0 meaning no emotional experience at all during the viewing of that particular movie and 6 meaning the strongest feeling for that emotion experienced in a long time. The mean scores for each emotional experience for all categories are shown in Table 1 below. By analyzing the behavioural ratings in terms of how successful the movies induced the target emotions, we perform an ANOVA using four *movies* (i.e. happy, sad, fear and neutral) and three *targets* of induction (i.e., happiness, sadness and fear). We found a significant interaction between *movie* and *target* emotion ($F > 90$, $df = 15$, $p < 10^{-6}$). Using a Newman Keuls-posthoc exploration of the interaction indicated, we found that the happy, sad and fearful movies were rated significantly higher along their respective emotional category than along the two other categories (all at $p<0.001$). In addition, the neutral movies were considered less happy than the happy, less sad than the sad, and less fearful than the fear evoking movies (all $p<0.001$). In line with earlier reports (Phillipot et al. 1993; Gross and Levenson 1995; Beauregard et al. 1998), these data shows that the emotional movies were indeed significantly capable of inducing the target emotions, while the neutral movies in the present experiment showed a lack of emotional content.

2.4.2. Table 1. Mean scores and SEM of subjective ratings of emotional experience of the two emotional movie clips for each emotion condition on a 0-6 likert scale.

Type of emotion	Happiness	Sadness	Fear
Happiness	4.56±0.24	1.71±0.06	0.18±0.06
Sadness	0.9±0.18	3.96±0.3	0.66±0.18
Fear	0.96±0.24	0.84±0.24	3.24±0.36
Neutral	1.68±0.24	0.24±0.12	0.3±0.12

2.4.3. Common Activations

Table 2 shows the regions with significant activations for all emotions as revealed by a conjunction analysis between happiness versus (vs.) neutral. sadness vs. neutral and fear vs. neutral at the random effects level. Subjects demonstrated a significant activation in a number of expected areas, including the cingulate regions (anterior cingulate 'ACC', posterior cingulate 'PCC'), occipito-parietal areas, paracentral lobule, and visual areas consisting of activation clusters in the fusiform gyrus, cuneus primary visual cortex (see Figure 2.1 and Table 2). Although the primary focus of this report is not to investigate the areas specifically involved during the processing of individual emotions, we included data of these findings by demonstrating both the trends for exclusive selectivity (Table 3) for descriptive purposes.

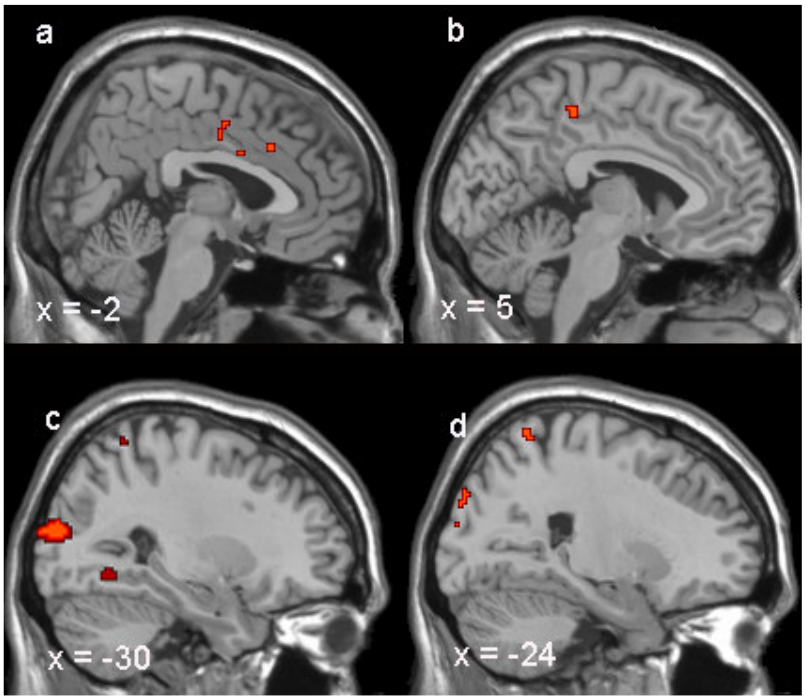

Figure 2.1. Common activations during all emotional conditions relative to the neutral condition. A conjunction map of the ACC (a) and posterior cingulate (b) showing activations during the viewing of happiness, sadness and fear. (c) and (d) shows activations in the paracentral lobule and visual areas. The results are set at a statistical threshold of $P < 0.01$, which is equivalent to $p^3 = 0.000001$ with minimum cut-off points set at $k > 5$ voxels.

2.4.4. Table 2. Common activations during happiness, sadness and fear

	MNI Coordinates					
Brain Region	X	Y	Z	Voxels	*T*Value	*Z*Value
PCC	-14	-22	40	5	2.77	2.53
ACC	-2	6	28	10	2.75	2.75
ACC	-2	20	34	11	2.78	2.54
ACC	-4	-6	40	6	2.74	2.51
Cuneus	-24	-88	34	12	2.82	2.57
Fusiform	-32	-60	-6	30	3.43	3.02
Middle Occipital	-30	-86	16	127	3.68	3.20

Results above are areas of activation common in the following contrasts; happiness > neutral. sadness > neutral and fear > neutral as obtained in a conjunction analysis at the random effects level at $p < 0.01$ uncorrected for multiple comparisons, with the likelihood of false positives in the three way conjunction being equal to $p^3 = 0.000001$; k >= 5 voxels.

2.4.5. Table 3. Specific activations during the viewing of happiness, sadness or fearful movies

	MNI Coordinates					
Brain Region	X	Y	Z	Voxels	*T*Value	*Z*Value
Happiness Specific						
Inferior temporal gyrus	-54	-68	-2	5	4.06	3.45
Inferior temporal gyrus/ Middle occipital gyrus	46	-68	-2	16	4.42	3.68
Insula	-42	-8	-14	5	4.11	3.48
Post central gyrus	-62	-24	34	17	3.89	3.34
Sadness specific						
Middle occipital gyrus	-32	-96	6	18	4.18	3.53
Fear Specific						
ACC	0	34	28	26	4.09	3.47
Fusiform gyrus/ Parahippocampal gyrus	22	-42	-14	328	7.50	5.17
Fusiform Gyrus/ Parahippocampal gyrus	-24	-42	-8	255	6.94	4.95
Superiro parietal lobule/ Precuneus	18	-70	60	39	4.97	4.00
Precuneus	12	-80	50	10	4.74	3.86
Superior occipital gyrus/ Angular gyrus/Cuneus/ Precuneus	32	-82	30	37	5.18	4.11

Results above are areas of activation specific to the viewing of happiness, sadness or fearful movies each masked at a p-value of 0.05 exclusively with the other two at a threshold p-value of 0.001 uncorrected for multiple comparisons. The results obtained in this analysis shows areas that are dominantly recruited during each of the emotional viewing conditions over the other.

2.5. Discussion

To investigate the neural circuitry common to happiness, sadness and fear, we exposed subjects to ~2 minute clips taken from emotional major motion pictures, and contrasted the neural activity during the viewing of these movies against that caused by the viewing of a less emotional movie (neutral condition). We found cingulate and occipital cortices involvement common to the processing of happiness, sadness and fear relative to the neutral condition. Our results provide empirical evidence of common neural activations during the processing of happiness, sadness and fear. These include areas involved in the processing of arousing emotional stimuli i.e., ACC and cuneus (Carretie et al. 2004), with the cingulate regions, especially the ACC, being found earlier to be involved in the processing of both positive appetitive as well as negative social stimuli (Dolan et al. 1996; Lane et al. 1997: Davidson and Irwin 1999). Furthermore, a recent finding of an involvement of some of the areas found in the present study in the expectancy of emotional stimuli (Bermpohl et al. 2006) supports the idea that, by watching emotional movies, subjects might be strongly involved in cognitive processes regarding the outcome of the social interactions in our emotionally laden movie clips. In this vein, our findings of common neural substrates responding to social interactions depicting happiness, sadness and fear may possibly be relevant in the social context, because innate fear (e.g. fear of heights or specific phobias) may likely involve different underlying neural substrates compared to the social fear we investigated here.

In the present study, we compared dynamic movie excerpts containing highly emotional social interactions to movies of social interactions lacking emotional arousal. Furthermore, our stimuli contain whole body views making it possible for the subjects to see whole body gestures in all movie clips as the emotional interactions enfold. These could partly explain our findings of common activations during the viewing of emotional movies in areas strongly known to be involved in arousal and autonomic processes in cingulate areas, as well as integration of emotional facial expression and body language in the fusiform cortex and adjacent visual areas (Critchley 2005; de Gelder 2006). On the other hand, these areas, especially the cingulate areas and amygdala are reported extensively in studies examining various aspects of emotional processes with or without putting the general arousal nature of the stimuli into account (Phan et al. 2002; Murphy et al. 2003; Critchley 2005). Our failure of finding any common or specific amygdala involvement in all emotions studied here could be explained by the fact that our stimuli were longer movie clips (~2 minutes), which may have lead to habituation in this structure Winston et al. 2003; Phillips et al. 2001; Wright et al. 2001; Glascher et al. 2004; Zald et al. 2003).

By studying emotions in the laboratory using complex social stimuli like films or pictures, several processes encompassing affective, perceptual, mnemonic and experiential processes are recruited during exposure to such stimuli (Davidson et al. 2003; Dolan 2002). Each of these different subcomponents is implemented in different, but overlapping and interconnected circuitries (Davidson et al. 2003). When we use stimuli to arouse emotions in humans or in animals that have a fairly complex brain, it is important that we not unwittingly assume that we are activating a single process or program (Damasio 2000; Phan et al. 2002; Davidson 2003; Davidson and Irwin 1999). Thus, internal cognitions and prospective judgments of complex emotional stimuli may be closely coupled with changes in bodily arousal that reinforce, through feeling states, a sense of presence and a “feeling of what happens” (Damasio et al. 2000; Damasio 1999). In this light, emotions may be subsumed within

homeostatic mechanisms that underlie survival of the organism (Damasio et al. 2000; Damasio 1994; Damasio 1999; Critchley 2005).

Our present results demonstrate the existence of a common neural substrate involved in the processing of happiness, sadness and fear. The process of linking the perceived emotionally arousing interactions in a social environment with the resulting arousing state of the observer "see somatic marker hypothesis" (Damasio 1994), might bridge the dualistic divide and enable individuals to understand as well as empathise with others in a social environment (Gallese et al. 2004; Wicker et al. 2003; Critchley 2005; Wright et al. 2001; Decety and Jackson 2004; Parr et al. 2005).

Here, due to technical difficulties and factors beyond our control (i.e., the availability of the MR scanner), only eight subjects were included in our final analysis. Although small sample sizes like this has been shown to reveal results similar to those of larger samples sizes in terms of activated areas using the same experimental paradigm (Murphy et al. 2003), this limitation may be the reason for the relatively low statistical power of our observations, as evidenced by the low T and Z-scores and low cluster sizes. Nonetheless, significant activations were found in our study in areas well known to be involved in emotional processing despite the limited number of subjects. This suggests that the activations evoked by our movies were robust enough to survive statistical comparison with large residual errors. Furthermore, it might be beneficial for future research to acquire physiological measures of the intensity of the emotional states over time (e.g., electroencephalography, galvanic skin responses, and startle potentiation) in conjunction with fMRI, and to use these curves instead of boxcar functions to model the BOLD signals in GLM.

In summary, while a growing number of studies are looking into the common activation maps during the perception of different emotional conditions (Phan et al. 2002; Winston et al. 2003), we believe this to be the first study that uses dynamic movie excerpts to identify overlapping neural activations during the perception/induction of more than one basic emotion. Physiological changes in bodily state directly influence emotional expression, cognitive functions such as decision-making and memory (James 1894; Damasio 1994; Bechara et al. 1997; Cahill et al. 1997; Critchley et al. 2001; Anderson and Phelps 2000; Craig et al. 2002; Craig et al. 2003). In every day situations, we express different emotions in similar manners depending on the context; tears of both joy and sorrow are a known phenomenon; our hairs raise on end not only when we are joyful or excited but also when we are sad, dead scared or simply imagining some of these emotional experiences. These latter activities are likely involved in integrative processes during perception and experience "serving as somatic cues" of emotional stimuli rather than specifically involved in one type of emotion, underscoring the multifunctional nature of some brain areas in emotion related processes.

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Chapter 3

Empathy for Positive and Negative Emotions in the Gustatory Cortex

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3.1. Abstract

Anterior insula and adjacent frontal operculum (hereafter referred to as IFO) are active during exposure to tastants/odorants (particularly disgusting ones), and during the viewing of disgusted facial expressions. Together with lesion data, the IFO has thus been proposed to be crucial in processing disgust related stimuli. Here, we examined IFO involvement in the processing of other people's gustatory emotions more generally by exposing participants to food related disgusted, pleased and neutral facial expressions during functional magnetic resonance imaging (fMRI). We then exposed participants to pleasant, unpleasant and neutral tastants for the purpose of mapping their gustatory IFO. Finally, we associated participants' self reported empathy (measured using the Interpersonal Reactivity Index, IRI) with their IFO activation during the witnessing of others' gustatory emotions. We show that participants' empathy scores were predictive of their gustatory IFO activation while witnessing both the pleased and disgusted facial expression of others. While the IFO has been implicated in the processing of negative emotions of others and empathy for negative experiences like pain, our finding extends this concept to empathy for intense positive feelings, and provides empirical support for the view that the IFO contributes to empathy by mapping the bodily feelings of others onto the internal bodily states of the observer, in agreement with the putative interoceptive function of the IFO**.**

3.2. Introduction

When we see the facial expressions of other individuals, we can often intuitively feel what they are feeling. The neural basis of this process has received intense interest. A family of theories that could jointly be labelled 'simulation theories' (Keysers et al. 2003; Singer et al. 2004; Lawrence et al. 2005; Botvinick et al. 2005; Jackson et al. 2005; Saarela et al. in press, for reviews see Damasio 1996; Calder 2000; Preston and De Waal 2002; Gallese et al. 2004; Critchley 2005; Adolphs 2006; Keysers and Gazzola, 2006) proposes that this process involves the following: (a) observing the states of others activates representations of similar states in the observer; (b) these activations, which represent a form of simulation of the observed states, are sensed by a network of brain areas that represent bodily states and (c) the sensed states are interpreted and attributed to the other individuals distinguishing them from the observer's own emotions.

The distinction between these sub-processes relates to one made in psychology. Young babies, while witnessing the distress of other individuals, often cry as if they were unable to distinguish their own distress from that of others (for a review see Singer et al. 2006). This phenomenon has been termed 'emotional contagion'. In contrast, while more mature individuals are not immune to emotional contagion, they are increasingly able to attribute the shared distress to the other individual, leading to an empathic *understanding* of the state of others (for reviews see Preston and de Waal 2002; Gallese 2003; Gallese et al. 2004; Decety and Jackson 2004). The processes of simulating and sensing the simulated state of others, hypothesised by simulation theories, would be common to emotional contagion and empathic understanding (for reviews see Critchley 2005; Adolphs 2006). Only the third process of attribution differentiates early contagion from more mature empathic understanding (for reviews see Frith and Frith 1999; Frith and Frith 2003; Singer 2006).

At present, the quest to provide empirical evidence for simulation theories has focussed on providing evidence for the fact that the brain creates a simulation of the states of other individuals, with current evidence suggesting that the observation of the *negative* states of others triggers neural activations that resemble those associated with experiencing similar negative states. Both the observation of disgusted facial expressions and the experience of disgust activate the anterior insula and the adjacent frontal operculum, which will jointly be referred to as IFO (Phillips et al. 1997; Zald et al. 2002; Small et al. 2003; Wicker et al. 2003; Dapretto et al. 2006). The IFO is also activated when subjects observe facial expressions of pain, know a loved one is in pain or experience pain themselves (Singer et al. 2004, 2005; Decety and Jackson 2004; Botvinick et al. 2005; Jackson et al. 2006; Lamm et al. in press; Saarela et al. in press). In addition, lesions in the IFO impair both the experience of disgust and the understanding of other people's disgust (Calder et al. 2000; Adolphs et al. 2003), and subjects that report having more empathic concern activate their IFO more strongly while aware of other's pain (Singer et al. 2004, 2005). Together these experiments converge to ascribe a pivotal role for the IFO in the network of brain areas that underpin the process of simulating observed states of others. This process may be important both for emotional contagion and empathic understanding.

The IFO has also been identified as essential for sensing one's own visceral bodily state (Craig et al. 2000; Critchley et al. 2001, 2002, 2003, 2004, 2005; for reviews see Damasio 1996, Craig 2002; Craig 2003; Critchley 2005), with people more able to sense their own heart beat showing stronger IFO responses (for a review see Critchley 2005). Altogether, the IFO might therefore be engaged in two aspects that are key to simulation theories: the activation of simulated states, and the sensing of one's own state, be it simulated or experienced (Keysers and Gazzola, 2006). In addition, the IFO has been shown to have the pattern of efferent and afferent connections necessary for performing both tasks (Mesulam and Mufson 1982a, 1982b; Mufson and Mesulam 1982).

Is the IFO confined to the processing of negative states, such as pain and disgust, or does it also process positive states, as long as the latter are associated with the visceral sensations that the IFO is thought to represent? The ingestion of pleasant foods and liquids, associated with such positive bodily states, provide a way to test this prediction that has, to our knowledge, so far not been explored. We therefore scanned subjects while they viewed short movie clips of actors sipping from a cup and displaying either an intensely pleased, intensely disgusted or neutral facial expression. Subsequently, we then scanned the same participants while ingesting pleasant (sucrose), unpleasant (quinine) and neutral (artificial saliva) solutions to map their gustatory IFO.

Individuals differ in their sensitivity to the feeling states of others, and these differences can be measured using self-report questionnaires, such as the Interpersonal Reactivity Index (IRI, Davis 1980). Here, we measured participants' IRI scores and then searched for regions that respond more strongly to the gustatory experiences of others in subjects with higher IRI scores. We restricted such a search to subjects' functionally defined gustatory IFO. As argued previously (Singer et al. 2004, 2005, Gazzola et al. 2006) this approach searches for areas underpinning our inter-individual variation in transforming the states of other people into our own, a process thought to be essential for emotional contagion and empathic sharing.

3.3. Materials and Methods

3.3.1. Subjects and procedures: The institutional review board of the University Medical Center Groningen approved the study. Thirty three healthy volunteers free from any known gustatory, olfactory, visual, neurological or psychiatric disorders gave written informed consent and participated in a screening and training sessions. Participants were screen for their taste sensitivity using labeled magnitude scaling (LMS) (Green et al. 1996), for the goal of excluding super/non tasters during the initial rating of the quinine and sucrose solutions as reported earlier (Small et al. 2003). We used quinine and sucrose for the taste screening with the participants reporting their perceived taste intensity on the LMS scale, ranging from 0 (barely detectable) to 100 (strongest imaginable). As we examine the influence of empathy on interindividual differences in brain activity, it is important keep other sources of variance in check. In accordance with other studies (Small et al. 2003), we therefore restricted our experiments to subjects in the normal range of tasting. Normal tasters were defined as those whose score for sucrose fell within the range of 15-75; while the normal tasting for quinine was define by scores ranging from 30-75. Normal tasting scores were obtained for all but 10 participants (9 non tasters and 1 super taster) who were excluded from fMRI. Of the remaining 23 subjects that were scanned, two were excluded because of excessive movement, two for not being able to follow the taste and swallow instructions and one because of a vomiting spell. The final sample included in the analysis consisted of 18 right-handed healthy individuals (10 females, mean age 24; SD 2.64) as classified by the Edinburgh scale (Oldfield 1971). Subjects were questioned to ensure they were ignorant about the aim of the study before the event related fMRI sessions (see Figure 3.1 & 3.2).

3.3.2. Visual Runs: Visual runs consisted of the observation of disgusted, pleased and neutral facial expressions (see Figure 3.1). Actors were recruited from the Noord Nederlands toneel and the Jeugd theatre school, Groningen. They were asked to taste the content of a cup and express their resulting emotion in a naturally vivid manner (see Figure 3.1). A separate group of 16 individuals rated the facial expressions of all the edited movies in terms of the intensity, naturalness and vividness of pleased, disgust, and the neutral expressions they recognized for each movie on a 7 point likert-scale. The 10 best clips for each emotional category in terms of the intensity, naturalness and vividness of expression of the emotions (as rated by the 16 individuals) for the three emotional conditions were selected for the final experiment. Each visual run contained all of the final selected 30 movie clips (3s each, 10 movies per condition x 3 conditions) presented in a randomized event related fashion with a red fixation cross between two movie clips.

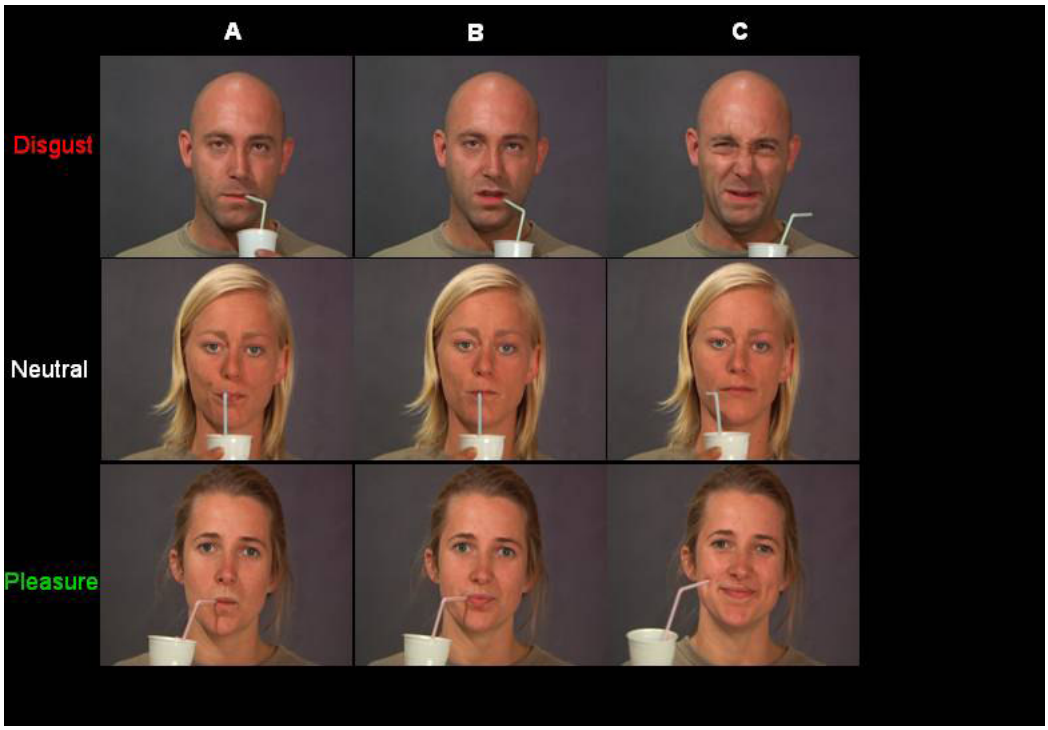

Figure 3.1. Gustatory visual stimuli. Above are picture frames of three sample movies portraying actors while tasting concentrated citric acid (shown in the first row) leading to the experience (expression) of disgust, water (middle row) portraying an neutral gustatory experience and orange juice (last row) leading to the experience (facial expressions) of pleasure. Each movie shows an actor holding a cup and eventually sipping the content by means of a plastic straw (~1s) as can be seen in column (A). This was followed by the actor releasing the straw and simultaneously swallowing the liquid (column B), and finally, the actor then expresses a neutral. pleased, or disgusted facial expression (~2s) shown in column C. During the visual experiment, 10 movie clips for each emotional category was presented with a red fixation cross between each movie lasting for a variable duration of 8-12s in a fully randomized event related fashion. Two repetitions of the original run were present in a fully randomized event related design.

3.3.3. Gustatory Runs: Subjects sampled and rated the intensity and pleasantness/unpleasantness of quinine (unpleasant taste) with a concentration of 1.0×10^{-3}M and sucrose (pleasant taste) with a concentration of 1.8×10^{-2} M as used previously (Small et al. 2003). The neutral taste consisted of artificial saliva (Saliva Orthana; Farmachemie BV Haarlem, the Netherlands; art no. 39.701.130) diluted with distilled water to a subject-specific neutral taste (subjects chose the most neutrally tasting solution from concentrations of 5, 10 and 20 % artificial saliva in water). In the fMRI experiment, taste solutions were delivered as a 0.5 cc bolus over a 5 s period (Small et al. 2003). The solutions were delivered by an experimenter standing beside the MRI scanner using a tubing system that consisted of a syringe (for each taste condition) connected to a 45 cm infusion tube that was inserted into a pacifier through a perforation at the top with the tip of the tube protruding slightly through the sucking tip of the pacifier (see Figure 3.2). Taste instructions were given before scanning using water. Regardless of the strong unpleasant sensations as a result of the quinine delivery, the data obtained from the realignment procedure confirmed that all but two subjects (excluded from the study for this reason) did not move their heads in reaction to the taste solutions for more than 1 mm.

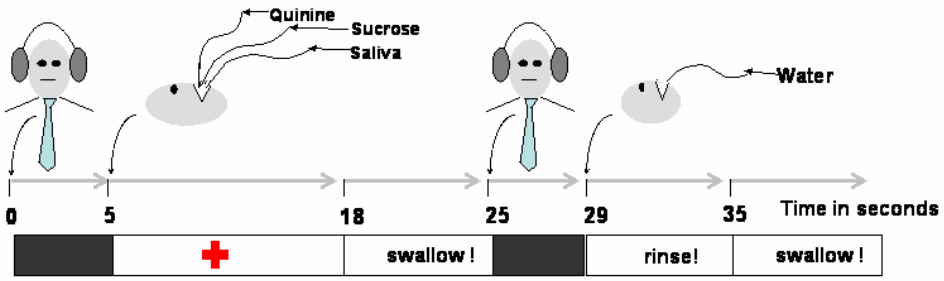

Figure 3.2. Gustatory procedures. Procedures involving presentation of auditory instructions to the experimenter (shown with a tie). Visual instructions were presented to the subject (shown supine). At the start of the experiment, subjects saw a black screen (lowest row), the experimenter simultaneously hears a voice instructing him to deliver either quinine, sucrose or artificial saliva (depending on the trial at hand) by means of the 5ml syringe connected to the tubing (upper row). Timing of delivery of the taste and rinsing solutions was controlled with a voice counting from three to start as in (Keysers et al. 2004) (middle row). For each individual taste stimulus, delivery of the solutions coincides with the presentation of a red cross that follows the black screen, instructing subjects to keep the liquid in their mouth and taste it. This is followed by the text "swallow!" appearing on the screen, instructing subjects to swallow the liquid. The experimenter then receives another auditory instruction to deliver the rinsing water, the delivery of which coincides with a visual instruction to the subject to rinse their mouth, followed by an instruction to swallow the rinsing solution. For each run, a total of three deliveries for each taste condition were carried out, and a total of four taste runs were presented in a fully randomized slow event related fashion with a black screen being presented between two consecutive stimuli with variable durations of 4-6 s. A total of 12 conditions (3 x 4) were modeled for final analysis (i.e., 3 tasting conditions for quinine, sucrose and saliva; 3 swallowing of these tasting conditions; 3 rinsing of these tasting conditions and 3 swallowing of the rinsing solutions).

3.3.4. Self-Report Measures: We rated participants' subjective reaction to the sight of others' gustatory emotions by asking how willing they are to drink some of the beverage the individuals in the movies just tried (from –6 'absolutely not' to 6 'very much') see Figure 3.3. Furthermore, we asked subjects to rate the quinine, sucrose and neutral solution that they had to ingest during the experiment on a scale ranging from –6 'extremely disgusting' to 6 'extremely delicious'. Both scales measure participants' evaluation of the beverages involved in the third person (He tastes) and first person perspective (I taste) of the experiment, and thus allow a direct comparison of these two perspectives. Additionally, the IRI (Davis 1980) was administered to assess participant's interpersonal reactivity index.

3.3.5. fMRI Acquisition: Images were acquired using a Philips 3T whole-body scanner (Best, The Netherlands) equipped with a circular sense head coil. For functional imaging, we used a T2*-weighted echo-planar sequence with 39 interleaved 3.5 mm thick axial slices with 0 mm gap (TR = 2000, TE = 30 ms, flip angle = 80°, FOV = 224 mm, 64 × 64 matrix of 3.5 × 3.5 × 3.5 mm voxels. At the end of the functional scans, a T1-weighted anatomical image (1 × 1 × 1 mm) parallel to the bicommisural plane, covering the whole brain was acquired.

3.3.6. General fMRI data Analysis: Data were preprocessed and analyzed using Statistical Parametric Mapping (SPM2, Wellcome Department of Cognitive Neurology, London, UK; http://www.fil.ion.ucl.ac.uk). All functional volumes were realigned to the first acquired volume. Images were coregistered to the subject's anatomical space and spatial normalization was then carried out on all images (Friston et al. 1995) to obtain images with a voxel size of 2 × 2 × 2mm). All volumes were then smoothed with an 8 mm full-width half-maximum isotropic Gaussian kernel. For time series analysis on all subjects, high-pass filters with cut-off points at 106s and 310s for the visual and gustatory conditions respectively, were included in the filtering matrix in order to remove low-frequency noise and slow drifts in the signal, which could bias the estimates of the error. Condition-specific effects at each voxel were estimated using the general linear model. Single subject's t contrast maps and random effects analyses were carried out (Wicker et al. 2003). Furthermore, a simple regression analysis (using the individual scores of each IRI subscale and individual disgust sensitivity scores as a predictor) of the activations of the whole brain was carried out at the second level using the basic models function in SMP2 and a statistical threshold of $p < 0.005$ (uncorrected).

3.3.7. Functional definition of the gustatory IFO: We defined the gustatory IFO functionally using the data from the taste experiment. We calculated at the second level, the contrasts quinine-saliva and sucrose-saliva, thresholded both contrasts individually at $p < 0.005$ (uncorrected) and applied a logical OR (figure 3.4). The resulting map thus included all voxels involved in tasting (be they positive of negative) relative to saliva. Since this map included more than the IFO, we additionally required that voxels had MNI coordinates in the following range: X = (22 to 70 or -70 to -22), Y = (-4 to 40) and Z = (-20 to 18). The resulting map was used as an inclusive mask for the correlation analyses between participant's IFO activation during the viewing of facial expressions of other's gustatory experiences and their own tendency to empathise with others as measured by the IRI scale.

3.3.8. Correlation analysis: To identify correlations between empathy and visual activations in the IFO, for each subject, we calculated the parameter estimates for viewing disgusted, pleased and neutral facial expressions separately. Using a separate simple regression model at the second level, we then searched for voxels in which a correlation between the individuals IRI score and parameter estimate for each of the facial expressions existed. These maps where inclusively masked with the gustatory IFO mask. This analysis was performed for the total IRI score (composite IRI), and separately for each of the four subscales (i.e., empathic concern 'EC', Fantasy 'FS', perspective taking 'PT' and personal distress 'PD').

Additional analyses within identified clusters of correlation were performed using Marsbar (http://marsbar.scourceforge.net; M. Brett, J.-L. Anton, R. Valabregue, and J.-B. Poline, 2002, Region of Interest analysis using an SPM toolbox, abstract).

3.4. Results

3.4.1. Ratings

In order to determine how much the emotional facial expressions in the movies affect the subjects, we rated their subjective reaction to the gustatory emotions depicted in the films by asking how willing they would be to drink some of the beverage the individuals in the movies just tried (from –6 'absolutely not willing' to 6 'very much willing'); see Figure 3.3. Furthermore, we asked subjects to rate the quinine, sucrose and neutral solutions that they had to ingest during the experiment on a scale ranging from –6 'extremely disgusting' to 6 'extremely delicious'. Both the willingness and the direct taste rating scales measure participants' evaluation of the beverages (albeit from different perspectives), and were therefore directly compared using a repeated measurement ANOVA with 2 perspectives (tasting vs. viewing) x 3 beverages (unpleasant, pleasant and neutral).

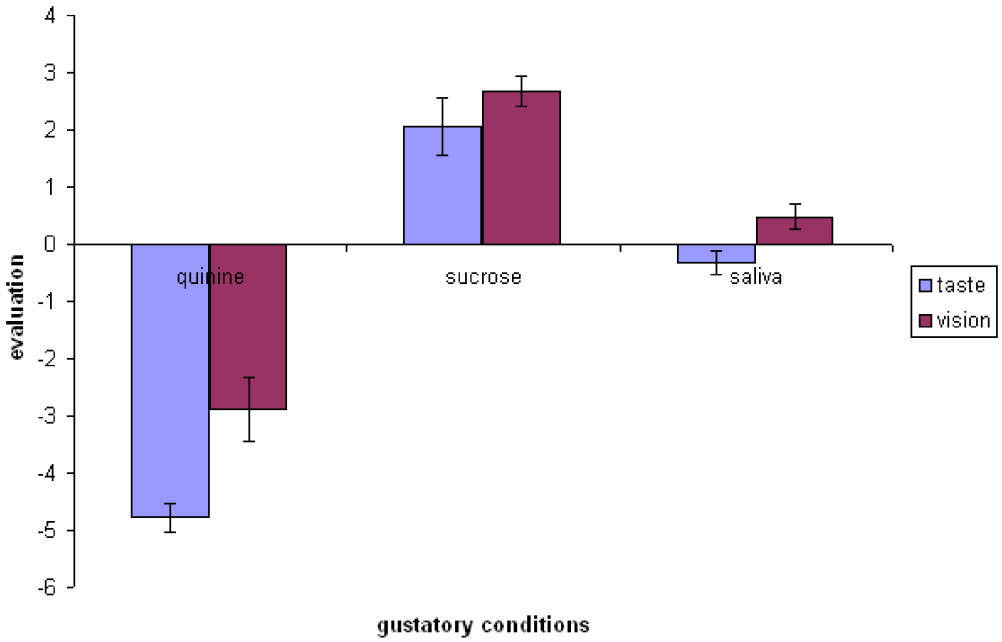

Figure 3.3. Mean subjective ratings ± SEM of how disgusting/delicious the liquids were perceived by participants during tasting of quinine, sucrose and artificial saliva (taste ratings blue bars) and ratings of ones' willingness to drink the liquids they saw the actor drinking (visual ratings red bars). Participants were asked to rate the taste experience of others in their own person-relevant perspective (i.e., how willing they were to taste the drink they saw the actors just took), this way, we could judge the affective states the facial expressions induced in them by means of their willingness/unwillingness ratings as a result of the perceived pleasure/disgust.

This analysis revealed a main effect of perspective ($F(1,17)=9.9$, $p<0.006$), with tasting leading to more negative ratings than observing and a main effect of beverage ($F(2,34)=184$, $p<10^{-18}$) were observed. Importantly, there was no interaction of perspective and beverage ($p<0.386$), suggesting that the difference between the beverages was similar for the two perspectives. During tasting however, quinine was rated as more disgusting than sucrose as pleasant ($p<0.001$, after comparing absolute values by changing the negative scores for quinine into positive scores). This phenomenon has been reported earlier (Wicker et al. 2003; Nitschke et al. 2006), and explained as related to differences in perceived intensity as well as valence (Small et al. 2003; Liberzon et al. 2003). Finally, post-hoc examination (Newman-Keuls) shows that negative and positive stimuli were rated as more negative and more positive respectively than neutral stimuli (all $p<0.001$).

3.4.2. Neuroimaging Results

To identify the relationship between individual's gustatory IFO responses to the gustatory experience of others during the witnesses of these experiences, we correlated individuals' IRI scores with their brain activations during the viewing of other's gustatory emotions. The IRI is a 28-item with a 5-point Likert-type scale (0 = does not describe me well to 4 = describes me very well) that assesses four dimensions of empathy: FS, PD, PT and EC (for an indepth explanation of the IRI scales see supplementary materials). To restrict our analysis to the gustatory IFO we masked these correlation maps with the functionally defined gustatory IFO (see methods).

As a first step, we analysed correlations with the composite IRI obtained from summing the scores obtained on the four distinct subscales. Figure 4 shows that positive correlation exist between this composite IRI and the parameter estimates obtained during the vision of disgusted facial expressions, in both the right and left gustatory IFO. In the left IFO, similar correlations were observed between the vision of pleased facial expressions and the composite IRI. Correlations with neutral facial expressions were smaller, and restricted to the right hemisphere.

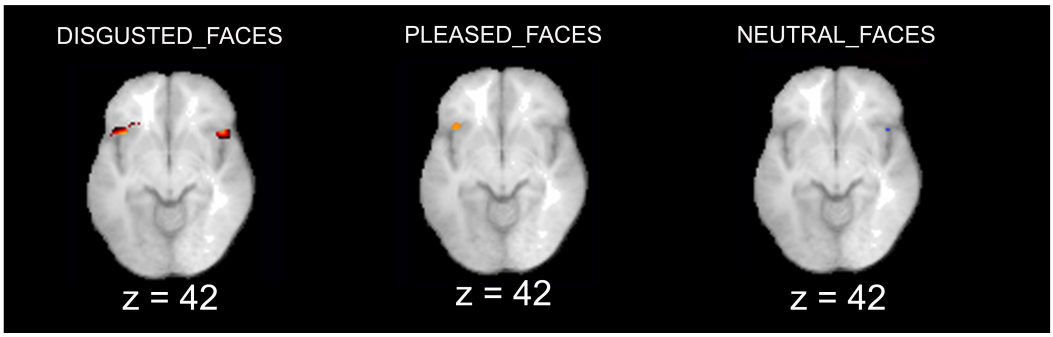

Figure 3.4. Composite IRI scores (i.e., total scores on the whole IRI questionnaire) predicted IFO activations during the observation of different facial expressions. Results show voxels where the correlation between the parameter estimate obtained during the vision of the facial expression and the composite IRI was significant at $p<0.005$, uncorrected, and the cluster contained at least 5 contiguous voxels. Results were masked with the gustatory IFO. Results are overlaid on the mean T1 image of the 18 subjects.

The composite IRI pools data from four different subscales measuring different aspects of empathy, and it has been advocated that a better approach stems from examining the individual contribution of each subscale (D'Orazio 2004). We therefore examined the pattern of correlation observed for each individual subscale.

Both the PD and FS scales correlated significantly with visual activations during the observation of *both* the disgusted and pleased facial expressions of others in bilateral gustatory IFO (Figure 3.5a-b). The center column of Figure 5a-b shows this spatial pattern of correlation. Voxels shown in red correlated with the empathy scores while viewing disgusted faces, but not while viewing neutral or pleased faces; voxels shown in white correlated with the empathy scores during both the vision of pleased and disgusted facial expressions, but not during the vision of neutral facial expressions. No other combination of correlations was observed within the IFO for the PD and FS scales. To examine the nature of the correlational relationship further, we extracted the mean BOLD signal from the compound clusters of correlations (combining the white and red voxels circled in the figure), and calculated parameter estimates for all visual and gustatory conditions (see graphs in the right and left column of Figure 3.5). For both scales, and both hemispheres, parameter estimates for

the vision of pleased and disgusted facial expressions correlated significantly with the empathy measures at $p < 0.005$ if the entire cluster was considered (Figure 5a and b, left column). The right column illustrates the overall trend in these clusters if the low and highly empathic subjects are combined, and shows that over all, subject's mean activations to pleased and disgusted facial expressions were extremely similar.

For PT and EC scores, the link to the visual brain activations was less clear. None of the voxels in the gustatory IFO had visual activations that correlated with the EC scale during the vision of any facial expressions. For PT, only one cluster (relatively smaller than the ones predictive for PD and FS) in the right gustatory IFO correlated significantly with the parameter estimate for viewing disgust and neutral. but not pleased, facial expressions (shown in red in Figure 3.5c).

Within all five empathy-correlating clusters outlined in Figure 3.5, during vision, pleasant and disgusted facial expressions seemed to cause similar average activations, both of which exceeded that observed during the viewing of neutral faces. We performed a 5 cluster x 3 faces ANOVA to examine this effect. The analysis revealed no significant interaction between cluster and faces indicating that the effect of stimulus was similar in all clusters. A planned comparison between pleased and disgusted faces showed no significant difference ($F(1,17)<0.33$, $p>0.57$), while both emotional faces caused stronger activations than the neutral faces ($F(1,17)>6.56$, $p<0.02$). During tasting, the same analysis revealed a different pattern of activation: quinine exceeded both saliva and sucrose in activation (both $p<0.02$), and sucrose did not differ from saliva ($p>0.6$).

3.5. Discussion

Here, we examined whether the gustatory IFO activations during the vision of pleased and disgusted facial expressions correlated with how participants scored on a self report measure of their sensitivity to other people's feeling states (IRI). We found that for both pleased and disgusted facial expressions, subjects that obtained higher scores in the PD and FS subscales of the IRI activated their functionally defined gustatory IFO more strongly than participants obtaining lower scores. This relationship was less prominent in the case of PT and EC scores.

These results demonstrate for the first time, that during the vision of facial expressions of other people's gustatory emotions, the amplitude of activations in the gustatory IFO go hand-in-hand with differences in self reported interpersonal reactivity. This finding extends our previous demonstration, that the IFO contains voxels involved in the experience and the observation of negative states such as disgust (Wicker et al. 2003), and provides further support for the hypothesis that the IFO activation during emotion observation may represent a transformation of observed states into experienced emotional states (Wild et al. 2001; Hess & Blairy 2001; Lawrence et al. 2005; Saarela et al. in press; for reviews see Damasio 1996; Craig 2002; Gallese et al. 2004; Critchley 2005; Singer 2006). In addition, by using a correlation approach, we provide the first demonstration that the IFO is involved in the processing of positive states of other individuals.

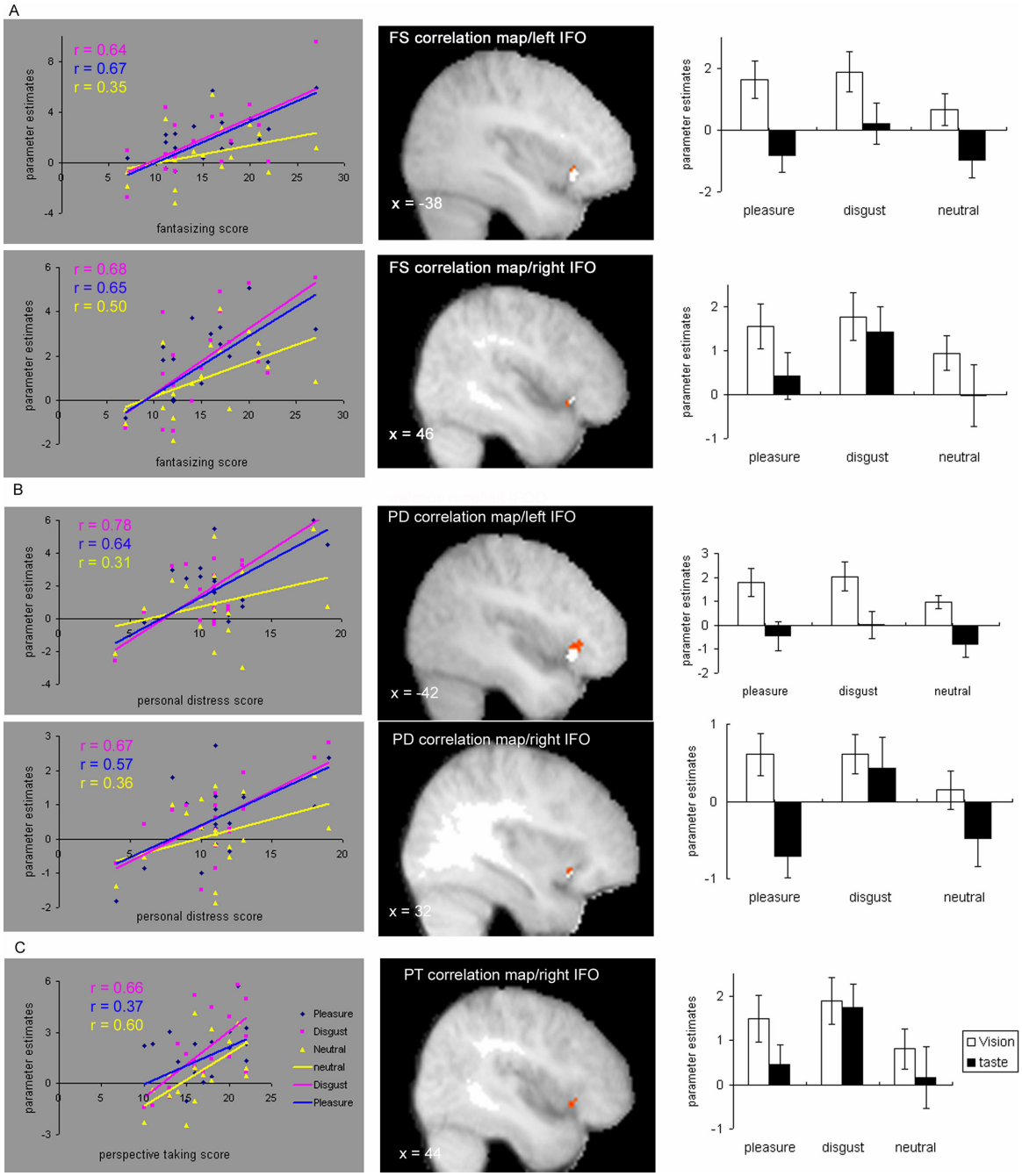

Figure 3.5. Empathy and the IFO. Center column: voxels showing significant correlations between individuals' IRI subscales and parameter estimates derived during the observation of disgusted, pleased or neutral facial expressions (see text for colour coding). Results were thresholded at p<0.005 uncorrected at the voxel level, and masked with the gustatory IFO. The mean signal in the circled compound cluster of correlation was then extracted, and further analysed in the left and right column, showing the relationship between subjects' IRI score (x-axis) and visual parameter estimates (y-axis) together with regression line and correlation value on the left, and mean parameter estimated during vision and taste (±SEM) on the right. The color-coding for the right and left columns defined in panel C applies to all panels. Our results of FS and PD predicted activations in IFO during the viewing of disgusted faces, and PD predicted cluster during the viewing of pleasure survived FDR correction at p < 0.05. The FS predicted activations during the viewing of pleased faces also survived FDR correction at p < 0.075 while the PT predicted IFO activation during the viewing of disgusted and neutral faces did not survive FDR corrections.

A number of studies have suggested that the insula is particularly involved in the processing of negative states, including disgust and pain (Phillips et al. 1997; Calder et al. 2000; Adolphs et al. 2003, Wicker et al. 2003; Singer et al. 2004, 2005;

Jackson et al. 2005; Botvinick et al. 2005; Lamm et al. in press, for reviews see Calder et al. 2001; Adolphs 2003). In contrast, others have shown that the insula is also important for the experience of positive visceral states such as positive tastes and flavours (Zald et al. 2002, Small et al. 2003; O'Doherty et al. 2006; Nitschke et al. 2006; for a review see Phan et al. 2002) and a PET study has shown preliminary evidence for the fact that the vision of bodily pleasure in others causes activations of the Insula (movies of pleasant heterosexual coitus, Stoleru et al. 1999). Our current study extends these findings to positive food related pleasure and suggests a link between these activations and interpersonal reactivity. It thereby confirms the involvement of the IFO in the processing of both the positive and negative states of others.

The pain, disgust and bodily pleasure that have been found to activate the insula so far all involve sensations triggered by strongly somatic stimuli such as food, electroshocks or sexual stimulation. Further research will be needed to test whether states such as primary disgust/distaste, pain, food- and sex-related pleasure do rely on the IFO because they are associated with a somatic *trigger.* On the other hand, facial expressions of sadness, fear and smiles without context may not rely on the IFO, simply because they are not typically triggered by somatic stimuli (although once triggered, these emotions can result in changes of the somatic state).

Our finding that different subscales of the IRI differ in their correlation with visual activations requires some consideration (see Supplemental Materials for a complete IRI questionnaire). According to simulation theories, as outline in the introduction, the sight of the states of others: (a) triggers similar states in the observer, which are then (b) sensed, and eventually (c) attributed to the other individuals. The IFO is thought to contribute mainly to the first two stages of this process. If this is true, IFO activity should correlate primarily with subscales of the IRI that tap into the sharing of feeling states (i.e. emotional contagion). The FS subscale taps directly into respondents' ability to transpose themselves into the feelings and behaviors of actors in movies (Davis 1980; Davis 1994). The PD subscale measures people's emotional arousability related to both *negative* and *positive* emotions (Eisenberg et al. 1991; Davis 1994). Both scales thus measure interindividual differences in emotional contagion and the fact that these scales were found to correlate with the activations in the IFO thus supports the view that the IFO may be part of a network that maps the states of others onto similar states in the observer. As the scales are self report scales, they rely on the subject's capacity to sense their own states, and these correlations are thus also in agreement with the idea that the IFO is involved in sensing the (simulated) states of others (Critchley et al. 2002, 2003, 2004: for reviews see Gallese et al. 2004; Keysers and Gazzola, 2006; Crichtley 2005). Based on our results, it is impossible to conclude whether the role of the IFO is primarily to activate a state resembling that of the observed subject or to sense and feel that simulated state.

The EC and PT scales on the other hand tap into aspects that go beyond the sharing of feeling states of others. The EC subscale focuses on sympathy for 'victims' (people less fortunate, people that are taken advantage of, people treated unfairly). Subjects scoring high on EC thus do not primarily respond to the sight of the misfortunes of others with states directly resembling those observed (which would be rage, pain or fear), but with sympathy. This involves a process that according to simulation theories would occur after a simulated state has been sensed and attributed to another individual. While negative findings should always be interpreted with caution, the stronger correlation between PD and IFO activation compared to EC might relate to two aspects. First, EC might be more related to the function of other

structures. Second, our actors were not prototypical victims: they voluntarily tried a variety of drinks, making most of the questions in the EC subscale irrelevant to our movies. In support of this latter interpretation, Singer et al. (2004) showed that when the stimuli relate to the pain inflicted by someone else to a loved victim (i.e., an electroshock), EC scores do predict activations in a region that overlaps with our IFO findings. Finally, the PT subscale mainly measures interpersonal reactivity from a more cognitive, emotionally neutral perspective that taps into people's voluntary attempts to understand the goals and motivations of other people. Here we focused on the gustatory IFO to examine the function of this structure. The stronger correlation with PD and FS and the weaker correlation with PT and EC suggest that this stucture is primarily involved in the involuntary sharing of observed states common to emotional contagion and empathic sharing (as measured by PD and FS). The IFO appears less involved in the more deliberate concern or cognitive perspective taking that requires an explicit and mature concept of self and other. Other areas of the insula, the anterior cingulate and other limbic structures appear more involved in these latter processes.

By capitalizing on inter-individual differences in empathy, we show that the regions of the IFO involved in the processing of our own sensation of drinking are activated both when participants witness other individuals drinking pleasant and disgusting beverages. These findings suggest that the role of the IFO in the representation of bodily states of others is broader than previously thought (Craig et al. 2000; Critchley et al. 2001, 2002, 2003, 2004, 2005; Krolack-Salmon et al. 2003; for reviews see Damasio 1996; 1999; Craig 2002; Churchland 2002; Preston and De Waal 2002; Gallese et al. 2004; Critchley 2005; Adolphs 2006; Keysers and Gazzola, 2006) and not limited to negative emotions. The fact that our activations depended on differences in interpersonal reactivity expands the original observation of a link between IFO activity and empathy for pain (Singer et al. 2004, 2005) to gustatory pleasure and disgust/distaste and strengthens the link between activations in these areas and our ability to share other people's emotions.

The human bilateral IFO may constitute a critical component of the neural mechanism that allows the mapping of the bodily states of others onto our own inner states and thereby facilitate our understanding of the social environment and ultimately survival. How strongly individuals mirror socially relevant bodily experiences may depends on the reactivity of their inner milieu (as measured by disgust sensitivity scales etc.) or their capacity to sense their inner milieu (as measure for instance by alexithymia scales). Future experiments will need to disentangle these processes and to determine the neural basis of the interaction between emotional contagion and empathic understanding.

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Chapter 4

COMT polymorphism and susceptibility to major depression modulates psychological stress response

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4.1. Abstract

The stress response is related to both physiological and psychological factors and is strongly marked by a neuroendocrine component. Genetic factors are believed to underlie individual differences in the degree of stress resilience and thereby contribute in determining susceptibility to stress-related pathologies like major depressive disorder (MD). To explore the genetic influence on the endocrine and behavioural stress response in relation to MD, we sought to examine the effects of the catechol *o*-methyltransferase polymorphism (*COMT*) on psychological stress in three groups of individuals with different degrees of susceptibility to MD (i.e., healthy controls, healthy high risk probands to MD and those suffering from MD). This genotype is involved in the metabolism of catecholamines (dopamine, norepinephrine "NE" & epinephrine "EP"). Allelic variations of this polymorphism were found to influence the degree of plasma EP response and subjective experience of stress. Interactions between *COMT* and diagnostic groups in measures of plasma EP, cortisol and subjective responses to psychological stress were also found, with the influence of the different alleles on these measures differing between healthy controls relative to MD patients and high risk probands (HRP). These observations support a possible role for *COMT* in the endocrine and subjective response to psychological stress and thus may qualify as a possible candidate gene involved in the pathogenesis of MD.

4.2. Introduction

Traumatic and stressful life-events are potential risk factors for the onset of certain psychiatric disorders (Selye 1936; Brown and Harris 1978; Ormel et al 2001). Differences in human resiliency and genetic determinants of the stress responses are therefore believed to influence the degree of individual's vulnerability to stress-related disorders (Caspi et al 2003; Charney 2004; Robbins 2005).

Traditionally genetic research in complex diseases like mental disorders has been guided by the assumption that genes cause diseases. In the face of evidence of both negative and positive associations between genes and psychiatric disorders, the expectation that direct paths will be found from gene to disease has not yet proven fruitful for complex psychiatric disorders (Colhoun et al 2003; Massat et al 2005; Munafo et al 2005; Moffitt et al 2005). The following reasons may explain the conflicting findings regarding gene-disorder association in psychiatric populations. First, many complex diseases like MD are thought to be inherited because they tend to run in families, but they do not show typical Mendelian pedigree patterns (Gambaro et al 2000). Second, a large fraction of prior studies has not considered the role of physiological and psychological responses to environmental pathogens in gene expression (Ormel et al 2001; Moffitt et al 2005).

A relationship between genetic processes and physiological and behavioural reactions to cognitive, pharmacological and emotional challenges has been suggested many times (Zubieta et al 2003a; Oswald et al 2004; Uhart et al 2004; Smolka et al 2005; Szegedi et al 2005; Goldberg and Weinberger 2004). Additionally, genetic differences as determined by *COMT*, have been shown to underlie individual differences in response to psychological and physically stressful (pain) challenges (Zubieta et al 2003a). It was found that within the *COMT* genotype, those homozygous for *met/met* allele reported a reduced subjective and neurochemical

response to pain, which may also be applicable for other types of stressors (Zubieta et al 2003a). This genotype is a frequent functional polymorphism of catechol-O-methyltransferase that codes the substitution of valine (*val*) by methionine (*met*) at codon 158 ($val^{158}met$) and is associated with a difference in thermo stability leading to a three- to fourfold reduction in the activity of the *COMT* enzyme in catabolizing dopamine (DA), EP, and NE (Lotta et al 1995; Lachman et al 1996). The alleles are co-dominant so that individuals with the *val/val* genotype have the highest activity of *COMT*, those with the *met/met* genotype have the lowest activity of *COMT*, and heterozygous individuals are intermediate (Scanlon et al 1979). Furthermore, this polymorphism has been associated with cortical response to negative emotional stimuli (Smolka et al 2005), altered μ-opioid receptor binding potential during the experience of a pain stressor (Zubieta et al 2003a), cortical response to the performance of cognitively demanding tasks (Goldberg and Weinberger 2004), response to anti depressive treatment (Szegedi 2005) and the risk for several neuropsychiatric conditions (Karayiorgou et al 1999). Taken together, experimental studies that expose participants to physiological and psychological challenges in relation to candidate genes may assist in elucidating the role of genes in the pathogenesis of psychiatric disorders.

Here, we address this problem by examining the role of *COMT* in endocrine and subjective response to psychological stress. Psychological stress is perceived as a valid environmental pathogen for affective disorders like MD (Munafo et al 2005; Sapolsky 2000). Even though psychological stress is believed to be a valid environmental pathogen for emotional disorders such as MD (Munafo et al 2005), little is known about the direct influence of *COMT* or any other candidate gene on endocrine and subjective responses to psychological stress in relation to this disorder.(Charney 2004; Sapolsky 2000).

We exposed human adolescents and young adults (ages ranging from 15-31 years) having different degrees of susceptibility to major depression to acute psychological stress in a laboratory setting. Healthy controls (defined as absence of psychiatric diagnosis in self as well as first and second degree relatives), patients suffering from early-onset-major depression (MD group) first diagnosed between ages 15-30, and healthy high risk probands (HRPs) of major depression (defined as having more than one first degree relative being affected with MD) were included. We label this grouping as diagnostic status for explanatory reasons even though members of the HRP group has no diagnosis other than the fact of a higher familial loading for MD (i.e., more than one first degree relative being diagnosed with the disorder). Thus, the MD and HRP groups were included alongside healthy controls because directly characterizing the genes expressed in 'normalcy' and disease might enable us to circumvent the need to sort through complex genetic variation by finding the small minority of important sites (Weinberger et al 2001; Weiss and Terwilliger 2000).

We hypothesize a moderating influence of *COMT* on the plasma catecholamine: EP and NE; and glucocorticoid: adreno-corticotropic-hormone 'ACTH' and cortisol responses to laboratory induced stress. Given the role of the met allelic variation in emotional and pain response (Smolka et al 2005; Zubieta et al 2003a), we expect the degree of *met* allelic loading of this genotype to predict higher endocrine and subjective stress responses. Furthermore, we expect a possible interaction between *COMT* polymorphism and diagnostic status during acute stress, with the degree of endocrine and subjective stress responses differing between diagnostic groups as a function of *COMT* allelic variation.

4.3. Materials and Methods

4.3.1. Subjects: Participants in this study were recruited from local and regional sources (North Netherlands Population Registration) as volunteers for the Neurobiological and Epidemiological cohort-study of Adolescents at Risk of Anxiety and Depression (ARIADNE) as described elsewhere (Landman-Peeters et al 2005). In brief, ARIADNE is a large prospective study into the development and course of depression and anxiety among adolescent and young-adult offspring of psychiatric patients with MD, panic disorder, and/or obsessive compulsive disorder.

Members of the HRP group were recruited from the ARIADNE cohort group that is currently being studied longitudinally (Landman-Peeters et al 2005). Our HRP group consisted of a sample of individuals with a predominantly high familial loading for MD. Healthy volunteers were recruited from families without a history of neuropsychiatric disorders residing in the same geographical area (Northern Netherlands). In the present study, an MD patient conforms to the criteria of the Diagnostic and Statistical Manual for Mental Disorders (DSM-IV) for the diagnosis of MD. Thus, MD individuals were all at the time of the study in/out patients at the psychiatry department of the UMCG or other institutions. All participants were interviewed by a trained clinician according to the DSM-IV. The healthy individuals (control and HRP groups) were free of any neuropsychiatric diagnosis as assessed by the DSM-IV interview. Additionally, subjects were interviewed to control for life-time depression using the Composite International Diagnostic Interview (CIDI) WHO-2000 version (Wittchen 1994). It is a structured interview to assess mental disorders designed for use by trained interviewers who are not clinicians. By means of computerized algorithms, it provides diagnoses according to accepted criteria such as the International Classification of Diseases (ICD-10) and the DSM-IV. The CIDI is used in samples of 14 years and older and has been shown to be reliable and valid (Wittchen 1994; Kessler et al 1994).

To control for the presence/absence of family history of MD, we developed a questionnaire (ARIADNE questionnaire) that is designed to detail individuals' and their family's neuropsychiatric and general health history, individuals' life style regarding participation in shift work, intensive sport, smoking, alcohol and drug use (i.e. frequency of cigarettes and/or alcohol consumption and history of drug abuse). For the healthy controls, those with an indication for a family history of neuropsychiatric disorders were excluded. All participants signed a consent form granting permission to contact their first degree relatives or general practitioner for further enquiries should it be necessary. For participants younger than 18, one of their primary care givers also filled in the ARIADNE questionnaire.

To control for confounding variables, only subjects of Dutch Caucasian origin were included. Other than the MD group (who were all taking selective serotonin reuptake inhibitors; SSRI), only subjects free of medication were included. Other exclusion criteria included obesity (as measured by their body mass index) and diagnosis of neuroendocrine and other related disorders. Additionally, state-trait-anxiety inventory (Van der Ploeg 1979), profile of mood states (McNair et al 1971, 1981, 1992), and the temperament inventory (Cloninger 1994) were assessed. The results of these questionnaires however, will not be discussed further in the present study. The Beck depression inventory (Beck et al 1961) was used as an inclusion criterion, with the acceptable scores for the depressed individuals being placed at 12 and above, while that of the controls and HRP group was limited to 8 and below. Since preclinical and clinical studies have shown that nicotine is able to activate the

HPA axis and the neuroendocrine response to psychological and neuroendocrine stressors, we assessed quantity of smoking through self-report (Pomerleau et al 2004; Mendelson et al 2005). Only subjects who smoked less than 20 cigarettes per day were included. Participants' level of education, as determined by the number of years of post primary education, was assessed alongside their recent life events and social support status. Diagnostic groups were matched as much as possible on age; gender and level of education (see Table 1 for more details on the group demographics). DNA assessment was carried out after the experimental procedure; therefore, genotype groups were not matched according to demographics.

The final cohort group that took part in the experimental procedures consisted of 68 individuals (22 healthy controls '9 females'; 25 HRP '14 females'; and 21 MD patients '15 females'). Women were tested within a week after the completion of their last menstruation and all participants gave written informed consent as required by the local ethics committee of the UMCG Groningen, in accordance with the declaration of Helsinki principles. For participants younger than 18 years, informed consent was given by a primary care giver in addition to their own permission. All participants received 32 Euros compensation and complete reimbursements for travel expenses.

4.3.2. Laboratory Stress Test: Subjects arrived at 12:30 PM for experimental sessions after completing the evaluations about 2 weeks prior to the study. They were instructed to fast the morning of the experiment, and refrain from intensive physical exercise and use of alcohol and tobacco, and consumption of any caffeine-containing beverage/foodstuffs 12 hours prior to the experiment. Subjects were escorted to the clinical research centre and an intravenous catheter (saline drip) was inserted into an antecubital vein at approximately 13:00 hours. They rested in a dental chair (semi-supine) for 45 min watching a documentary film about Antarctica for the purpose of getting used to the unfamiliar environment.

Subjects were then exposed to the Groningen Acute Stress test (GAST). The GAST is a modified version of the Trier Social Stress Test (Kirschbaum et al 1993), mainly consisting of a free speech and the speech preparation (SP) task, whereby participants were asked to defend themselves in an embarrassing situation (imagined accusation of shoplifting), and a forced mental arithmetic task under time pressure (Van der Pompe et al 2001). These three stress conditions (SP, speech and mental arithmetic) were conducted in the presence of a small audience (consisting of three experimenters) while participants were being video-taped. They were informed that the video tapes would be used for further psychological analysis by a group of behavioral experts for the purpose of selecting the individual with the most convincing story and that person would be rewarded with a small travel grant (see Figure 4.1 for a detail of the GAST). Prior to stress exposure and immediately after each stress condition (baseline plus three stress conditions and at the very end of the resting condition i.e., five in total), participants were asked to indicate on a 10-point Likert scale (1 representing none and 10 an intense experience) how much negative excitement they felt each time, i.e., during baseline rest, during the speech, mental arithmetic, computer tasks with both monetary gain and loss and during the final recovery period. An average of these measures taken after each of the three stress tasks (Forced speech, mental arithmetic and computer task) were used as an indicator of the degree of *subjective stress experience* during the experimental challenge.

4.3.3. Determination of Endocrine Levels: ACTH plasma levels were assayed using the Nichols Advantage enzyme Immunoassay for the 10 samples collected during different stress and rest conditions http://www.nicholsdiag.com/products. Plasma cortisol levels were assayed using a validated automatic analyzer system (Elecsys® 2010, Roche, Switzerland) whereby serum cortisol levels were determined by means of a specific and highly sensitive enzyme immunoassay. Plasma EP and NE levels were assayed by high performance liquid chromatography (HPLC) with electrochemical detection, as described by Smedes and colleagues (Smedes et al 1982).

4.3.4. DNA extraction and COMT genotype analysis: 10ml EDTA anticoagulated blood was collected and centrifuged at 800g for 10 minutes at 4 °C. The Buffy coat was collected and stored at – 20 °C until DNA isolation. After thawing, DNA was isolated with the help of the Qiamp Mini Blood Kit (QIAGEN Benelux B.V., Venlo, The Netherlands). Genotyping of the *COMT val*^{158}*met* polymorphism (1947 G/A; GenBank Z26491; dbSNP: rs4680) was performed with the allelic discrimination technique on an Applied Biosystems 7500 HT Real-Time PCR system (Applied Biosystems, Nieuwekerk a/d Ijssel, The Netherlands) according to the protocol supplied by Applied Biosystems. We used primers COMT-GAF (5'-CGAGATCAACCCCGACTGT-3') and COMT-GAR (5'-CAGGCATGCACACCTTGTC-3'), as well as minor grove binding (MGB) probes VIC-5' –TTTCGCTGGCGTGAAG-3'-NFQ (G), and FAM-5'-TCGCTGGCATGAAG-3'-NFQ (A). Additionally, a Taqman universal PCR master mix, all supplied by Applied Biosystems was also used.

4.3.5. Statistical analyses. Between groups comparisons of the demographics data were performed using ANOVA and chi-square tests (χ^2) on the final 68 subjects of which both DNA and hormone data was available (see table 1 b). Association between *COMT* and diagnostic status was analyzed using a case-control analysis by comparing the allele frequencies between diagnostic groups with an x^2 test. The genotype frequency distribution was tested for Hardy Weinberg equilibrium using an x^2 test. No deviations that could point to missed alleles or recent population admixture were found. The Hardy-Weinberg test is also valid as test for association, but has limited power.

Principal component analysis (PCA) was performed using the raw values of the ten time points of blood measurement for the analysis of cortisol, ACTH, EP and NE plasma levels during the GAST using SPSS 12.0.1 (Chicago, IL). PCA is a data-reduction technique that reduces a number of correlated variables into fewer uncorrelated factors. Two factors were extracted for each hormone. The first factor coincided with the stress measures, i.e., from baseline sample 1-sample 7, which we called the *stress-related component* (PCA1) and the second coincided with the rest measures, i.e., samples 8-10, which we called the *rest-related component* (PCA2), together accounting for 90.1% of the variance for cortisol, 87.4% for ACTH, 87.7% NE and 78.9% EP. This analysis is ideal for an experimental paradigm like our own because it reduces ten original variables to only two that capture most of the variability due to the physiological response to the experimental challenge. Given the fact that the baseline measure was collected 40 min after venapuncture, the plasma endocrine levels at this point might indeed be affected by physiological responses to venipuncture and catheterization as shown by the PCA analysis (Rose and Hurst 1975).

The area under the curve (AUC) was calculated with trapezoidal approximation from log transformed values for all hormonal measures (i.e. from baseline to last resting period) (Pruessner et al 2003). The latter measures integrated secretory effects over the full course of the experiment, which might be informative especially when applied to the slowly responding cortisol (Abelson et al 2005).

We conducted a *COMT* genotype by diagnostic group, *COMT* by Sex and Sex by diagnostic group analysis of variance (ANOVA) with smoking (in terms of number of cigarettes a day) as a covariate on hormonal response measures (PCA and AUC data), subjective stress experience and performance. Earlier studies have shown smoking to influence secretory patterns of some of the hormones under study (Pomerleau et al 2004; Mendelson et al 2005).

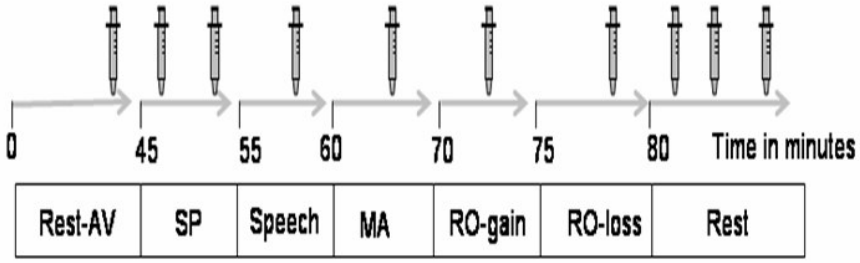

Figure 4.1. Experimental procedures. The syringes represent the moments of blood sampling during each stress task with the arrows representing the duration of the tasks shown in the lowest row. Time 0 is the moment of venipuncture which took place at about 13:00 for all participants, with the numbers from 0-80 and beyond showing the time schedule in minutes of the reactivity session during GAST. The lowest row represents the various tasks starting at time 0 with a rest period after venapuncture (Rest-AV) with the blood sampling during this period considered as our baseline measure. The GAST mainly consists of a speech preparation (SP) and a free speech, a mental arithmetic task (MA), rank-ordering of numbers including monetary gain (RO-gain) and loss (RO-loss) consecutively, and a resting period where a movie was shown of an expedition to a remote region of Siberia(rest). In the speech session, participants imagined a serious accusation of shoplifting and prepare their defense in 8 min (SP) and defend themselves verbally in a 6 min Speech. This was followed by a mental arithmetic task under time pressure for 10 min (MA). After MA, participants carried out a rank-ordering of numbers (from small to large) by filling in the number of steps needed to get the right order of a presented series of numbers on a computer screen placed in front of the them. This task was divided into two parts, the first consist of a situation where participants can will a lot of money, followed by a similar condition with increased task difficulty wherein almost every participant ended up losing all the money they earned. This task was followed by the rest condition. A total of 11 blood samples (9 ml per sample) were collected during the experiment, 10 for endocrine measures and 1 for DNA analysis. The pre-task baseline period (Rest-AV: at time = 40 min), speech preparation (SP: 48 and 52 min), speech (58 min), mental arithmetic (MA: 63 min), rank-ordering with financial gain (RO-gain: 73 min), rank-ordering with financial loss (RO-loss: 78), and recovery (Rest: 98, 118 and 138 min). The amount of money participants earned was used as a function of their performance. The 10^{th} and final blood sample was also considered as the final rest point.

4.3.6. Table 1

Table 1 a. Diagnostic group demographics

Variable	Diagnostic groups HRP	Patients	Controls
Age	19.81 *(2.06)*	20.41 *(5.15)*	20.27 *(2.90)*
Gender (*M/F*)	*11/14*	*6/15*	*13/9*
Education years	12.57 *(1.90)*	11.90 *(2.11)*	12.50 *(1.36)*
BDI	7.80 *(8.75)*	16.28*¶ *(6.67)*	4.53 *(3.16)*
Stress experience	18.40 *(5.33)*	19.39 *(5.35)*	17.33 *(5.75)*
Total	**(n = 25)**	**(n = 21)**	**(n = 22)**

Table 1 b. *COMT* By diagnostic group

	HRP	Patients	Controls	Total
COMT				
met/met	8 *(F = 4)*	7 *(F = 5)*	5 *(F = 4)*	**20**
val/met	11*(F = 7)*	10 *(F = 8)*	14 *(F = 5)*	**35**
val/val	6 *(F = 3)*	4 *(F = 2)*	3 *(F = 0)*	**13**
Total	**25** *(F = 14)*	**21** *(F = 15)*	**22** *(F = 9)*	**68**

Table 1. (a) Mean (*SD)* of group demographics of all the subjects that took part in this experiment. (b) Number of subjects and *gender* per *COMT* genotype by diagnostic status of subjects; * the MD group is significantly different compared to controls regarding their BDI scores ($p<0.0001$); ¶ the MD group is significantly different compared to HRP regarding their BDI scores ($p<0.001$); *F* = female & *M* = male.

4.4. Results

4.4.1. Demographics: A one way ANOVA showed no significant differences across diagnostic groups in all remaining demographics variables (gender, age, smoking, and level of education) nor were there any significant *COMT* genotype differences in these variables. After estimating the allelic frequency distribution of *COMT* in our population, we found genotype frequencies in accordance with the Hardy Weinberg Equilibrium using x^2 test, ($p > 0.12$), suggesting no missed alleles and no population admixture. Additionally, x^2 tests showed no significant association between gender and diagnostic status ($p > 0.323$), gender and *COMT* ($p = 0.150$) and diagnostic status and *COMT* ($p = 0.764$), see Table 1b. In the demographics data, there was no significant difference in BDI scores between the *COMT* genotypes. However, there were significant BDI scores as determined by a student's t-test between the MD group relative to both HRP ($t = -3.715$; $df = 46$; $p < 0.001$) and healthy controls ($t = -7.98$; $df = 46$; $p < 0.0001$), which might simply be a result of our selection bias (see methods section and table 1a). There was no significant difference between HRP and controls BDI scores ($t = 1.793$; $df = 50$; $p = 0.079$).

4.4.2. Endocrine and Subjective stress response: Main effects of *COMT* were found in the *stress-related* PCA1 values of plasma EP ($F = 5.0$, $df = 2$, $p < 0.01$, figure 4.2a) and in the subjective stress experience scores ($F = 3.19$, $df = 2$, $p < 0.049$, see Figure 4.2b). Regarding diagnostic status, we found a main effect of diagnostic group in the *rest-related* PCA2 (recovery) of plasma cortisol measures ($F = 3.50$, $df = 2$, $p < 0.037$). AUC analysis for both cortisol and NE showed significant main effects of diagnostic status ($F = 5.61$, $df = 2$, $p < 0.006$; and $F = 3.20$, $df = 2$, $p < 0.048$ respectively), however, since the primary focus of this report is the genotype-related

effects on our measurements during stress, we report the group and sex effects only for descriptive purposes, (table 2 & 3).

Additionally, main effects of sex were found in Baseline measures of EP ($F = 4.095$, $df = 1$, $p < 0.048$), *stress-related* PCA1 measures of EP ($F = 12$, $df = 1$, $p < 0.001$), *rest-related* PCA2 (recovery) measures of EP ($F = 6.091$, $df = 1$, $p < 0.011$) and AUC measures of EP ($F = 8.131$, $df = 1$, $p < 0.006$), with the male individuals showing higher baseline plasma EP levels than the females. The PCA1 and AUC findings of a male dominance in plasma EP levels prior to the stress challenge were significant at $p < 0.05$ using a Bonferroni correction (table 2 & 3).

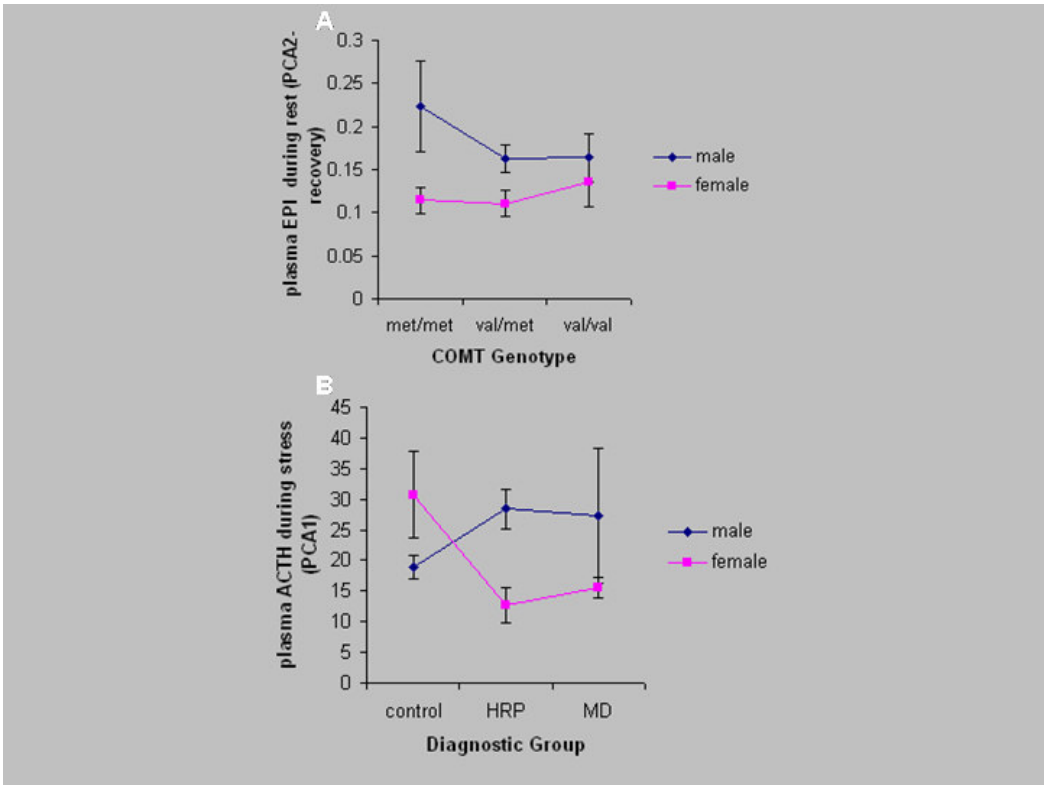

Figure 4.2. (A) Plasma EP level in nanogram/litre during the last three (i.e. sample 8-10) resting conditions. Figure shows the male individuals having a higher level of plasma EP during resting with met allelic load being predictive of these levels only in males. (B) Plasma ACTH in nanogram/litre showing a female dominance in this measure with the healthy control group. However, the same measure shows a significant male dominance within the high risk and MD groups.

An interaction between *COMT* and sex was found relating to the *rest-related* (recovery) PCA2 measures of EP ($F = 5.229$, $df = 2$, $p < 0.009$), showing a significantly higher plasma EP levels in males individuals homozygous for the met allele than their female counterparts ($p < 0.05$ Bonferroni correction, figure 4.2a). Furthermore, an interaction between sex and diagnostic group in both *stress-related* PCA1 and AUC measures of ACTH were found ($F = 6.903$, $df = 2$, $p < 0.009$; $F = 4.645$, $df = 2$, $p < 0.014$ respectively). Regarding this interaction, only the PCA1 result was significant after Bonferroni correction ($p < 0.05$), with healthy female controls showing the highest plasma ACTH response to stress, whilst within the male participants, it was the healthy controls that have the lowest ACTH response (figure 2b and table 2). Additionally, an interaction between *COMT* and diagnostic status was found in the *stress-related component* (PCA1) of plasma EP levels, with the control

group showing a more theoretically compatible overall *COMT* expression (i.e., in line with earlier findings, higher mean plasma level of EP was determined by *met* allelic loading (Scanlon and Raymond 1979; Wittchen 1994; Kessler 1994). On the other hand, the HRP and MD groups showed deviant endocrine responses from the theoretical expectation of *COMT* expression ($F = 7.59$, $df = 4$, $p < .0001$, figure 3a, 4a & 4c). Participant's report of subjective stress experience also showed significant group by *COMT* interactions ($F = 2.745$, $df = 4$, $p < 0.037$, figure 4.3b).

AUC assessment showed an interaction between *COMT* and diagnostic status regarding plasma cortisol levels ($F = 2.83$, $df = 4$, $p < 0.032$); and EP ($F = 4.27$, $df = 4$, $p < 0.004$), figure 4.4a-4.4f. Regarding the AUC measures of EP, the interaction between *COMT* and diagnostic group was significant after Bonferroni correction ($p < 0.05$), showing the differences in the influence of the *COMT* allelic variations on overall plasma EP response during the challenge to be evident only within the healthy control individuals (table 2 figure 4.4).

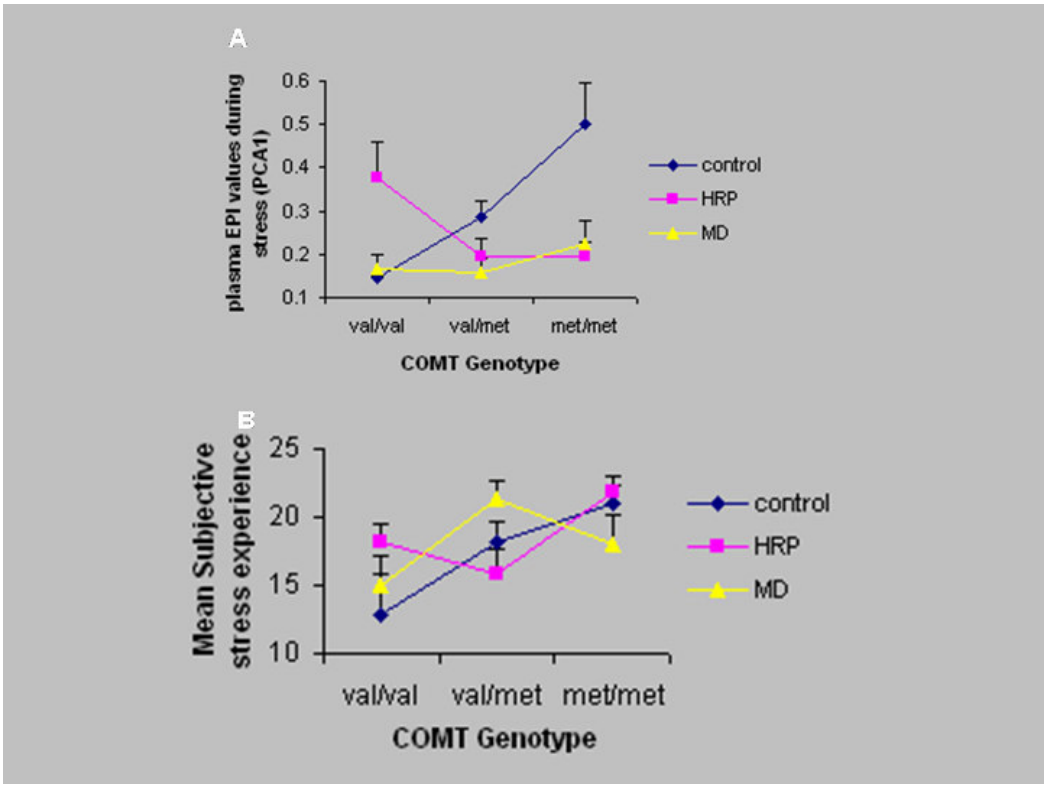

Figure 4.3. (A) Plasma EP responses to the stress challenges ([Mean (SEM)] across *COMT* and diagnostic groups in nanograms per litre, with the *met* allelic loading being predictive of EP stress response only markedly in healthy controls. (B) Subjective response to the stress challenge ([Mean (SEM)] across *COMT* and diagnostic group. Similar to the trend in mean EP responses to the stress challenge, participant's report of experienced distress showed an increase as a function of *met* allelic loading.

Because our patients were under SSRI medication which is known to influence neuroendocrine function (Nemeroff and Owens 2004; Jongsma et al 2005), we did the same analysis excluding the major depressive patients. As shown in supplementary table 1, the exclusion of the MDD group did not affect our major findings reported here, giving no indication for an internal validity problem as a result of SSRI treatment in the MDD group (table 3).

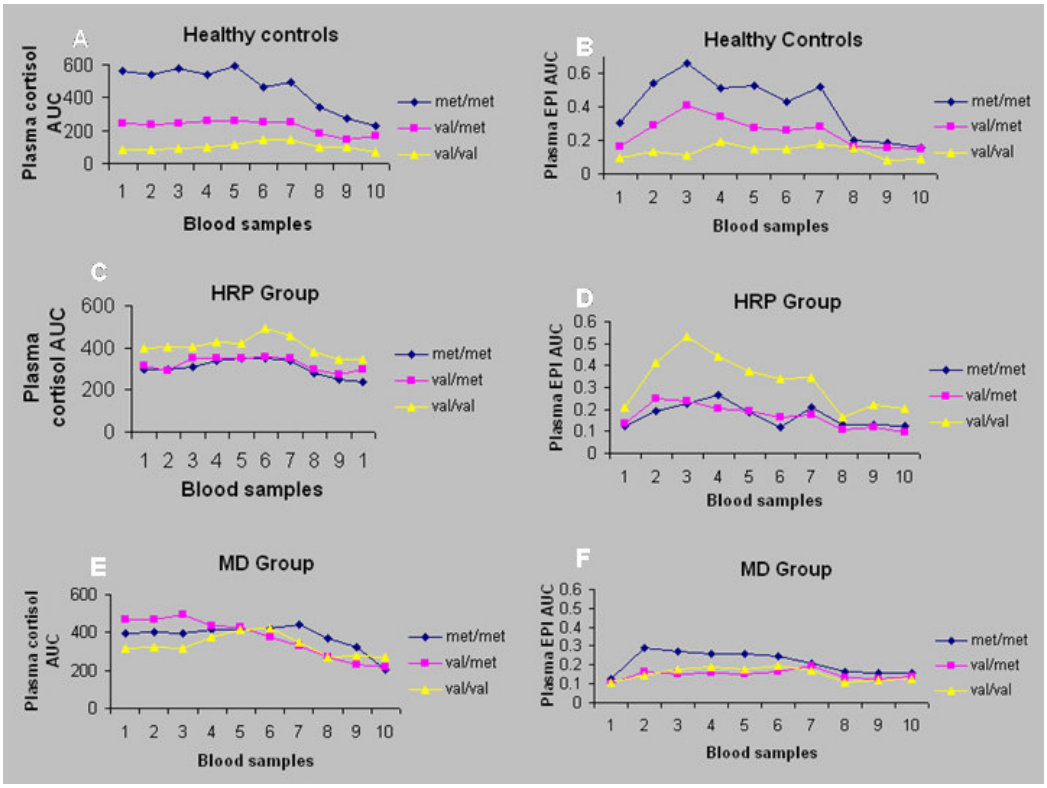

Figure 4.4. AUC graphs are meant to classify the different reaction time curves between the diagnostic groups as a function of COMT allelic variance. (A & B) represents AUC of the mean plasma cortisol (in nanomols per litre) and mean plasma EP (in nanograms per litre) responses to the laboratory challenge in controls respectively. (C & D) represents the same measures of plasma cortisol and EP responses in the HRP group while (E & F) showed the same measures in the MD group. While (A & B) showed a consistently higher reactivity of cortisol and EP to the whole experimental challenge as a function of *met* allelic loading in the healthy controls, (C-F) showed a mixed pattern in this regard in the HRP and patient group.

4.4.3. Table 2

Table 2 *COMT val*^{158}*met* genotype by diagnostic group MANOVA (total) N = 68

Analysis	variable	F-scores	p-value significance
Main effect of *COMT*			
	PCA1-EP	5.005	0.010
	Subjective stress experience	3.186	0.049
Main effect of diagnostic			
Group	PCA2-cortisol	3.505	0.037
	AUC-cortisol	4.35	0.006*
	AUC-NE	3.201	0.048
Main effect of Sex			
	Baseline EP	4.095	0.048
	PCA1-EP	12.147	0.001*
	PCA2-EP	6.091	0.011
	AUC-EP	8.131	0.006*
COMT BY			
Diagnostic Group	PCA1-EP	7.599	0.0001*
	AUC-cortisol	2.737	0.037
	AUC-EP	4.209	0.005*
	Subjective stress experience	2.745	0.037
COMT By Sex	PCA2-EP	5.229	0.009*
Sex By Diagnostic group			
	PCA1-ACTH	6.903	0.002*
	AUC-ACTH	4.645	0.014

Table 2. Main effects and interactions between *COMT* genotype and diagnostic groups. The results are considered significant at the 0.05. P values with an asterix (*) indicates significance at $p < 0.05$ after Bonferroni correction.

4.5. Discussion

This report provides the first evidence of a moderating effect of the functional polymorphism of *COMT* on downstream neuroendocrine and subjective responses to acute psychological stress, with individuals homozygous on the *met* allele showing higher endocrine and reported subjective stress response. Here, we observed an influence of *COMT* on subjects' diagnostic status with regards to MD with the genetic findings differing between controls, HRP and MD groups as measured in plasma glucocorticoid (cortisol) levels during the whole experimental procedure and plasma catecholamine (EP) levels during stress.

In line with our hypothesis, the *met* allelic loading was being identified in the present study as a marker for excercebated endocrine and subjective response to stress in the healthy individuals. This common polymorphism has been shown to result in a significant change in *COMT* enzyme activity in measures of catecholamines in peripheral blood and in liver (Scanlon et al. 1979; Weinshilboum and Dunnette 1981; Boudikova et al. 1990; Lotta et al. 1995). The *COMT met* allele is known to lead to lower *COMT* activity and has also been associated with anxiety and increased cortical response to negative emotions (Enoch et al 2003; Smolka et al 2005). Individuals with *met* genotypes are more sensitive to pain stress and, as shown by C11 Carfentanil imaging, and have a diminished ability to upregulate opioid release after pain/stress

(Zubieta et al 2003a & b). The *met* allelic variation was also found to influence plasma glucocorticoid release as a response to the treatment of Naloxone, while it was the *val* allele that was associated with individual's susceptibility to develop early onset major depression (Oswald et al. 2005; Massat et al. 2005, respectively).

4.4.4. Table 3

Table 3 Same analysis as Table 2, with the MD group being excluded. N = 47

Analysis	variable	F-scores	p-value significance
Main effect of *COMT*			
	PCA1-EP	4.464	0.018
	Subjective stress experience	3.763	0.032
Main effect of diagnostic			
Group	PCA2-cortisol	6.116	0.018
	PCA1-NE	4.941	0.032
	AUC-cortisol	11.141	0.002*
	AUC-NE	6.037	0.019
Main effect of Sex			
	Baseline EP	4.266	0.046
	PCA1-EP	11.612	0.002*
	AUC-EP	10.043	0.003*
COMT BY			
Diagnostic Group	PCA1-cortisol	4.668	0.015
	PCA1-EP	14.639	0.0001*
	AUC-cortisol	5.528	0.008*
	AUC-EP	10.844	0.0001*
COMT By Sex	PCA2-EP	5.460	0.009*
Sex By Diagnostic group	Baseline ACTH	7.580	0.009*
	PCA1-ACTH	12.163	0.001*
	AUC	10.172	0.003*

Table 3 consists of only the control and HRP group, to evaluate if the effects found when all three groups are included is partly due to the SSRI medication in patients or not. The results are considered significant at the 0.05. P values with an asterix (*) indicates significance at $p < 0.05$ after Bonferroni correction.

Interestingly, sex differences played a role in the present findings. Our observed ACTH response (a measure of HPA axis functioning), was shown to be strongly under the influence of sex, with the female individuals showing higher HPA axis response to the stress challenge. On the contrary, it was the male participants that showed higher EP response to the stress challenge. These results may be informative in the light of several findings demonstrating a sex differences in the pathogenesis and susceptibility to stress related emotional disorders (Kudielka et al 1998; Alsobrook et al 2002).

Diathesis-stress theories of depression predict that individuals' sensitivity to stressful events depends on their genetic makeup (Brown and Harris 1978; McEwen 1998; Ormel et al 2001; Caspi et al 2003; Charney 2004; Charmandari et al 2005). Behavioural genetics research supports this prediction, documenting that the risk of depression after a stressful event is elevated among people who are at high genetic risk and diminished among those at low genetic risk (Caspi et al. 2003; Kendler 2005). However, the majority of findings implicating *COMT* in the pathogenesis of affective disorders have been associative in nature (Massat et al 2005; Karayiorgou et al 1999). Although the relationship between a candidate genotype (GABAR6 polymorphism) and HPA-axis response to psychological stress has been examined once (Uhart et al

2004), to our knowledge, only one study has examined the role of *COMT* in plasma endocrine response to a pharmacological challenge (Oswald et al 2004). This report showed a relationship between *met* allelic loading and higher HPA-axis response to Naloxone challenge in healthy individuals (Oswald et al 2004). The present study is thus the first to examine the influence of *COMT* allelic variation on the endocrine stress response. However, *COMT* has been implicated in moderating psychological and neurophysiological correlates of pain (a valid physical stressor) processing (Zubieta et al 2003a; Rakvag et al 2005; Zubieta et al 2003b).

The *COMT* genotype has been implicated in cognitive processes relevant for psychopathology, especially for schizophrenia (Goldberg and Weinberger 2004; Weinberger et al 2001), and in cortical responses to negative emotional affect (Smolka et al 2005). This finding of an involvement of *COMT* in cortical response to negative emotional affect is relevant for our present data because recently, a relationship between cortical and negative emotional response to psychological stress has been reported (Wang et al 2005). Of interest, COMT is implicated earlier in neural response to negative emotions (Smolka et al 2005), and in both neural and behavioural response to noxious stimuli (Zubieta et al 2003a). These findings are particularly relevant in the context of *COMT* involvement in the aetiology of MD, because this disorder is strongly associated with both negative emotionality and somatic symptoms (Simon et al 1999). Moreover, decades of cross-sectional surveys have shown that chronic pain, MD, and anxiety often coexist (Max et al 2006). Thus, the convergence of our present findings with previously mentioned reports of a moderating role of *COMT* in central and peripheral response to physical (pain) as well as emotional and psychological stress, strongly suggests this genotype to be part of an interface that modulates physiological and emotional stress responses (Drolet et al 2001; Craig 2002; Ribeiro et al 2005).

Here, by examining the role of *COMT* in the stress related (PCA data) as well as overall endocrine response (AUC data) to a psychological stress challenge in individuals with different degrees of susceptibility to MD, our results suggests for the first time, a group dissociation with respect to *COMT* gene expression levels as measured in the endocrine stress response. We observed a higher EP response during stress (as shown in the PCA1 analysis) and a higher overall (AUC) cortisol and EP responses to be a function of *met* allelic loading in the healthy controls. The HRP group on the other hand, showed a higher endocrine stress response in those homozygous for the *val* allele compared to other genotypes. In the MD group, while no difference in overall cortisol response was found between genotypes, individuals homozygous for the *met* allele tend to show higher EPI response during the experimental challenge compared to other genotypes. Additionally, the reported subjective experience of stress was found to be associated with *met* allelic loading within the healthy control group. However, the HRP and MD groups showed different *COMT* effects on subjective stress experience. These findings of endocrine and subjective stress responses with respect to the *COMT* genotype support the hypothesis that *COMT* contributes to physiological and psychological mechanisms relevant for the stress response. Our findings of a different *COMT* expression regarding EP and subjective stress response between the healthy controls and HRP group may be the first genetic indication of the existence of abnormal HPA axis function in non affected family members of depressed probands, which was suggested to represent genetic vulnerability factors that predispose to and/or exacerbate the course of mood disorders (Modell et al 1998). However, we did not find a clear trend of *COMT* allelic variation regarding the stress response in individuals diagnosed with MD in the present study,

probably due to the effects of SSRI treatment. This is in line with earlier evidence that a neuroendocrine dysregulation often normalizes with antidepressant treatment, with a lack of normalization being associated with early relapse (Arborelius et al 1999; Barden 2004; Holsboer 2000).

Although our main findings confirms our hypothesis by showing an aberrant endocrine response to stress as a function of *met* allelic loading, the question of whether *met* allelic loading is a moderator of stress resilience, or rather, a vulnerability factor during the chronic phase of stress, has yet to be resolved, because gene expression levels are phenotypes that can be in 'cause' or 'effect' relationships to disease exposure (Weiss and Terwilliger 2000; Kendler 2005; York et al 2005). One should bear in mind that associating complex phenotypic variables like *stress resilience* or a likely susceptibility factor to a mental disorder with an allelic variation of a single polymorphism should be interpreted with caution (Plomin et al 1994; Kendler 2005; York et al 2005), because complex traits like mental disorders, whose genetic basis has evolved neutrally, are likely to have even 'noisier' genetic architecture than traits like malaria resistance, because multiple loci are often involved with a plethora of risk alleles and epistasis (Weiss and Terwilliger 2000; Raser and O'Shea 2005). With evidence implicating many different genetic and epigenetic factors directly or indirectly in the pathogenesis of mood disorders (Karayiorgou et al 1999; Caspi et al 2003; Uhart et al 2004; Massat et al 2005), focusing on single nucleotide polymorphisms, by concentrating on marginal effects as targets of influence in clinical studies, may shed limited light into complex genetic involvements in mental illness (Strohman 2002; Craddock et al 2006).

In summary, our results demonstrate an involvement of *COMT* in human endocrine and subjective response to psychological stress, suggesting a likely role for this genotype in vulnerability to stress related disorders like MD. Future genetic research may perhaps benefit from combining experimental manipulation of cognitive and physiological responses to disease related pathogens with observational association studies. Further work will be required to determine if pathways influenced by this genotype are suitable targets for pharmaceutical intervention.

4.6. Acknowledgements

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Chapter 5

Convergent genetic modulation of the endocrine stress response involves polymorphic variations of 5-HTT, COMT and MAOA

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5.1. Abstract

Highly prevalent stress related disorders such as major depression (MD) are characterized by a dysregulation of the neuroendocrine system. Although heritability for these disorders is high, the role of genes in the underlying pathophysiology is poorly understood. Here, we show that polymorphic variations in genes coding for *5-HTT, COMT* and *MAOA* as well as sex differences influence the regulation of HPA axis response to acute psychological and endocrine challenges. In our sample, the effects of *COMT* on the release of ACTH depend on the presence of the low expression *MAOA* variant in the same individual. By including individuals varying in their degree of susceptibility to MD, we showed evidence of interactions between *5-HTT* and MD susceptibility in baseline cortisol, and between *MAOA* and MD susceptibility in baseline ACTH measures, indicating a role for these genotypes in stable state endocrine regulation. Collectively, these results indicate that the simultaneous investigation of multiple monoaminergic genes in interaction with gender have to be measured to understand the endocrine regulation of stress. These findings points towards a genetic susceptibility to stress related disorders.

5.2. Introduction

Appropriate responsiveness to daily life stressors is crucial for adequate functioning in a natural environment (Charmandari et al. 2005). Conversely, depending on individual's genetic makeup, prolonged stress, coupled with inappropriate responsiveness may lead to physiological and psychiatric disorders (Charmandari et al. 2005; McEwen et al. 1998; Charney et al. 2004). Central to the stress-responses are the activation of the cerebral noradrenergic system, the peripheral sympathetic nervous system and the hypothalamic—pituitary—adrenal (HPA) axis (Sapolsky 2000), with corticotrophin releasing hormone (CRH) being identified as a critical coordinator of these processes (Wong and Lucinio 2001; Berton and Nestler 2006 , see figure 5.1).

Despite evidence mounting for a major genetic contribution to psychopathology, attempts at directly linking genotypes to psychopathological states remains largely unsuccessful (Moffitt et al. 2005; Caspi and Moffitt 2006; Meyer-Lindenberg and Weinberger 2006; Caspi et al. 2003). At present, results from studies relating psychopathology with the three most tested common monoaminergic polymorphic variations in the serotonin transporter (*5-HTT*), catechol-O-methyl transferase (*COMT)* and monoamine oxidase A (*MAOA*) have led to inconsistent results (Meyer-Lindenberg and Weinberger 2006). One reason for this could be that most studies involve simple associations between 'genetically complex' psychiatric or behavioural phenotypes and single genetic variables whose individual roles may be minimal. However, there is a growing body of literature advocating the examination of gene-gene and gene-environment interactions, which are both believed to be additive and epistatic making their inclusion in single studies relevant (Moffitt et al. 2005; Meyer-Lindenberg and Weinberger; Kendler 2005).

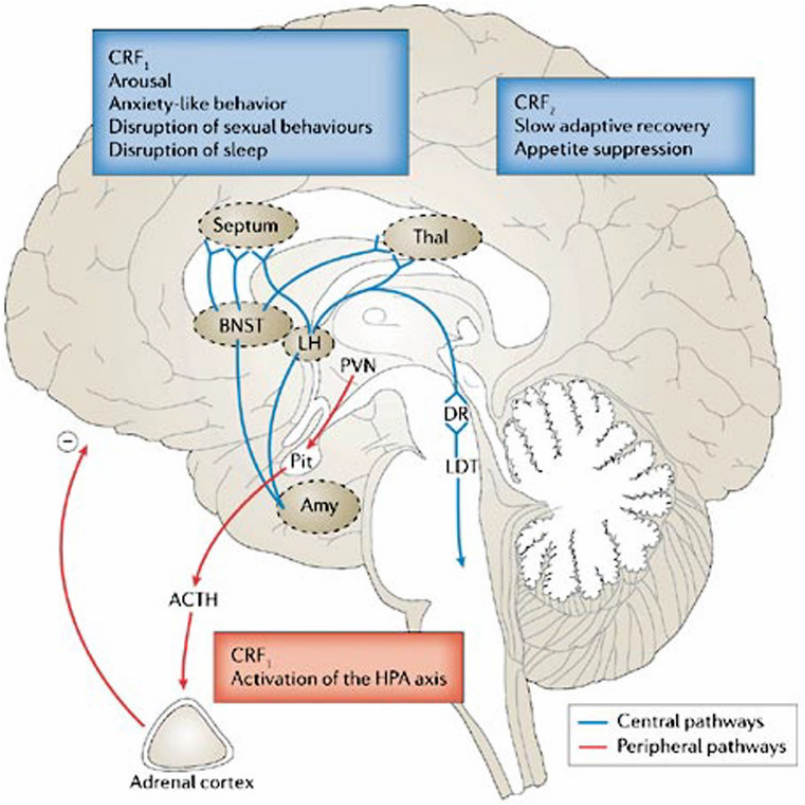

Figure 5.1. Adapted from Berton and Nestler. The Corticotrophin-releasing hormone system in depression. CRH from the paraventricular nucleus (PVN) of the hypothalamus is released into the hypophyseal portal system and triggers the release of corticotrophin (ACTH) from the anterior pituitary via stimulation of the CRHR1. ACTH, in turn, stimulates the secretion of glucocorticoid hormones (cortisol in humans or corticosterone in rodents) from the adrenal cortex. Increased glucocorticoid levels suppress hypothalamic CRH expression via negative feedback through hippocampal and hypothalamic glucocorticoid receptors. The neurotransmitter action of CRH on CRHR1 receptors throughout the limbic system mediates anxiogenic effects of stress. By contrast, its neurotransmitter action on CRHR2 receptors in more discrete regions of the brain might reduce anxiety-like behaviour in a delayed fashion. Amy, amygdala; BNST, bed nucleus of the stria terminalis; DR, dorsal raphe; LDT, laterodorsal tegmental nucleus; LH, lateral hypothalamus; Pit, pituitary; Thal. thalamus (see Berton and Nestler 2006, for a review).

A large body of both clinical and preclinical studies supports a role of monoaminergic genes in psychopathology (Wong and Licinio 2001). Studies examining functional polymorphic variations relating to monoamine degrading enzymes such as monoamine oxidase A (*MAOA*), catechol-methyltransferase (*COMT*), or the serotonin transporter (*5-HTT*) display alterations in emotional and physiological stress responses (Youdim et al 2006; Zubieta et al. 2003; Smolka et al. 2005). The gene coding for *MAOA* is mapped to the short arm of the X chromosome, with a variable-number-tandem-repeat (VNTR) polymorphism in the promoter region of the MAOA (Zhu et al. 1992). The polymorphism consists of 30-bp repeated sequence present in 2, 3, 3.5, 4, or 5 repeats (R), with the 3.5R or 4R, which together are called *MAOA-H* (high activity) transcribed 10 times more efficient than those with 2R, 3R or 5R also known as *MAOA-L*, (low activity) in *MAOA* functioning (Sabol et al. 1998). On the other hand, a common functional polymorphism in the *COMT* gene, which is the result of a G to A mutation that translates into a valine (*val*) to methionine (*met*) substitution at codon 158, has been shown to account for a fourfold decrease in enzyme activity (Meyer-Lindenberg and Weinberger 2006)**.** Furthermore, the serotonin transporter (*5-HTT*) gene, located at 17q11.1–q12, plays a central role in the regulation of the serotonin synaptic function and is considered to be a promising candidate involved in various psychiatric disorders (Lesch and Mossner 1998).

Even though these monoaminergic genotypes are implicated in emotional regulation and psychopathology (Meyer-Lindenberg and Weinberger 2006; Smolka et al. 2005; Tunbridge et al. 2006), there are virtually no reports of functional interactions between these genes and valid intermediate phenotypes of emotional disorders (Moffitt et al. 2005; Meyer-Lindenberg and Weinberger 2006; Kirschbaum et al. 1993; Segman et al. 2005). To test the hypothesis that allelic variations of *MAOA, COMT* and *5-HTT* will collectively influence endocrine and behavioural response to psychosocial stress, we exposed seventy individuals with varying degree of susceptibility to MD (as determined by their mental health status and familial loading; see also the methods section) to an acute psychological stress challenge in the laboratory (Charney et al. 2004; Moffitt et al. 2005; de Kloet et al. 2005). We next ask how these genes will affect HPA axis regulation to an endocrine challenge because due to the acute nature of our stress challenge, one can only interpret our findings as relating to the acute psychological stress. Instead of including a placebo day, we test if these genotypes influences HPA axis reactivity to an endocrine challenge that is known to induce neuroendocrine states similar to stress related clinical phenotypes; we exposed 64 individuals (most of whom also underwent the stress challenge) to the combined Dexamethason and corticotrophin releasing hormone (dex/CRH) challenge (Heuser et al. 1994; Schule et al. 2006).

5.3. Materials and Methods

5.4.1. Participants: Participants in this study were recruited from local and regional sources (North Netherlands Population Registration) as volunteers for the Neurobiological and Epidemiological cohort-study of Adolescents at Risk of Anxiety and Depression (ARIADNE) described in our paper in submission (Jabbi et al. in press; Landman-Peters et al. 2005). For the psychological stress challenge, 70 participants took part in the laboratory stress challenge (see TableS1). For the dex/CRH challenge, 64 individuals were included, a majority of whom were tested in the stress challenge. The Healthy controls, healthy high risk probands (HRP) (i.e., subjects who have at least two first-degree relatives suffering from MD), and patients diagnosed with early onset MD before the age of 30 years (Mean age = 20.14; ranging from 15-32) took part in the study.

Psychiatric assessment was performed according to *DSM IV* Revised, with life-time depression being determined with the Composite International Diagnostic Interview (CIDI) WHO-2000 version. The healthy controls and HRP individuals were free of medication and psychiatric/neurological disorders. MD patients were free of neurological disorders and under SSRI medication.

5.3.2. Laboratory Tasks: Subjects arrived at 12:30 PM for experimental sessions after completing the evaluations about 2 weeks prior to the study. For both the stress experiment and dex/CRH challenge, they were instructed to fast the morning of the experiment, and refrain from intensive physical exercise and use of alcohol 24 hours prior to the experiment. Prior to the stress test, an intravenous catheter (saline drip) was inserted into an antecubital vein at approximately 13:00 hours. Subjects rested in a dental chair (semi-supine) for 45 minutes (min) watching a documentary film about Antarctica to acclimatize. Subjects were then exposed to the Groningen Acute Stress test (GAST). The GAST is a modified version of psychological stress task used earlier in a similar experimental setting (Kirschbaum 1993; Uhart et al. 2006). Here we

added a computer task in which participants could make financial gains in the first block, but would subsequently lose all their gained money or a substantial part of it in the second block depending on their task performance (Jabbi et al. in press). Subjective stress experience scores during exposure to various stressors were acquired immediately after each stress tasks on a 10 point Likert scale: 1 signifying no stress experience at all and 10 extreme experience of distress. The procedure for the dex/CRH challenge was carried out as reported earlier (Heuser et al. 1994; Schule et al. 2006).

5.3.3. Endocrine Assessments: ACTH plasma levels were assayed using the Nichols Advantage enzyme Immunoassay for the 10 samples collected during different stress and rest conditions http://www.nicholsdiag.com/products. Plasma cortisol levels were assayed using a validated automatic analyzer system (Elecsys® 2010, Roche, Switzerland) whereby serum cortisol levels were determined by means of a specific and highly sensitive enzyme immunoassay.

5.3.4. Genotyping

5.3.4.1. *COMT* genotyping: 10ml EDTA anticoagulated blood was collected and centrifuged at 800g for 10 minutes at 4 °C. The Buffy coat was collected and stored at – 20 °C until DNA isolation. After thawing, DNA was isolated with the help of the Qiamp Mini Blood Kit (QIAGEN Benelux B.V., Venlo, The Netherlands). Genotyping of the *COMT val*^{158}*met* polymorphism (1947 G/A; GenBank Z26491; dbSNP: rs4680) was performed with the allelic discrimination technique on an Applied Biosystems 7500 HT Real-Time PCR system (Applied Biosystems, Nieuwekerk a/d Ijssel, The Netherlands) according to the protocol supplied by Applied Biosystems. We used primers COMT-GAF (5'-CGAGATCAACCCCGACTGT-3') and COMT-GAR (5'-CAGGCATGCACACCTTGTC-3'), as well as minor grove binding (MGB) probes VIC-5' –TTTCGCTGGCGTGAAG-3'-NFQ (G), and FAM-5'-TCGCTGGCATGAAG-3'-NFQ (A). Additionally, a Taqman universal PCR master mix, all supplied by Applied Biosystems was also used.

5.3.4.2. *MAOA* genotyping: *MAOA* genotyping was performed using blood samples as described earlier[15]. Some modifications in the methodological approach were made: we used 100-350ng of DNA. AmpliTaq Gold polymerase and 1U (instead of 0.5U) was used during PCR. Separation of PCR-products through gel electrophoresis was done with the Spreadex EL-1200 gel, and the acquired base pairs were made visible using GelStar (SYBR-green).

5.3.4.3. *5-HTT* genotyping: *5-HTT* genotyping was used using anticoagulated blood according to the general procedures described earlier (Cook et al. 1997). We used HTTp2A en HTTp2B primesets for the PCR analysis of 406/450bp. Instead of 10 µl, we used 50 µl PCR reaction mix using DNA ranging from 100-350ng. For the PCR reaction, an initial step of 12 min instead of 2 min was applied at 95ºC, followed by 40 cycles of 30 sec at 95ºC, 30 sec at 61ºC, 60 sec at 72ºC with the end extension being 7 min at 72ºC. Detection was done on a 2 % agarose gel containing 0.64 µg /ml ethidium bromide in TBE buffer using 50bp ladder instead of 100bp.

5.3.5. Statistical analysis: A chi-square test (χ^2) was used to associate genetic and demographic variables. Baseline values for each hormonal measure were analyzed using the first blood samples for each variable: cortisol, ACTH, EP, NE. Additionally, mean scores for subjective stress experience were used using the reported stress experience after each stressor on a 10 point likert scale. The Kolmogorov-Smirnov statistic was used to determine normality, allowing us to transform all non normal measures to the logarithmic scale.

Stress-related hormonal measures are presented as percent of baseline (i.e., the original variable at each sampling point in time divided by the baseline mean and multiplied by 100 using SPSS 12.0.1 (Chicago, IL) (Uhart et al. 2005). Out of the 10 blood sampling points, the two samples collected during speech preparation/anticipation (SP) were averaged. A similar measure was adopted by averaging the last three samples collected during rest/recovery for a measure of the overall percentage change during recovery from stress adding up to a total amount of six stress conditions (i.e., SP, public speaking (PS), mental arithmetic (MA), financial gain 'gain', financial loss 'loss', and recovery from stress). For the DEX/CRH challenge in experiment 2, peak responses of cortisol and ACTH were assessed (i.e., sample 4 for ACTH and 5 for cortisol. Furthermore, the area under the curve (AUC) was calculated with trapezoidal approximation from logarithmic transformed values for cortisol and ACTH (Preussner et al. 2003).

We included individuals with different degrees of MD susceptibility to test whether these differences will be predictive for any observed Gene by Endocrine response to acute psychological stress. Due to the fact that our MD patients were all under SSRI treatment, we assessed the effects of positive MD diagnosis on our observed stress response by redefining our groups into two, i.e., those with a presence of MD diagnosis (Patients) and those without MD diagnosis/treatment (healthy controls and HRPs). This new variable will be hereafter referred to as Diagnosis. We initially tested if the groups differed in behavioural and endocrine responses to the experimental challenges and found no differences between the HRP and MD groups in all of our measured variables. This led to our redefinition of the three groups into a *high* (HRP and MD individuals) and *low* risk (Healthy controls) groups, hereafter referred to as MDRisk.

An allelic variation of MAOA in terms of high expression MAOA variants as compared to the low expression variants was defined as described (Sabol et al. 1998). Main effects of Genotypes (*5-HTT, COMT and MAOA*), MDRisk, Sex and diagnosis as well as their interactions on mean baseline peripheral endocrine measures, mean subjective stress scores and mean percentage change in endocrine stress response in all hormonal measures were analyzed using ANOVA. Due to the limited number of subjects included in our experiments for the purpose of examining gene-gene interactions, we will focus our report on interaction between two variables (i.e. without looking at three way interactions) and only results that survived multiple testing using the Bonferroni correction at $p < 0.05$ are reported.

5.4. Results

We tested for significant association between the genetic and demographic variables and the only association we found was between *MAOA* and Sex ($\chi^2 = 5.833$, $p = 0.016$, $df = 1$), with a higher frequency of male representation within the group carrying the low activity *MAOA* while high activity allelic carriers were predominantly women. Initially, we analyzed the baseline measures and found an interaction between *5-HTT*

and MDRisk in baseline measures of cortisol ($F = 3.491$, $df = 2$, $p < 0.039$, Figure 5.2a), with the high risk individuals that are homozygous for the long allele showing the highest levels of baseline plasma cortisol. Regarding the same finding in low risk individuals, it was the short allelic load of the 5-HTT gene that led to higher baseline cortisol levels. An interaction between *MAOA* and sex were found in baseline plasma cortisol measures ($F = 7.529$, $df = 1$, $p < 0.009$; Figure 5.2b), which shows female dominance in baseline plasma cortisol levels, but only within the group of high expression *MAOA* variants. Additionally, an interaction between *MAOA* allelic variation and MDRisk was found in baseline ACTH measures ($F = 5.432$, $df = 1$, $p = 0.024$ Figure 5.2c), with the high risk individuals showing higher baseline ACTH levels but only in individuals with the low expression *MAOA* variation.

We rated participants' subjective experience to examine if the stress response during the psychological challenge significantly affected their experience of stress. An interaction between *MAOA* allelic variation and sex was found regarding the reported stress experience during MA ($F = 10.993$, $df = 1$, $p < 0.002$, Figure 5.2d).

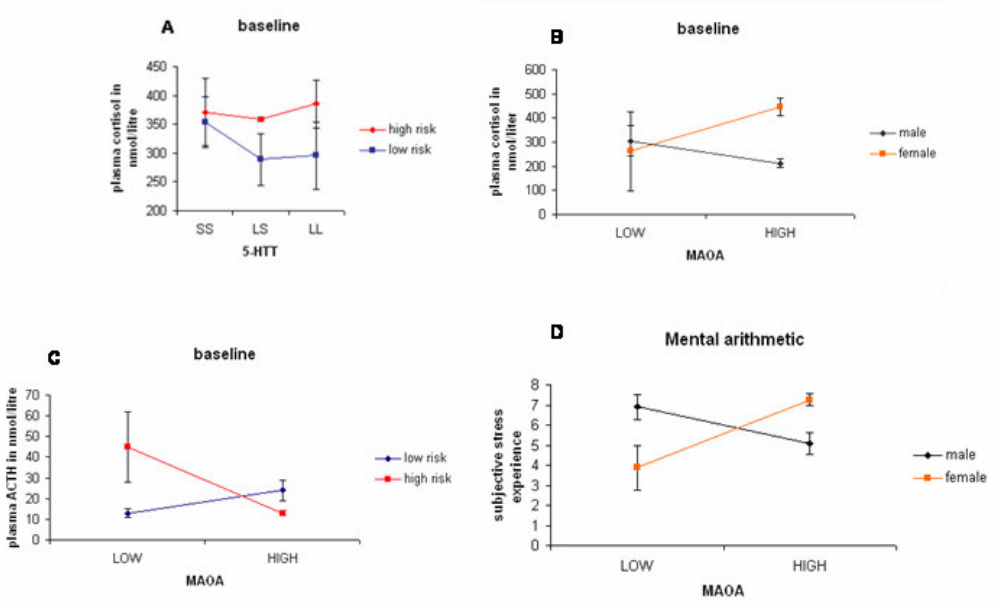

Figure 5.2. Baseline endocrine and subjective measures. (a) Mean (SEM) baseline differences in plasma cortisol levels in individuals with different variants of 5-HTT, with the gene determined baseline levels differing among individuals with different degree of MD susceptibility if they carry a long allele ($p<0.05$ corrected). (b) Mean (SEM) of baseline plasma cortisol levels in males and females as a function of their *MAOA* genotype, showing the sex differences to related to the high expression *MAOA* variant ($p<0.05$ corrected). (C) Mean (SEM) baseline plasma ACTH levels showing an interaction between MAOA and MD susceptibility, with the difference in degree of MD susceptibility being determined by the low *MAOA* expression variants ($p<0.05$ corrected). (d) Mean (SEM) differences in reported subjective experience of stress during MA between males and females as a function of their *MAOA* allelic variation, with only the low expression *MAOA* variants showing significant sex differences ($p<0.05$ corrected).

Endocrine regulation is central to the maintenance of homeostasis. Here, we tested the role of genes on peripheral cortisol and ACTH response to psychological stress. Most importantly, an interaction between the allelic variations of *COMT* and *MAOA* in percentage change in plasma ACTH response to PS ($F = 3.610$, $df = 2$, $p = 0.035$), MA ($F = 4.155$, $df = 2$, $p = 0.022$), gain ($F = 9.874$, $df = 2$, $p < 0.0001$), loss ($F = 5.692$, $df = 2$, $p < 0.006$) and during recovery ($F = 6.412$, $df = 2$, $p < 0.004$) (figure 5.3a and 5.3b) was found. An interaction between *5-HTT* and sex was found in the percentage change in plasma cortisol response during PS and MA ($F = 3.604$, $df = 2$, $p = 0.036$; $F = 5.759$, $df = 2$, $p < 0.006$ respectively, Figure 5.3c). Additionally, a main effect of *5-HTT* regarding percentage change in plasma ACTH response to PS ($F = 4.214$, $df = 2$, $p < 0.024$ and during MA ($F = 5.704$, $df = 2$, $p < 0.007$, Figure 5.3d) was found.

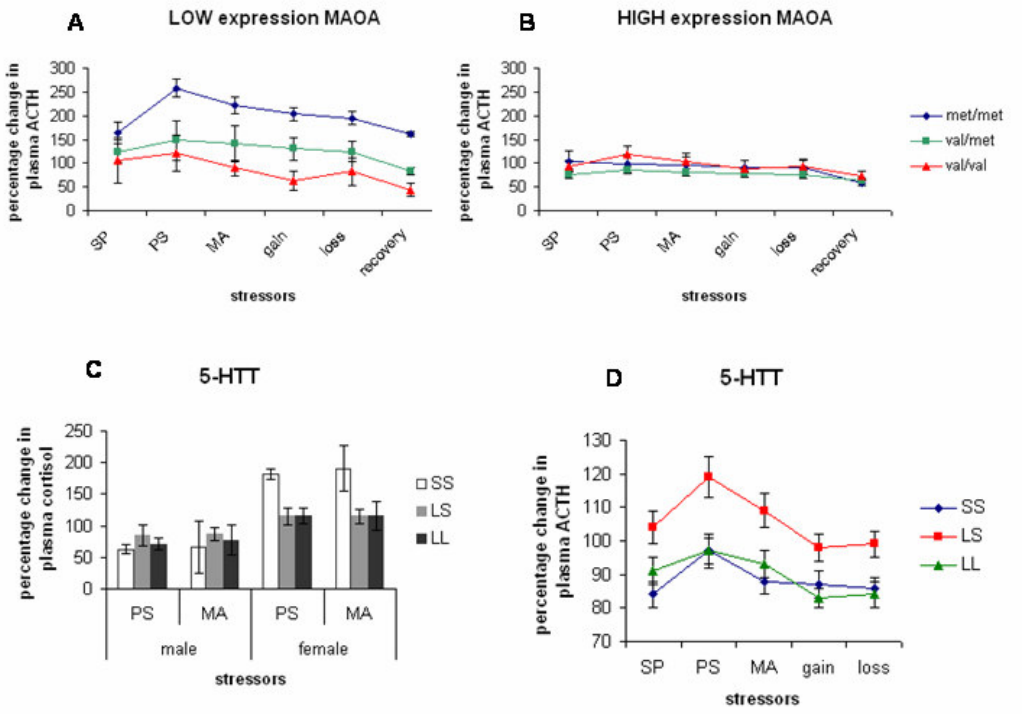

Figure 5.3. (a and b) Mean (SEM) of percentage change in plasma ACTH in allelic differences in *COMT* genotype as a function of *MAOA* during PS, MA, gain, loss and recovery, with the differences between met/met and val/val variants being the most significant only within the low expression *MAOA* variants ($p<0.05$ corrected). (c) Mean (SEM) of percentage change in stress-related cortisol response, showing an interaction between allelic variations of *5-HTT* and sex. Regarding this interaction, the individuals with the SS variant showed increased percentage change in plasma cortisol response to the stressors than those with the LS and LL variants but only in women ($p<0.05$ corrected). (d) Mean (SEM) of percentage change in plasma cortisol response to PS and MA as a function of individuals' *5-HTT* allelic variations, while the LL and LS variants did not differ in their response, both differed significantly from the SS variants during SP, PS and MA ($P<0.05$ corrected).

Regarding the sample of individuals that took part in the dex/CRH challenge, we found no associations between participant's genetic and demographic variables. A significant main effect of *MAOA* on cortisol peak response to the challenge was found ($F = 8.151$, $df = 1$, $p < 0.007$, figure 5.4a). Additionally, a main effect of Sex was found in the ACTH peak response ($F = 4.850$, $df = 1$, $p = 0.034$, figure 5.4b). Interestingly, we found a main effect of MAOA on the AUC response of cortisol during the endocrine challenge ($F = 10.31$, $df = 1$, $p < 0.003$, Figure 5.4c). A main

effect of sex in AUC response of ACTH was also found (F = 7.443, df = 1, p < 0.010, Figure 5.4d).

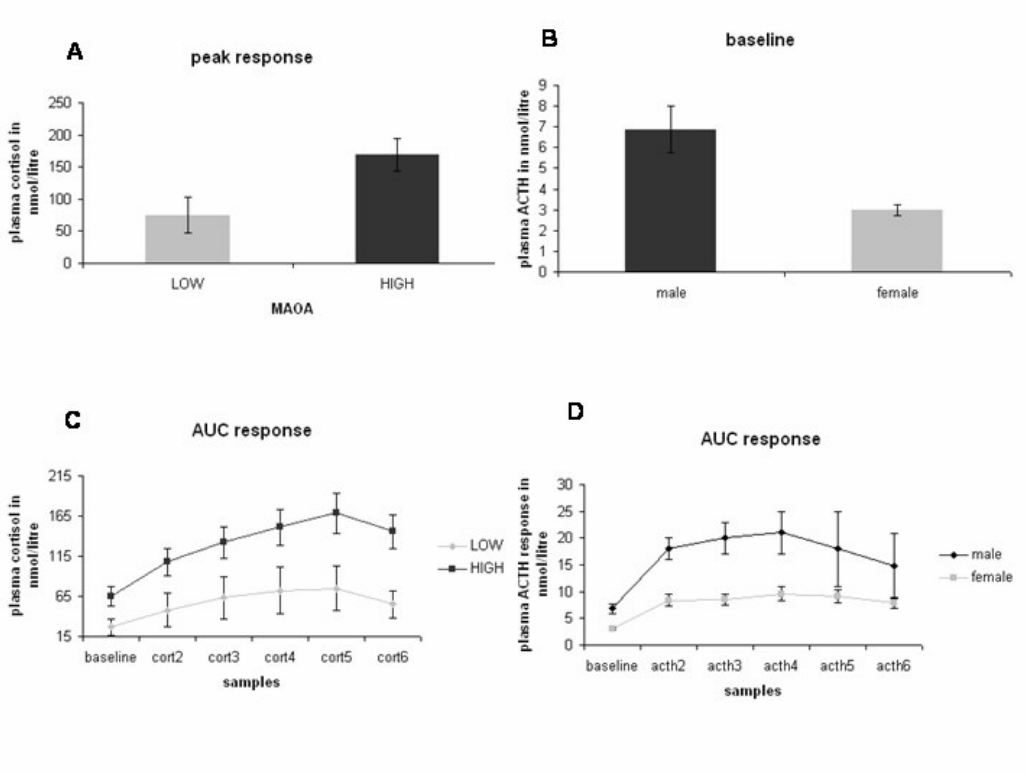

Figure 5.4. (a) Mean (SEM) plasma levels of cortisol, showing higher levels in high expression MAOA variants than low expression variants (p<0.05 corrected). (b) Mean (SEM) in peak levels of plasma ACTH showing male individuals having higher plasma ACTH peaks than controls (P<0.05 corrected). (c) Mean (SEM) of plasma cortisol levels, showing the overall response curve/AUC during the dex/CRH challenge, with the low expression MAOA variants showing the least glucocorticoid response to the endocrine challenges (p<0.05 corrected). (d) Mean (SEM) plasma ACTH response as measured in the whole experiment, with the male individuals showing more HPA axis response to the endocrine challenge (p<0.05 corrected).

5.5. DISCUSSION

The "stress-diathesis" theory of major depression predicts the contribution of multiple factors to MD pathophysiology (Jabbi et al. In press). The preclinical and clinical literature posits that adverse early life experiences triggers MD onset, with the vulnerability or sensitisation playing out differently depending on the genetic constitution of the individual and environmental variables such as duration of the adverse experience and the availability of social buffering (Charney 2004). Here, we found a main effect of *5-HTT* and an interaction between COMT and MAOA on plasma ACTH stress response.

Patients suffering from MD are known to exhibit hypersecretion of CRH, coupled with an elevated CRH concentration in the cerebrospinal fluid (CSF) and a blunted ACTH response to exogenous CRH administration (Carmandari et al. 2005). By further examining the role of these genes in an endocrine challenge, that may be seen as a simulation of the intermediate endophenotypes of prolonged long term stress experience (de Kloet et al. 2005), we found main effects of both sex and *MAOA* in ACTH and cortisol responses to the dex/CRH challenge respectively.

Our findings of sex interactions with *MAOA* in baseline measures of cortisol and subjective experience during mental arithmetic may suggest a role of this genotype in sex related differences in prevalence of disorders like MD. With *MAOA* being an X-linked gene however, such findings should be interpreted with caution. Additionally, sex by *5-HTT* interaction in cortisol response to psychosocial stress was made. Given the putative role of the SS allelic variation of this genotype in MD susceptibility in the face of adversity, our findings of higher endocrine response in SS female individuals may also likely contribute to the sex related differences in MD prevalence. Indeed, the same *5-HTT* genotype also interacted with MD susceptibility in baseline measures of cortisol. Taken together, these findings suggest a role for these genotypes in the susceptibility to stress related disorders like MD.

Activation of the HPA axis is an important adaptive mechanism that enables the maintenance of homeostasis. Additionally, reciprocal reverberatory neural connections as well as a functional relationship exist between the CRH and noradrenergic neurons of the central stress system (Charmandari et al. 2005). It has been shown that both CRH and noradrenergic neurons receive stimulatory innervations from serotonergic and cholinergic systems, inhibitory input from γ-aminobutyric acid-benzodiazepine and opioid peptide neuronal systems of the brain, as well as from the end-product of the HPA axis, the glucocorticoids (Charmandari et al. 2005). Taken together, our findings of a main effect of *5-HTT* on the one hand, suggests a functionally unique role of the serotonergic system in the HPA axis functioning that might be mediated by CRH. Furthermore, our only observed stress related gene-gene interaction between these genotypes in HPA axis (i.e. ACTH) response to stress, may suggest an important contribution of catecholamines in the regulation of homeostasis and maintenance of health.

Glucocorticoids play an important contribution in the regulation of basal activity of the HPA axis, as well as in the termination of the stress response by acting at the extra-hypothalamic centres, the hypothalamus, and pituitary gland (Charmandari et al. 2005). Thus, the negative feedback of glucocorticoids on the secretion of CRH and ACTH serves to limit the duration of the total tissue exposure of the organism to glucocorticoids, minimizing catabolic, lipogenic, antireproductive, and immunosuppressive effects of these hormones (Charmandari et al. 2005). Our findings of significant main effects of *MAOA*, showing that individuals carrying the low expression variants are less able to achieve adequate negative feedback of the HPA axis response to the endocrine challenges, resulting in lower glucocorticoid response to the dex/CRH challenge, suggests that this allelic variation may play a role in aberrant HPA axis response to environmental challenges. Interestingly, this finding is in line with a recent report showing low expression variants of the BDNF gene leading to similar HPA axis response to the dex/CRH challenge in severely depressed (Schule et al. 2006). Earlier, polymorphic variations in the *COMT* gene have been shown to predict brain response to negative emotional and painful stimuli[12,13], as well as HPA axis response to various endocrine challenges as a function of *COMT* or GABA polymorphic variations (Jabbi et al. in press; Uhart et al. 2006).

Earlier, it was shown that *COMT* may mediate HPA axis functioning (Jabbi et al. 2003). *MAOA* allelic variation on the other hand, specifically the low expression *MAOA* variant, has been implicated in complex phenotypic variables such as aggression, impulsivity and violent behaviour (Caspi and Moffitt 2006; Meyer-Lindenberg and Weinberger 2006) and both *COMT* and *MAOA* genotypes have been associated with psychiatric samples and treatment response to antidepressants (Meyer-Lindenberg and Weinberger 2006). Longitudinal studies showed that patients

with persistent HPA axis disturbance responded poorly to treatment and when remitted, still maintain a high risk of relapse (de Kloet et al. 2005). Our findings of a main effect of *5-HTT* and an interaction between *COMT* and *MAOA* in HPA axis regulated endocrine stress response to psychological stress, whereby *COMT* mediated stress response seemed to be determined by individuals *MAOA* genotype, was in line with our findings of a functional influence of *MAOA* on endocrine response to the dex/CRH challenge. Together, these findings indicate an involvement of these common monoaminergic genes in the modulation of peripheral endocrine regulation. Such a system may be relevant for the maintenance of homeostasis and thereby physical and mental well being.

The monoamine hypothesis of depression was based upon the postulated deficiency or imbalance in noradrenalin or serotonin (Youdim et al. 2006). However, hyperactivity in the HPA axis in MD patients has been shown to be affected by long-term antidepressant treatment (Wong and Licinio 2001; Youdim et al. 2006). Our current findings of a *COMT* by *MAOA* interaction, as well as a mian effect of *5-HTT* on HPA axis response to psychological stress is in line with evidence that attenuation of HPA axis activity is a direct pharmacological effect of antidepressants (Wong and Licinio 2001; Youdim et al. 2006). Our findings of a strong genetic modulation of ACTH and not cortisol in response to stress may relate to the possibility that ACTH is more sensitive to the stress experience because of the direct influence of the hypothalamus on the secretion of ACTH which in turn triggers cortisol secretion (Cook et al. 1997; Slawik et al. 2004). One explanation for our lack of finding a significant interaction between *5-HTT* and other genes could be the fact that NE and their metabolizing enzymes are present in different cellular compartments. So the catecholamines could be affected by intercellular actions of either *MAOA* or *COMT* separately or in combination, whereas serotonin degradation by *MAOA* is confined to cells expressing *5-HTT* (Slawik et al. 2004; Dodson et al. 2004). In sum, despite the acute nature of our stress challenge, it might be likely that a combination of these low activity variants of *COMT* and *MAOA* genes may become a vulnerability trait if stressful experiences persist.

Here, the sample used is rather small given the goals, making the need for a replication with a larger sample size highly necessary to further allow the assessment of complex genetic contributions without compromising statistical power (Meyer-Lindenberg and Weinberger 2006; Kendler 2005; Uhart et al. 2006). In conclusion, we analysed the effects of monoamine-associated genes on endocrine and psychological stress challenges. Our findings successfully demonstrated the potential of investigating complex genetic response to valid disease pathways. If replicated, our findings of significant gene-gene interactions whereby no marked response related to the *COMT* genotype in terms of peripheral endocrine response to psychological stress in individuals carriers of the high expression *MAOA* variation, supported by a stronger role of the MAOA genotype in HPA axis response to an endocrine challenge may lead to beneficial therapies for stress related disorders.

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Chapter 6

6.1. Summary and Conclusions

6.1.1 Summary

Over recent decades, research evidence showed that the brain not only controls the so-called autonomic nervous system, but that it also has considerable influence on physiological endocrine and immunological responses[1]. This knowledge was used to design the experiments discussed in this thesis. By examining the individual differences in central and peripheral responses to psychosocial stress and social emotional behaviour, we argued in line with the conjecture that the neuroanatomical substrates for subjective emotion in humans are based on an (Meta)-representation of a physiological state of the body[2]. In this thesis, we examine individual differences in emotional and stress experience in terms of genetic and interpersonal reactivity differences.

In *chapter 2*, we demonstrated that certain brain regions like the anterior cingulate are commonly recruited during the induction of sadness, happiness and fear, although some brain areas tend to be more selectively recruited during the processing of individual emotions. This intriguing finding does indeed underscore a very important point of the present thesis: that emotion, to some extent, generally alters the homeostatic balance of the body leading to heightened attention and autonomic arousal. The argument for this reasoning is that increased arousal and attention are able to also recruit the cingulate cortices[3-8]. In line with our findings, it has been argued that by studying emotions in the laboratory using complex social stimuli like films or pictures, several processes encompassing affective, perceptual, mnemonic and experiential processes are recruited during exposure to such stimuli[9]. Each of these different subcomponents is likely being implemented in different, but overlapping and interconnected circuitries. Thus we tentatively interpreted our findings of the existence of a common neural response to the processing of sadness, happiness and fear as likely demonstration of a mapping of the bodily arousal mechanisms that may underlie these emotions. Such an empirical finding converges with an earlier speculation of William James when he wrote that: *any classification of the emotions is seen to be as true and as 'natural' as any other,* if it only serves some purpose; and such a question as "what is the real'' or 'typical' expression of anger, or fear?" is seen to have no objective physiological meaning at all[2].

In *chapter 3*, we investigated the role of individual differences in empathic evaluation of other people's emotional experiences on the pattern of neural activations while watching gustatory emotions of others. Earlier reports have shown disgusting gustatory and olfactory stimuli, but also pain related noxious stimuli that are strongly related to bodily feeling states, recruit the human anterior insula and frontal operculum[10]. We capitalized on these findings and tested the hypothesis that individuals high on social empathy will also recruit their brain areas that map their bodily experiences during the observation of similar experiences in others more so than individuals low on empathy. As expected, we found that the degree of activation of the insula/frontal operculum during the observation of other people's gustatory emotions to be a function of their ability to empathize with others.

These results demonstrate for the first time that when people reported a higher tendency of being aroused by the emotions of others, they in fact activate their insula

more during the actual witnessing of emotionally arousing experiences of others than those who reported a lower tendency. Given the common phenomenon that emotions are contagious in a social context, such a finding may suggest that individuals may simulate the emotional states of others by generating similar arousing bodily states in themselves, in order to feel/sense what the others were feeling. In other words, if perception of somebody else's feelings can change my physiological state (and thereby homeostasis), I may feel what you that person is feeling (literally). Such a mechanism may be part of the biological basis of emotional contagion.

We found that the endocrine and behavioral response to psychological stress was modulated by allelic variations in *COMT* genotypes as described in *chapter 4*. We showed that the modulatory role of this genotype in endocrine stress response differed as a function of the degree of susceptibility to MD. In line with our *COMT* related findings during stress, this genotype has been implicated in modulating μ-opioid neurotransmitter responses to a pain stressor[11]. Of interest to this finding, the μ-opioid receptor gene polymorphism was also independently found to contribute to interindividual variation in the manner in which pain produces depression or anxiety[12]. Furthermore, *COMT met* allelic load was found to influence the degree of functional neuroanatomical response to negative emotional stimuli[13,14]. This genotype has been shown to be critical for prefrontal dopamine flux, prefrontal cortex-dependent cognition and activation, and has been implicated in the pathogenesis of emotional disorders and schizophrenia[15-18].

These results show for the first time that genetic differences do not only determine vulnerability to psychiatric disorders, but that genes might likely mediate vulnerability through interaction with valid environmental pathogens (in this case exposure to psychosocial stress). These findings thus suggest a strong functional role for *COMT* allelic variation in human homeostatic maintenance, a dysfunction of which may underlie a series of disorders.Taken together, experimental studies that expose participants to physiological and psychological challenges in relation to candidate genes may assist in elucidating the role of genes in the pathogenesis of psychiatric disorders.

In the final study of this thesis described in *chapter 5,* we demonstrated a main effect of *5-HTT* and an interaction between *COMT* and *MAOA* on the peripheral ACTH stress response. Specifically, we found that during experience of acute psychological stress, individual's peripheral plasma ACTH response was modulated by the allelic variations of *5-HTT* alone, as well on a combination of allelic variations of both *COMT* and *MAOA*. Part of this gene-environment interaction on the observed HPA axis response to stress was found to be sexually dimorphic. This sexually dimorphic nature of *MAOA* expression, whereby the dominance of endocrine stress response in low expression as compared to high expression *MAOA* variant in only males, may relate to recent findings of higher amygdala and hippocampal response during emotional memory retrieval and higher anterior cingulate activation during response inhibition[19-21]. Such a finding of an interaction between two polymorphic variations of monoaminergic genes that were earlier identified to metabolize catecholamines in affecting the glucocorticoid stress response is striking. For the first time, we showed that the presence of a high expression *MAOA* variant led to a silencing of *COMT* function in endocrine regulation of psychosocial stress. Indeed, only when individuals carry a low expression *MAOA* variant, does their *COMT* gene play an important role in the hormonal regulation of stress. Such a finding may have relevant pharmacological implications. Furthermore, this finding was supported by the follow up study using a pharmacological challenge by showing that only the role

of *MAOA* in HPA axis regulation of the stress response survived multiple comparisons. Together, these results showed that even though multiple genes play a role in disease phenotypes, the effects of the various genes, though collective, may differ in significance.

In line with our findings, several recent studies have shown the association of genetic variation of *MAOA* with various psychiatric conditions[22-26]. Studies in mice deficient in *MAOA* were shown to have elevated norepinephrine and serotonin levels in the brain, compared with normal wild-type mice. Additionally, these *MAOA* knock-out mice exhibited enhanced aggression (e.g., in the resident-intruder test), and aberrant emotional behaviour[27]. The enhanced aggressive behaviour exhibited by the *MAOA* mutant mice is consistent with impulsive aggression reported many times in humans[28-33].

Most importantly, low activity *MAOA* allelic variation, associated with increased risk of violent behaviour, was shown to predict pronounced limbic volume reductions and hyper responsive amygdala during emotional arousal. On the other hand, a diminished reactivity of regulatory prefrontal regions during emotional arousal was found in the low activity *MAOA* individuals compared to high expression variant[33]. Previous reports confirmed an association between low expression variant *MAOA* with aggression and aberrant emotional regulation[33]. In combination with our finding of an interaction between *COMT* and *MAOA* in HPA-axis response to psychological stress, whereby a combination of the low expression variants of both genotypes leads to elevated endocrine stress response, together, these findings suggests that low expression monoaminergic gene variants in general, may mediate vulnerability factors in the pathogenesis of emotional disorders.

Interestingly, co-occurring diagnoses of substance abuse and mental disorders (e.g., schizophrenia, depression, or bipolar, anxiety, personality, conduct, or attention-deficit/hyperactivity disorders) are highly prevalent[34]. Genetic variation including those involving monoaminergic genes, may partially underlie complex personality and physiological traits—such as impulsivity, risk taking and stress responsivity—as well as a substantial proportion of vulnerability to addictive diseases[35]. Specifically, low expression MAOA variation was shown to be associated with antisocial and anxious-depressive traits in alcoholic males[36]. Such findings make it apparent that a motivation or physiological drive stronger than our conscious concerns is at work fuelling our addictive behaviours[37]. Furthermore, comorbidity is known to be common between anxiety and depressive disorders, associated with other psychiatric disorders and frequently found coexisting with long-standing chronic medical conditions such as cardiovascular disease and diabetes mellitus[38,39].

Perhaps certain genetic markers may result to endophenotypes like HPA axis dysfunction, leading to susceptibility for some of these co-occurring neuropsychiatric disorders[38,40]. Given the strong evidence that multiple genetic mediation are involved in co-occuring neuropsychiatric conditions[38,40], it might be important to investigate the roles some common underlying genetic mechanism play in homeostatic regulation, in the face of adverse environmental circumstances.

Of importance to our present findings, gene expression profile of peripheral blood mononuclear cells (PBMCs) collected from trauma survivors, were recently shown to identify a gene expression 'signature' for post traumatic stress disorder (PTSD)[41,42]. In Segman *et al*[41], patients were studied immediately following a traumatic event, 1 month, and 4 months later. Their results provide evidence that patterns of gene expression profile in PBMCs can identify survivors who either persistently manifested full criteria for acute and chronic PTSD or remained healthy at

follow up. Such a finding of a psychiatric correlate in peripheral blood, in convergence with our own finding is important. It shows that peripheral markers can be used to determine symptom/disease outcome in psychiatry when they are meticulously analyzed during disease onset[42].

In line with Segman *et al*[41], our findings showing evidence of monoaminergic gene's modulatory role in peripheral endocrine response to psychological stress may have both theoretical and pharmacological implications. HPA-axis involvement in the maintenance of homeostasis during stress has been reported many times. One might speculate that an interaction between *COMT* and *MAOA* on peripheral endocrine responsivity to stress may be related to a combined influence of these genotypes on central catecholaminergic functioning, that might in turn affect CRH functioning. This has been supported by our finding of a stronger *MAOA* involvement in HPA axis regulation to the dex/CRH challenge than the other genotypes under study. Although future research is needed to verify this hypothesis, our findings of an additive effect of these genotypes on peripheral ACTH response to psychological stress strongly supports this idea.

If replicated, such findings may stimulate the development of psychopharmacological agents that might be effective in targeting the underlying genetic vulnerabilities. For example, individuals suffering from stress-related disorders like MD who are homozygous carriers of the *COMT met* allele and the low activity *MAOA* allele, may in fact benefit more from pharmacological agents that will enhance the reuptake of their central catecholaminergic and serotonergic availability.

6.2. Is Stress an Emotion?

Grief and fear,
when lingering,
provoke melancholia
Attributed to Hippocrates fifth century BC

Stress is a response to aversive stimuli and defined as "the nonspecific response of the body to any demand"[43]. In both animals and man a longstanding idea has been that uncertainty, lack of information, or the absence or loss of control produce alarm states[43]. Conversely, the presence of information in the form of clear and salient safety signals and behaviours, that lead to positive outcomes or result in control, reduce or eliminate the alarm state with a concomitant reduction of physiological responses[1]. The stress system receives and integrates a diversity of cognitive, emotional, neurosensory, and peripheral somatic signals that arrive through distinct pathways[44]. Such a conceptualization of the stress response makes it an integral part of an adaptive biological system, similar to the general emotional response.

The stress experience in complex organisms like humans, is however more complex than that of a single emotion. Activation of the stress system therefore leads to behavioural and physical changes that are remarkably consistent in their qualitative presentation and are collectively defined as the stress syndrome[44]. These changes are normally adaptive and time limited and improves the chances of survival. However, it is now well-documented that exposures to uncontrollable (inescapable and unpredictable) stress in adulthood can have profound effects on brain and behaviour and eventually lead to disease of various aetiologies[45].

By now we can relate to the fact that the Metmetians described in the introductory chapter are not weaklings. They are not soft hearted at all, but rather, they appear to be normal functioning people with genetic traits that make them vulnerable for a variety of stressors thereby increasing their vulnerability to stress-

related emotional disorders when exposed to adverse circumstances like the disaster on the island of Hetzygon. As Kipling expressed it over a hundred years ago, treating triumph and disaster just the same is not a question of choice for Metmetians. Disaster, unlike triumph, causes a series of physiological conditions to the Metmetian body that result in long term mental and physiological health consequences simply due to their genetic makeup. Our empirical findings reported in the present thesis, coupled with numerous earlier findings, have shown that an individual's genetic makeup does indeed influence their physiological and behavioral responses to environmentally challenging stimuli.

> If you can meet with triumph and disaster
> And treat those two impostors just the same…….
>
> If you can force your heart and nerve and sinew
> To serve your turn long after they are gone,
> And so hold on when there is nothing in you
> Except the Will which says to them: "Hold on"…….
>
> Yours is the Earth and everything that's in it…
> Rudyard Kipling 1895

Acute experience of a stressful encounter like the Metmetian disaster, can trigger a cascade of emotional and physiological responses. If we take an average Metmetian survivor of the event that disturbed the innocence and peace of their island, e.g., losing many loved ones in a single traumatic event leads to unbearable *grief*. While the visible damages to both structural and social fabrics of society will constantly remind an average survivor of his loss and thereby repeatedly triggering a complex of emotions varying from the *pain* of loss, the *disgusting* reminder of cadavers of loved ones, the feeling of *helplessness* for not being able to do any thing about the pains and deaths of loved ones coupled with some form of *guilt*, unspecified *anger*, extreme *sadness* and *frustration* about the turn of events, will complicate the coping process even further. Moreover, an average Metmetian survivor may be constantly haunted with *fear* of an impending disaster looming from the mountain ranges. The question then *if stress is an emotion*, may not be easy to answer. Intuitively however, and based on the observations outlined above, stress might be the mother of a cascade of emotional experiences (see figure 6.1 below).

Neuroendocrine markers activated by stressors include the increased secretion of EPI and NE from the sympathetic nervous system and adrenal medulla. The release of CRH and vasopressin from paraventricular neurons of the hypothalamus into the portal circulation, and seconds later, the secretion of ACTH, leading to secretion of glucocorticoids by the adrenal gland[47], are all part of this cascade of response. CRH coordinates the endocrine, autonomic, behavioural and immune responses to stress and emotional experience, as well as acting as a neurotransmitter or neuromodulators in the amygdala, dorsal raphe nucleus, hippocampus and locus coeruleus, to integrate subcortical and cortical responses to these experiences[47].

In the present thesis, we did not map the neural representation of the human stress response, however, independent observations of the involvement of the anterior insula in the regulation of the human stress response[48] converges with our findings of a central role of these regions in processing emotions during the experience and social observation of pleasant and unpleasant gustatory stimuli (see chapter 4).

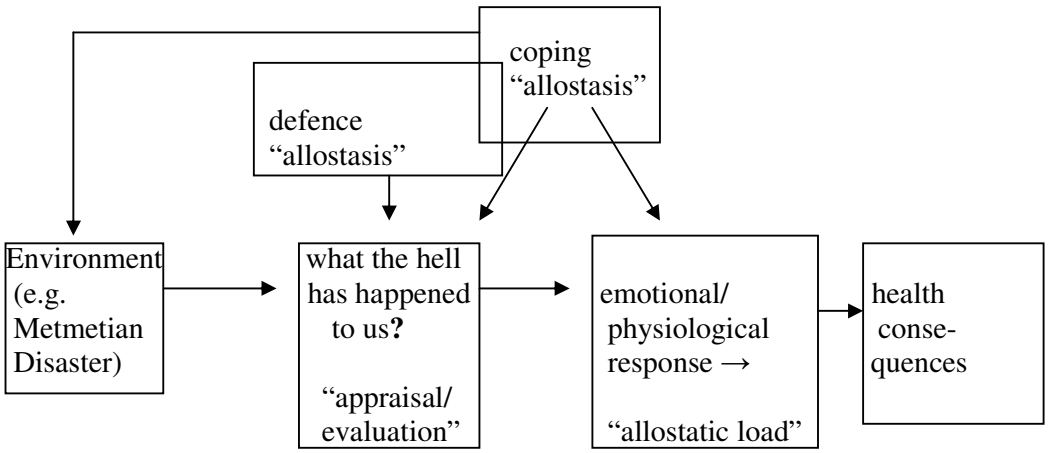


Figure 6.1. A modified model of the role of defence and coping in the stress-health chain as adopted from Ursin and Olff with the inclusion of the allostasis theory of McEwen as illustrated in the context of the Metmetian disaster[1,46]. This modified model is indeed valid for the experience and eventual health consequences of present day stressful life experiences. For example given the disease burden of psychiatric illnesses like major depression (believed to be the highest in the developed countries according to the latest WHO report), it is almost always a fact that the onset of the depressive episodes are triggered either directly or indirectly by adverse environmental circumstances relating to socio-economic issues (loss in terms of material. social status, social relationships, dead of a loved one that results to grief etc.), physical health (chronic disease of the physical body), long term uncertainties or difficulties.

It is important to recognize that this neural/sensory system is part of an entire network involved in homeostasis, relating to the autonomic, hormonal (sensory) and behavioral mechanisms that maintains optimal physiological conditions of the body. Thus, although the stress experience may not be an emotion as we know it, it does indeed results to a cascade of emotional responses. In essence, unravelling the pathways and neurobiological mechanisms that underlie the regulation of physical and emotional stress responses in humans is of critical importance in our attempt to understand vulnerability and resilience factors relevant to the development of a number of complex physical and psychopathological states[49]. Together, the available evidence suggests similar bodily mechanisms underlying the human homeostatic maintenance both in response to psychosocial stress and social emotional interactions. These sensory mechanisms are strongly embodied and under the influence of individual's genetic make up[50].

6.3. Conclusions and Future Directions

In recent years, there has been rapid progress in identifying neurobiological systems that are essential in the regulation of negative affective states and in the maintenance of homeostasis. Bodily arousal responses to emotional stimuli, especially negative mood states, and stressful life experiences that recruit the HPA-axis response, in conjunction with the context of maintaining homeostasis, have emerged as neurobiological mechanisms plausibly linked to stress and emotional resilience and vulnerability to mood disorders. We showed that when individuals are exposed to socially relevant emotional stimuli, some brain regions that include cingulate and somatosensory regions are active regardless of the valence of the emotional exposure. Subsequently, we have demonstrated that by inducing emotional experience by means of gustatory stimuli, individual's tendency of responding to the emotional experiences of others, with their own gustatory cortex, is strongly influenced by their degree of emotional arousability.

Furthermore, our identified mechanism of the degree of endocrine and behavioral stress response being a function of *met* allelic loading is in convergence

with earlier reports of increased limbic reactivity to unpleasant stimuli. Thus the observations of increased limbic reactivity to unpleasant stimuli, in conjunction with increased subjective, endocrine and neurochemical responses to both psychological and physiological stressors, might be part of the underlying cause of the lower emotional resilience against negative mood states observed in individuals with higher *met* allelic load within the *COMT* genotype. We then demonstrated that individuals heterozygous of the *5-HTT* gene showed exacerbated ACTH response to stress. Additionally, when higher *met* allelic loading (i.e., low expression variant of the *COMT* genotype) occurs in combination with low expression variant of *MAOA*, individuals show elevated ACTH response to psychological stress. Such expression patterns regarding our genetic variables in response to stress may imply that higher endocrine responses during acute psychological stress could be seen as an adaptive mechanism and may therefore represent adaptive stress response and not a vulnerability factor. It remains for future research to examine if these low expression gene variants, that lead to higher availability of monoaminergic neurotransmitters, are resilience factors to stressful experience or not.

Ceteris paribus, the results of our studies that examined brain response to social emotional induction suggests that activations during the observation of social emotional stimuli may be correlates of body states that are similar to what is been observed in the other person. On the other hand, our genetic findings show that studies to date, designed to examine the role of genes in the aetiology of stress related disorders like major depression, may not be informative enough in examining the functional role of a single nucleotide polymorphism. Complex psychological traits like mental disorders have noisier genetic architectures like say malaria, and should therefore be treated as such. This has been supported by our findings of an interaction between *COMT* and *MAOA* in human endocrine response to psychological stress. These interactions showed the existence of additive effects of two monoaminergic genes in human endocrine stress responses. Such findings underscore the complex nature of gene-environment interactions and their roles in the pathogenesis of psychiatric disorders. These results may be seen as a small, but valuable contribution in understanding the physiological pathways to homeostatic regulation.

In sum, humans are deeply social animals. A vast majority of humans today live in urban or semi urban settlements, making other humans the potential sources of stress and emotional experience. Evolutionary functions of stress and emotional experience are therefore mostly implicating social interaction, making social interactions critical for our emotional milieu and well being. We all know the phenomenon associating the stress experience with aversive social encounters, say an aggressive attack. Likewise, very positive social interactions with higher emotional values such as a wedding could none the less be surprisingly stressful. An important factor that governs these experiences is the differences between individuals in their reactivity to social environment.

Here, our experiments spans interindividual differences in neural and endocrine response to social emotional and stressful stimuli to contribute to our awareness of how the human body deals with socially relevant environmental challenges. As our results have shown, tapping into neurobiological substrates underlying individual differences in social emotional cognitive concepts such as empathy, in conjunction with the mediatory roles of genes on peripheral bodily response to environmental challenges, may provide a window into the physiological mechanisms involved in the regulation of homeostasis and the maintenance of physical and mental health.

6.4 Nedelandse Samenvatting

Samenvatting

Gedurende de laatste decennia is duidelijk geworden dat de hersenen niet alleen het zogenaamde autonome zenuwstelsel controleren, en tevens een belangrijke rol spelen bij de regulatie van endocriene en immunologische processen[1]. De experimenten die beschreven worden in dit proefschrift zijn ontworpen met als doel individuele verschillen te onderzoeken in homeostatische processen die verstoord kunnen raken tijdens psychologische stressoren en emotioneel gedrag. Het argument om dit in samenhang te onderzoeken is gebaseerd op het gegeven dat subjectieve gevoelens bij de mens gebaseerd zijn op een (meta)representatie van een fysiologische toestand in het lichaam[2].

In dit proefschrift zijn een aantal experimentele onderzoeksdesigns beschreven waarin wij een aantal (1) individuele verschillen bestuderen tijdens verschillende emoties en (2) de subjectieve en neuroendocriene respons op experimentele stressoren in kaart brengen, waarbij vooral aandacht besteed wordt aan genetische verschillen die de subjectieve en neuroendocriene verschillen bepalen tussen individuen.

In *hoofdstuk 2* werden de resultaten beschreven van een onderzoek met behulp van functionele MRI (fMRI) naar de gebieden in de hersenen die geactiveerd worden bij de inductie van de emoties verdriet, geluk en angst. Hersengebieden zoals de anterieure cingulate cortex zijn bij al deze subtypen geactiveerd, terwijl andere hersengebieden meer geactiveerd worden gedurende het verwerken van meer specifieke emoties. Het gegeven dat er een gemeenschappelijk neuraal substraat blijkt te bestaan voor emoties zoals verdriet, geluk en angst onderstreept een belangrijk uitgangspunt van dit proefschrift, namelijk dat bepaalde emoties de homeostatische balans van het lichaam veranderen wat aanleiding geeft tot o.a. een verhoogde autonome prikkeling/arousal. Het argument voor deze redenering is dat toegenomen prikkeling/arousal en aandacht eveneens in staat zijn om de activatie van de cingulate cortex te verhogen[3-8]. Consistent met onze bevindingen is eerder betoogd dat tijdens het bestuderen van emoties in het laboratorium, waarbij complexe sociale stimuli gebruikt worden (zoals filmclips of plaatjes met een emotionele inhoud), verschillende processen geactiveerd worden die een rol spelen bij affectieve en perceptuele processen, geheugenprocessen en subjectieve beleving[9]. Deze verschillende subcomponenten van onze emotionele respons worden gerepresenteerd in verschillende maar overlappende neurale circuits die een sterke interactie met elkaar vertonen. Wij hebben onze gegevens dan ook geïnterpreteerd conform het inzicht dat er een gemeenschappelijk neuraal substraat bestaat als representatie van de emoties verdriet, geluk en angst, waarbij de gemeenschappelijke lichamelijke arousal die deze verschillende emoties induceren, gerepresenteerd wordt in dit overlappende neurale circuit. Deze empirische bevindingen komen overeen met eerdere opvattingen van William James toen hij schreef: "*any classification of the emotions is seen to be true and as 'natural' as any other,* if it only serves some purpose; and such a question as 'what is the real' or 'typical' expression of anger, or fear?" wordt door hem opgevat als een verkeerde vraag[2].

In *hoofdstuk 3* werd de rol onderzocht van individuele verschillen in de empatische respons van proefpersonen tijdens het zien van de emotie 'walging' op het gelaat van anderen. Tevens werd in dit fMRI onderzoek gekeken naar neurale activatie tijdens het kijken naar gezichten die de emotie 'walging' uitdrukten. Eerder onderzoek heeft aangetoond dat tijdens aanbieding van aversieve reukstimuli en

smaakstimuli, die beiden de emotie 'walging' opwekten, vooral aanleiding geven tot toegenomen activatie in de anterieure insula en het frontale operculum[10]. Wij onderzochten de hypothese dat proefpersonen die sterke empathische vermogens bezitten, in sterkere mate hersengebieden activeren die de lichamelijke sensatie van walging representeren tijdens het observeren van dergelijke ervaringen bij anderen.

De hypothese van dit onderzoek was dat dit minder het geval zou zijn bij proefpersonen die een lagere score op empathieschalen vertoonden. Conform onze hypothese vonden wij dat de activatiegraad van de insula/frontale operculum tijdens het observeren van de emoties die anderen doormaakten bij het proeven van vieze drankjes een functie bleek te zijn van hun vermogen zich in anderen te verplaatsen (een hoge empathiescore). Deze resultaten laten zien dat wanneer proefpersonen zelf aangeven dat zij de neiging hebben om sterk mee te voelen met de emoties van anderen, zij in feite de insula in hun hersenen sterker activeren op het moment dat zij dergelijke emoties bij anderen waarnemen. Gegeven het feit dat emotionele belevingen nogal "besmettelijk" zijn in een sociale context, zou dit er op kunnen wijzen dat individuen de neiging hebben de emotionele toestand van anderen in hun eigen brein te simuleren doordat zij dezelfde fysiologische staat in hun lichaam oproepen ten einde hen in staat te stellen te voelen wat de ander ervaart. Met andere woorden, wanneer de waarneming van de gevoelens bij anderen mijn eigen fysiologische lichamelijke toestand (en dus de homeostatische processen in mijn lichaam) kan veranderen, is het mogelijk om bijna letterlijk te voelen wat de andere voelt. Een dergelijk mechanisme zou wel eens de biologische basis kunnen vormen van het aanstekelijke effect van verschillende emoties.

In *hoofdstuk 4* worden de resultaten beschreven van de endocriene en gedragsmatige respons op een psychologische stressor, waarbij gevonden werd dat deze respons gemoduleerd werd door een polymorfisme van het gen dat codeert voor Catechol-O-methyltransferase (COMT). Dit polymorfisme van COMT blijkt een modulerende rol te hebben in de endocriene respons tijdens een psychologische stressor en speelt mogelijk een rol in de kwetsbaarheid voor het ontwikkelen van een depressief syndroom. Eerder onderzoek naar de functie van het polymorfisme van COMT toonde aan dat dit genotype ook een rol speelt in het moduleren van de μ-opioid neurotransmissieprocessen als reactie op een pijnlijke stimulus[11], terwijl het μ-opioid receptor polymorfisme tevens van invloed bleek te zijn op interindividuele variaties in de wijze waarin pijn aanleiding geeft tot het ervaren van depressie of angst[12]. Bovendien bleek dat het COMT *met* allel bepalend is voor de functionele respons in de hersenen op negatieve emotionele stimuli[13,14]. COMT is betrokken bij de afgifte van dopamine in de prefrontale cortex, speelt een rol bij cognitieve processen, waarbij relaties gesuggereerd zijn met de pathogenese van emotionele stoornissen en schizofrenie[15-18].

Deze resultaten tonen aan dat genetische verschillen niet zozeer een directe samenhang vertonen met het ontstaan van psychiatrische stoornissen, maar dat de beschreven genetische polymorfismen een bemiddelende rol spelen bij de kwetsbaarheid voor omgevingsstimuli (in dit geval blootstelling aan psychosociale stress). Deze bevindingen suggereren een sterke functionele rol voor genetische variaties op het niveau van COMT ten aanzien van homeostatische processen, waarbij verandering hierin ten grondslag zouden kunnen liggen aan een breed spectrum van psychiatrische stoornissen.

In het laatste onderzoek dat in dit proefschrift beschreven wordt in *hoofdstuk 5,* beschrijven wij een overall effect van 5-HTT en een interactie tussen COMT en MAOA ten aanzien van de ACTH respons op een stressvolle stimulus. Wij vonden

dat tijdens acute psychologische stress, de plasma ACTH respons gemoduleerd wordt door polymorfismen van het gen voor de serotonine transporter (5-HTT). Wij vonden echter ook dat de ACTH afgifte afhankelijk is van de combinatie van genetische polymorfismen van zowel COMT als mono-amino-oxidase-A (MAOA). Bovendien vonden wij dat deze gen-omgevingsinteractie ten aanzien van de respons van de HPA-as op een stressvolle stimulus een seksueel dimorfe respons te zien gaf[19-21]. In dit onderzoek wordt voor het eerst aangetoond dat de aanwezigheid van de MAOA variant die leidt tot een hogere expressie van dit enzym, gepaard gaat met een lage activiteit van COMT functie ten aanzien van de endocriene regulatie tijdens psychosociale stress. Uitsluitend in het geval waarbij proefpersonen dragers zijn van de lage expressie MAOA variant (dus lage activiteit van het enzym MAOA) speelt het COMT gen een belangrijke rol in de hormonale regulatie van stress. Deze bevinding zou in de toekomst farmacologische consequenties kunnen hebben.

In een follow-up studie waarbij wij gebruik maken van een farmacologische provocatie test (DEX/CRH) werd gevonden dat vooral MAOA een rol speelt in HPA-as regulatie tijdens deze neuroendocriene stressor. Deze resultaten tonen niet alleen dat meerdere genen een rol kunnen spelen bij verschillende ziektebeelden en de respons op stress, maar tevens wijzen ze erop dat verschillende genen een onderscheidende rol kunnen spelen afhankelijk van de vraag welke type stressor aangeboden wordt.

In eerder onderzoek is getracht na te gaan of er een associatie bestaat tussen verschillende in dit proefschrift beschreven genetische polymorfismen zoals MAOA en verschillende psychiatrische ziektebeelden[22-26]. Studies bij muizen die geen MAOA enzym activiteit bezaten toonden toegenomen noradrenaline- en serotonine niveaus in de hersenen in vergelijking tot normale (wild-type) muizen. Deze zogenaamde MAOA knock-out muizen waren bovendien agressiever (bijvoorbeeld in de resident-intruder test) en toonden afwijkend emotioneel gedrag[27]. Het toegenomen agressieve gedrag bij deze MAOA knock-out muizen komt overeen met de impulsieve agressie die waargenomen is in families en patiëntenpopulaties met een verminderde MAOA functie[28-33]. Een lage activiteit van het enzym MAOA blijkt in combinatie met toegenomen risico op gewelddadig gedrag, een voorspellende waarde te hebben ten aanzien van een verminderd volume van het limbische systeem en een hyperresponsieve amygdala tijdens emotionele ervaringen. Anderzijds werd een verlaagde reactiviteit in prefrontale hersengebieden gevonden tijdens emoties bij dragers van het allel voor deze lage MAOA activiteit[33]. Ook eerder onderzoek kon de associatie bevestigen tussen lage expressie van MAOA en het optreden van agressie en afwijkende emotionele regulatie[33]. In combinatie met onze bevinding van het bestaan van een interactie tussen COMT en MAOA ten aanzien van de HPA-as respons op psychologische stress, zouden deze bevindingen kunnen suggereren dat een lage expressie van deze mono-aminerge genvarianten een rol kunnen spelen bij de kwetsbaarheid en aldus het risico op het ontwikkelen van een emotionele stoornis doen toenemen.

In dit verband is het interessant dat er een hoge graad van co-morbiditeit bestaat tussen middelenmisbruik en psychiatrische syndromen (bijvoorbeeld schizofrenie, depressie, bipolaire stoornis, angststoornissen, persoonlijkheidsstoornissen, gedragsstoornissen en attention-deficit/hyperactivity disorder)[34]. De genetische variatie die een rol speelt bij de regulatie van monoaminerge neurotransmissie ligt waarschijnlijk mede ten grondslag aan complexe persoonlijkheidstrekken (impulsiviteit, risicovol gedrag) en onze fysiologische respons op stressoren. Tevens speelt deze genetische variatie een rol bij de kwetsbaarheid voor het ontwikkelen van

verslavingsproblematiek[35]. Bijvoorbeeld de lage expressievariant van het MAOA gen blijkt geassocieerd te zijn met antisociale- en angstige depressieve trekken bij mannen die alcoholmisbruik vertonen[36]. Deze bevindingen illustreren dat zowel genetische polymorfismen die onze fysiologische "drive" beïnvloeden, tevens betrokken zijn bij het ontwikkelen van verslavingsproblematiek[37]. Bovendien is het zo dat co-morbiditeit tussen angst en depressie frequent voorkomt en tevens bestaat er een sterke co-morbiditeit bij angst en depressie met chronisch lichamelijke ziekten zoals cardiovasculaire problematiek en diabetes mellitus[38,39].

Mogelijk is het zo dat bepaalde genetische markers leiden tot een endofenotype dat gekenmerkt wordt door HPA-as disfunctie waardoor de kwetsbaarheid voor het ontwikkelen van deze neuropsychiatrische ziekten toeneemt[38,40]. Gegeven het feit dat er meerdere genen betrokken zijn bij het ontstaan van neuropsychiatrische syndromen is het van groot belang de rol te onderzoeken van gemeenschappelijke genetische mechanismen die een rol spelen bij homeostatische processen, vooral in het licht van aversieve omgevingsinvloeden.

In dit verband is het interessant te vermelden dat recent onderzoek aantoonde dat het genexpressieprofiel in perifere bloedcellen (mononucleaire bloedcellen) afkomstig van overlevenden van ernstig traumatische gebeurtenissen, een soort genetische 'handtekening' bleek te vertonen die samenhing met het ontwikkelen van posttraumatische stressstoornis[41,42]. In de studie van Segman en medewerkers[41] werden patiënten onderzocht onmiddellijk na het optreden van een ernstig trauma en tevens één en vier maanden later. De resultaten van dit onderzoek suggereren dat een bepaald patroon van genexpressie in mononucleaire cellen kan voorspellen welke patiënt na het doormaken van een dergelijk trauma daadwerkelijk posttraumatische stressstoornis zal ontwikkelen. Een dergelijke bevinding waarin een correlatief verband gevonden wordt tussen de respons op een ernstige stressvolle gebeurtenis en het optreden van een psychiatrisch syndroom is consistent met de bevindingen in dit proefschrift. Het laat namelijk zien dat perifere markers op basis waarvan een bepaald patroon herkend kan worden in de expressie van meerdere genen, gebruikt kan worden om voorspellingen te doen over het optreden van een psychiatrisch ziektebeeld na een ernstige traumatische gebeurtenis[42]. Ook de experimentele bevindingen die in dit proefschrift beschreven worden laten zien dat genetische polymorfismen een modulerende rol spelen ten aanzien van de monoaminerge neurotransmissie, die op zijn beurt een rol speelt in de perifere endocriene respons op psychologische stress. Dit heeft zowel theoretische als farmacologische implicaties.

De betrokkenheid van de HPA-as bij homeostatische processen gedurende stress is meerdere malen gerapporteerd. Op basis van onze bevindingen zouden wij kunnen postuleren dat de interactie tussen COMT en MAOA van invloed is op de perifere endocriene respons tijdens stressvolle gebeurtenissen, waarbij de invloed van deze polymorfismen op het centraal catecholaminerge functioneren de afgifte van corticotropine releasing hormone (CRH) mede bepaalt. Dit wordt gesteund door onze bevinding dat er een sterke betrokkenheid is van een polymorfisme van MAOA bij de regulatie van de HPA-as activatie tijdens de DEX/CRH provocatie test. Ook het gegeven van een additief effect van polymorfismen van MAOA en COMT op de perifere ACTH respons tijdens ons psychologisch stressexperiment steunt deze hypothese. Wanneer deze bevindingen gerepliceerd worden is het wellicht mogelijk via psychologische interventies de onderliggende genetische kwetsbaarheid te beïnvloeden. Het is bijvoorbeeld mogelijk dat individuen die een stressgerelateerde aandoening vertonen (major depression) homozygote carriers zijn van het COMT *met* allel en het lage activiteit MAOA allel. Deze patiënten zouden gebaseerd op het

profiel van hun genetisch polymorfisme baat kunnen hebben bij een farmacologische interventie die de terugopname van catecholamine en serotonine stimuleert in plaats van remt. Het argument hiervoor is dat zowel het COMT *met* allel als het lage activiteit MAOA allel beide de activiteit van het enzym (respectievelijk COMT en MAOA) afremmen, waardoor er een hogere concentratie van catecholaminen en serotonine in de synaptische spleet bestaat.

Is stress een emotie?

Grief and fear,
when lingering,
provoke melancholia

Toegeschreven aan Hippocrates vijfde eeuw v.Chr.

Stress is een respons op een aversieve stimulus en is gedefinieerd als "*the non-specific response of the body to any demand*"[43]. Zowel bij dieren als bij de mens weten wij dat factoren zoals onzekerheid, gebrek aan informatie, of de afwezigheid of het verlies van controle, zogenaamde *alarm states/alarm toestanden* kunnen uitlokken[43]. Omgekeerd is het zo dat de aanwezigheid van informatie over de buitenwereld in de vorm van signalen die veiligheid representeren en leiden tot een positieve uitkomst of resulteren in het gevoel van controle, deze "alarm state" tot verdwijnen kunnen brengen, hetgeen gepaard gaat met een vermindering van fysiologische arousal[1]. Het stresssysteem ontvangt en integreert een grote diversiteit van cognitieve, emotionele, neurosensorische en perifere lichamelijke signalen die allemaal via specifieke projectiegebieden de hersenen bereiken[44]. Conform een dergelijke conceptualisering van de stressrespons vormt deze een integraal onderdeel van adaptatieve biologische systemen, en is als zodanig vergelijkbaar met de responssystemen die betrokken zijn bij het ervaren van emoties.

Het ervaren van stressvolle gebeurtenissen bij complexe organismen zoals mensen is echter ingewikkelder dan die van een specifieke emotionele ervaring. Activatie van het stresssysteem leidt tot gedragsmatige en fysiologische veranderingen die ten aanzien van hun kwalitatieve presentatie een grote graad van consistentie vertonen en tezamen gedefinieerd zijn als het zogenaamde stresssyndroom[44]. Deze veranderingen tonen een adaptatief karakter, zijn begrensd in de tijd en verhogen de kans op overleving. Echter, er is veel empirische evidentie die er op wijst dat chronische blootstelling aan oncontroleerbare stress, zowel op kinderleeftijd als op volwassenenleeftijd ernstige consequenties kan hebben voor de hersenen en tevens kan leiden tot verschillende somatische en psychiatrische ziektebeelden[45].

Inmiddels begrijpen wij dat de denkbeeldige *Metmetians* beschreven in de introductie van dit proefschrift geen zwakkelingen zijn. Zij zijn normaal functionerende mensen met specifieke genetische eigenschappen die hen kwetsbaar maken voor een groot aantal stressoren, waarbij de kans op het ontwikkelen voor emotionele stoornissen bij blootstelling aan een trauma (zoals de ramp op het eiland Hetzygon) toegenomen is. Zoals Kipling het ruim een eeuw geleden al formuleerde, hebben de *Metmatians* niet de keuze om triomf en rampspoed op dezelfde wijze te verwerken. De in de introductie beschreven rampspoed veroorzaakt een cascade van fysiologische gebeurtenissen in het lichaam van de *Metmatians* die resulteerde in chronische, psychologische en fysiologische gezondheidsproblemen. Dit wordt voor een

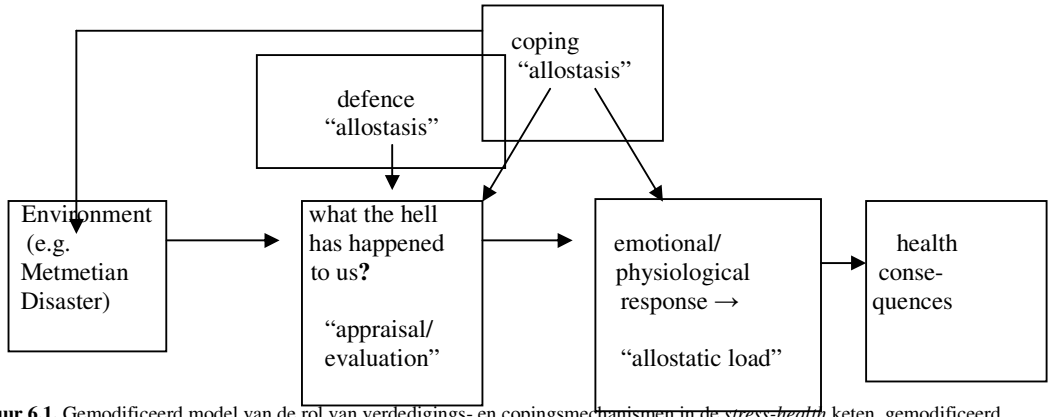
belangrijk deel veroorzaakt door de aanwezigheid van het beschreven genetisch polymorfisme. De empirische bevindingen waarvan verslag wordt gedaan in dit proefschrift tonen aan dat de genetische make-up van een individu de fysiologische en gedragsmatige respons op omgevingsstimuli sterk kan beïnvloeden.

If you can meet with triumph and disaster
And treat those two impostors just the same …….

If you can force your heart and nerve and sinew
To serve your turn long after they are gone,
And so hold on when there is nothing in you
Except the Will which says to them: "Hold on"……

Yours is the Earth and everything that's in it…
Rudyard Kipling 1895

De ervaring van een acute stressor zoals de *Metmatians* doormaakten, kan een cascade van emotionele en fysiologische responssystemen teweeg brengen. Het is mogelijk dat ten gevolge van het genetisch polymorfisme waarmee zij behept zijn, overlevenden die hun familieleden verloren hebben bij een enkele traumatische gebeurtenis ten prooi vallen aan een rouwproces dat zij niet kunnen verwerken. De herinnering van de gemiddelde overlevende aan een ernstige verlieservaring waarbij voortdurend complexe emoties gereactiveerd worden gerelateerd aan de pijn van dit verlies, de walging die opgeroepen wordt door de ontbindende lijken van familieleden, het gevoel van hopeloosheid en oncontroleerbaarheid van de omstandigheden waaraan zij zijn blootgesteld, hetgeen mogelijk gepaard gaat met schuldgevoelens, angst, verdriet en gevoelens van frustratie, zullen gezonde copingsmechanismen ernstig in de weg staan. Bovendien is het zo dat de gemiddelde overlevende het angstige gevoel kan hebben opgejaagd te worden omdat het gevoel van zekerheid verdwenen is en hij wellicht chronisch geconfronteerd wordt met anticipatieangst over een mogelijke herhaling van de ramp. De vraag of stress een emotie is, is niet eenvoudig te beantwoorden. Op intuïtieve gronden echter, en gebaseerd op de observaties hierboven beschreven, zou men kunnen stellen dat stress ten grondslag ligt aan een cascade van emotionele ervaringen.



Figuur 6.1. Gemodificeerd model van de rol van verdedigings- en copingsmechanismen in de *stress-health* keten, gemodificeerd naar Ursin and Olff. Tevens is in deze figuur de theorie over allostasis geïncludeerd van McEwen[1,46]. Dit model is geldig voor het ervaren van de schadelijke consequenties voor de gezondheid van stressvolle levensgebeurtenissen. Bijvoorbeeld, een ernstige psychiatrische ziekte zoals major depression (volgens het laatste rapport van de WHO het hoogste in ontwikkelingslanden) is vrijwel altijd het directe of indirecte gevolg van stressvolle omgevingsfactoren of levensgebeurtenissen die gerelateerd zijn aan gebrekkige sociaal economische omstandigheden (verlies van sociale status, uitholling van sociale relaties, verlieservaringen die leiden tot rouw). Dezelfde ervaringen kunnen aanleiding geven tot lichamelijke gezondheidsproblemen.

Neuroendocriene markers die geactiveerd worden door stressoren betreffen een toegenomen afgifte van noradrenaline en adrenaline in het sympatische zenuwstelsel en het bijniermerg. De afgifte van CRH en vasopressine uit paraventriculaire neuronen in de hypothalamus en enkele seconden later de afgifte van ACTH die aanleiding geeft tot een verhoogde vrijmaking van glucocorticoiden door de bijnierschors, zijn alle delen van deze cascade[47]. CRH speelt een rol bij het coördineren van de endocriene, immunologische, autonome en gedragsmatige respons op stressvolle en emotionele gebeurtenissen en speelt tevens een rol als neurotransmitter/neuromodulator in de amygdala, dorsale raphe kern, hippocampus en locus coeruleus[47].

In het huidige proefschrift hebben we geen beeldvormend onderzoek gedaan tijdens de experimentele stress testen. Echter ander onderzoek toonde aan dat de anterieure cingulate en insula cortex betrokken is bij de regulatie van de reactie op stressoren[48], hetgeen overeenkomt met onze bevindingen (beschreven in hoofstuk 4) waar gevonden werd dat ditzelfde hersengebied een rol speelt gedurende de ervaring en observatie (op het gelaat van anderen) van onplezierige smaakstimuli. Ditzelfde gebied in de hersenen is betrokken bij een netwerk dat een rol speelt bij het handhaven van homeostatische mechanismen en speelt zo een rol bij het handhaven van optimale fysiologische condities. De conclusie is dus verdedigbaar dat ook al zijn stressvolle ervaringen niet hetzelfde als emoties zoals wij deze kennen, deze tegelijkertijd resulteren in een cascade van emotionele responssystemen. De neurale architectuur en neurobiologische mechanismen die een rol spelen bij de regulatie van de fysiologische en emotionele stressrespons bij de mens is van groot belang bij het begrijpen van kwetsbaarheids- en weerbaarheidsfactoren die een rol spelen bij de ontwikkeling van een aantal complexe lichamelijke ziekten en psychopathologische syndromen[49]. De aanwezige empirische evidentie suggereert dat vergelijkbare fysiologische mechanismen ten grondslag liggen aan het handhaven van de homeostase, zowel in respons op psychosociale stressoren als op emoties die wij doormaken tijdens sociale interacties. Deze mechanismen zijn in ons lichaam verankerd en staan tevens sterk onder invloed van de genetische make-up van het individu[50].

Conclusies en suggesties voor toekomstig onderzoek

De laatste jaren is er grote vooruitgang geboekt met betrekking tot het in kaart brengen van neurobiologische systemen die een belangrijke rol spelen bij de regulatie van ons affectieve leven en het behouden van de homeostase in het lichaam. De lichamelijke arousal die optreedt tijdens het doormaken van vooral negatief geladen emoties en stressvolle levensgebeurtenissen die de HPA-as activeren in samenhang met de rol die zij spelen bij het bewaken van homeostatische processen, zijn gezamenlijk te zien als neurobiologische mechanismen die gekoppeld zijn aan de respons op stress en tevens een rol spelen bij emotionele weerbaarheid/kwetsbaarheid voor het ontwikkelen van stemmingsstoornissen (en andere stressgerelateerde psychopathologie). Wij hebben aangetoond dat wanneer proefpersonen blootgesteld worden aan sociaal relevante emotionele stimuli, hersengebieden zoals de cingulate en somatosensorische cortices geactiveerd worden, ongeacht het type emotie. Vervolgens werd in dit proefschrift aangetoond dat wanneer een emotionele toestand opgewekt wordt door smaakstimuli aan te bieden, de tendens van proefpersonen om op de emoties van anderen te reageren met hun eigen corticale gebieden die betrokken

zijn bij smaak, sterk beïnvloed wordt door de neiging die zij vertonen om in emotioneel opzicht aangedaan te raken.

Wij hebben tevens laten zien dat de mate van endocriene en gedragsmatige responsiviteit op een psychologische stressor een functie is van de aanwezigheid van het *met* allel. Hetgeen in overeenstemming is met eerder onderzoek waarin werd aangetoond dat er een toegenomen limbische acitivatie bestaat in respons op aversieve stimuli. De toegenomen activatie in het limbische systeem in reactie op aversieve stimuli, tezamen met de toegenomen subjectieve, endocriene en neurochemische respons op psychologische en neuroendocriene stressoren (hier de DEX/CRH provocatie test), zou ten grondslag kunnen liggen aan de verminderde emotionele weerbaarheid tijdens negatieve emoties bij individuen met het *met* allel van het COMT genotype. Tevens vonden wij aanwijzingen dat individuen die heterozygoot zijn voor het 5-HTT gen een toegenomen ACTH respons vertonen op stressvolle stimuli. Wanneer er een combinatie bestaat van het *met* allel (lage expressievariant van het COMT genotype) in combinatie met de lage expressievariant van MAOA, blijken proefpersonen een toegenomen ACTH respons te vertonen op een psychologische stressor. De vraag is of een dergelijk patroon van genetische polymorfismen die van invloed is op de respons op stress, in een acute situatie gezien moet worden als een adaptatief mechanisme, en niet zozeer als een kwetsbaarheidsfactor. Toekomstig onderzoek zal moeten uitwijzen of deze lage expressievarianten van COMT en MAOA, die gezamenlijk leiden tot een toegenomen beschikbaarheid van monoaminerge neurotransmitters, bij acute blootstelling aan stressoren juist een beschermde rol spelen voor het ontwikkelen van psychopathologie, terwijl de schadelijke effecten optreden bij chronische blootstelling aan stressoren.

Ceteris paribus, de resultaten van ons onderzoek waarin de respons van de hersenen onderzocht wordt tijdens sociaal emotionele stimuli suggereren dat bij observatie van dergelijke emoties op het gelaat van anderen, dezelfde lichamelijke toestand optreedt als in het geval waarbij de proefpersoon deze emotie zelf meemaakt. Tegelijkertijd illustreert ons onderzoek naar de rol van genetische polyformismen bij de etiologie van stressgerelateerde syndromen, dat deze wellicht nog niet informatief genoeg zijn ten aanzien van de rol van een enkel genetisch polymorfisme. Ingewikkelde psychologische trekken en psychiatrische stoornissen hebben een meer complexe genetische architectuur en zijn waarschijnlijk niet te herleiden tot veranderingen in een gen. Dit wordt ondersteund door onze bevinding van het bestaan van een interactie tussen COMT en MAOA ten aanzien van de endocriene respons op een psychologische stressor. Deze interactie illustreert het mogelijke bestaan van additieve effecten van twee monoaminerge genen in de humane endocriene respons op een stressor. Ook deze bevindingen onderstrepen de complexe aard van gen-omgevingsinteracties en hun rol in de pathogenese van psychiatrische stoornissen. Deze eerste resultaten kunnen niettemin gezien worden als een waardevolle bijdrage aan ons begrip over de fysiologische en neurale circuits die betrokken zijn bij homeostatische processen.

Mensen zijn sociale dieren. De meerderheid van mensen vandaag de dag leeft in een urbane of semi-urbane omgeving waarbij zij frequent blootgesteld zijn aan stressvolle gebeurtenissen en emotionele ervaringen. De respons op stressoren en onze emoties hebben een evolutionaire functie en spelen een belangrijke rol bij sociale interacties. De heftigheid van de lichamelijke en cerebrale respons op sociale stressoren zoals agressie is iedereen bekend. Echter ook positieve sociale interacties met een sterke emotionele component zoals een huwelijk kunnen niettemin zeer

stressvol zijn. Een belangrijke factor die een regulerende rol speelt bij deze ervaring wordt gevormd door de genetische en biologische make-up van het individu waardoor grote verschillen kunnen optreden in de fysiologische en gedragsmatige reactiviteit op dergelijke ervaringen. Onze experimentele studies waren vooral gericht op interindividuele verschillen in de neurale en endocriene respons op sociaal emotionele en stressvolle stimuli en kunnen een bijdrage leveren aan ons inzicht in de reactie van het menselijk lichaam op sociaal relevante omgevingsvariabelen. Onze resultaten laten zien dat het onderzoek naar het neurobiologische substraat dat ten grondslag ligt aan individuele verschillen in sociaal emotionele en cognitieve concepten zoals empathie, in samenhang met het bestuderen van de mediërende rol van genen op de lichamelijke respons op omgevingsstressoren, een bijdrage kan leveren in ons inzicht in de fysiologische mechanismen die betrokken zijn bij de regulatie van homeostatische processen. Deze zijn van belang bij het behouden van lichamelijke en geestelijke gezondheid.

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2007 May: Jabbi M, Bastiaansen JA, Keysers C. Neural correlates of imagining, experiencing and observing gustatory disgust and pleasure, *abstract, CNS to be held in New York.*

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