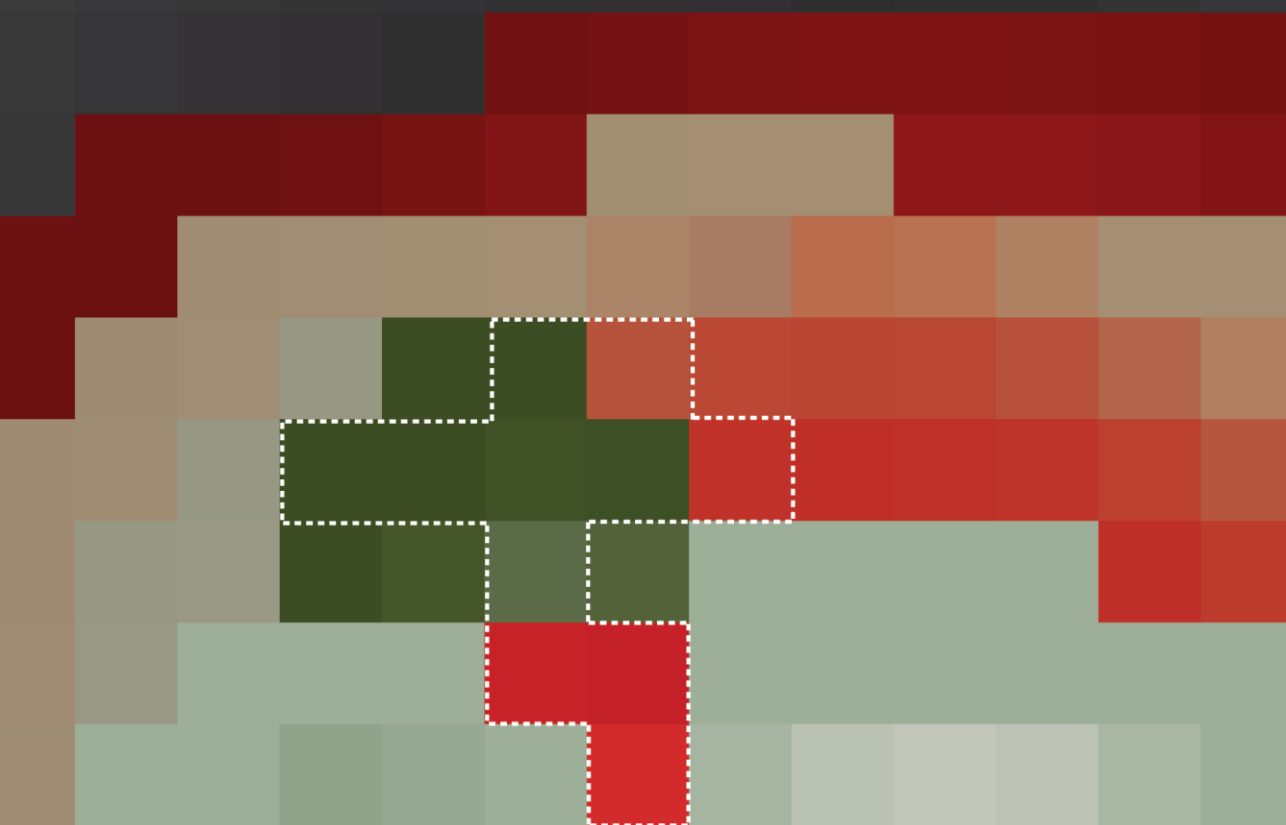
Performance monitoring in depression

Behavioural and neurophysiological correlates of feedback processing

Gabry Mies



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Performance Monitoring in Depression
Behavioural and Neurophysiological Correlates of Feedback Processing

Zelfmonitoren van prestatie bij depressie
Gedrags- en neurofysiologische correlaten van feedbackverwerking

Proefschrift

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Chapter 1

General introduction

Major depressive disorder

Unipolar major depressive disorder (MDD) is a common mental health disorder which has a lifetime prevalence of about 19% in the Netherlands (De Graaf *et al.*, 2010). This means that almost 1 out of 5 persons will experience a depressive episode at some point in their lives. Women are almost twice as often affected as men (Bijl *et al.*, 1997, De Graaf *et al.*, 2010). Worldwide, about 121 million of people are affected by depression, and it is one of the leading causes of disability (WHO, 2011).

MDD is a heterogeneous disease which is characterized by a depressed mood and/or anhedonia (loss of pleasure and interest). Other symptoms include sleep disturbances, appetite and weight changes, psychomotor disturbances, loss of concentration or indecisiveness, loss of energy, feelings of worthlessness or inappropriate guilt, and suicidal thoughts. According to the DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, American Psychiatric Association, 1994) at least five of these symptoms, including depressed mood or anhedonia, need to be present for the same two-week period in order to make a diagnosis of MDD.

The etiology of MDD is not fully understood. Several factors are known to be involved, ranging from purely biological to more psychological in nature. These factors include a genetic predisposition, a more broadly-defined family history of depression, neurotransmitter dysfunctions (serotonin, dopamine, norepinephrine), disturbances of the HPA-axis, adverse life events, and cognitive biases.

Cognitive theories of depression postulate that thoughts, attitudes, interpretations, and the way in which individuals attend to events and recall them, could render an individual vulnerable to develop depression (Gotlib and Joormann, 2010). One of the most influential cognitive theories of depression was developed by Beck (1979). He argued that dysfunctional 'schemas' may lead to depression. Personal life experiences can lead to negative attitudes, which subsequently influence the way depressed people interpret situations. Depressed individuals filter different information from the environment than healthy individuals. Their attention is drawn to information that is congruent with their dysfunctional or negative schemas, which is also known as 'negativity bias': more attention is drawn to negative information than to positive information, and neutral information is more often interpreted in a negative way. Another influential model, known as learned helplessness, was developed by Seligman (1972) on the basis of observed animal behaviour in response to uncontrollable stress situations. The lack of motivation associated with depression is often explained in terms of learned helplessness. Due to negative life experiences and a lack of control, depressed individuals feel that everything is futile. It can be difficult for depressed patients to get out of this vicious cycle of negative automatic thoughts, cognitive biases, and depressed mood without treatment.

Effective treatments for depression are psychotherapy (e.g., cognitive behavioural therapy), pharmacological treatment (antidepressants) and electro-convulsive therapy

(ECT). Although most patients respond well to treatment, unfortunately, the recurrence rate of depression is very high: most patients will experience another episode in their lives, and the recurrence rate increases with each subsequent episode (Boland and Keller, 2009).

Feedback processing

The negativity bias from which patients with MDD are thought to suffer is reflected in the way they interpret social situations, and may lead to social dysfunction. It is therefore of interest to investigate how patients process external feedback. Continuously monitoring your own performance is a prerequisite for optimal goal-directed behaviour. In everyday life, optimal error and feedback processing enable you to adjust your behaviour when necessary, i.e., to be flexible. In contrast to healthy individuals, who generally learn from their mistakes and use feedback to guide their future actions, depressed individuals have been found to respond abnormally to errors and negative feedback. When depressed patients make an error or receive negative feedback, their subsequent performance deteriorates drastically (e.g., Beats *et al.*, 1996; Elliott *et al.*, 1997). This maladaptive response to negative feedback has been suggested to be a key deficit linking the negative affect and the cognitive impairments associated with depression (Elliott *et al.*, 1997).

The disturbances in feedback processing in patients with MDD can be interpreted in several ways. In line with Beck's cognitive theory of depression, perceived failure due to negative feedback might lead to more negative thoughts, which interfere with subsequent performance (Eshel and Roiser, 2010). It is also possible that the patient simply does not use the negative feedback to adjust behaviour, possibly because the patient is less motivated to obtain positive feedback than others, or perceives a lack of control, in line with the learned helplessness model (e.g., Elliott *et al.*, 1997; Eshel and Roiser, 2010).

Their negativity bias may, therefore, play an important role in the way depressed individuals process feedback. It is likely that patients with MDD attend more to negative feedback than to positive feedback. Murphy *et al.* (2003), however, found that depressed patients were not impaired in the processing of *accurate* negative feedback, but were impaired in the processing of *misleading* negative feedback, i.e., negative feedback which does not call for a behavioural adaptation. People with MDD were more likely to make behavioural adjustments when it was not necessary. Murphy *et al.* suggested that negative feedback that is more affective in nature might disrupt performance, whereas negative feedback that is more informational might not. One of the purposes of this thesis is to investigate this hypothesis.

Many tasks have been developed to measure error and feedback processing. The task that we have used in all studies reported in this thesis, a time-estimation task, is a typical example of a task in which external feedback is necessary to become aware of an

error. This task was originally developed by Miltner *et al.* (1997). Participants are required to estimate an interval of 1 second. They press a button following a cue when they feel that the required interval elapsed. They receive positive feedback when their button press falls within a pre-specified window around the end of the interval and negative feedback when their response falls outside this window. The pre-specified window is adjusted from trial-to-trial by using a dynamic tracking algorithm: the window for the upcoming trial is shortened following a correct estimate and lengthened following an incorrect estimate. This leads to an almost equal amount of positive and negative feedback. It is therefore difficult to have strong expectations about the outcome of the response, and participants will have to rely on the (external) feedback in order to monitor their performance.

Several measures or techniques can be used to assess how the brain processes feedback. In the studies described in this thesis we have used behavioural measures, electro-cortical measures (event-related brain potentials; ERPs), heart rate, and functional Magnetic Resonance Imaging (fMRI).

Error and feedback processing have been extensively investigated in healthy volunteers by means of electroencephalography (EEG), from which ERPs can be derived. EEG has an excellent temporal resolution, making it possible to measure brain processes within milliseconds. Already within 50-100 ms after an error has been made, a negative-going ERP occurs, which is called the *error-related negativity* (ERN or Ne; Falkenstein *et al.*, 1991, Gehring *et al.*, 1993). A similar component is observed when a person receives negative feedback, the so-called *feedback-related negativity* (FRN), which occurs around 200-350 ms after feedback onset (Miltner *et al.*, 1997). ERP studies in depression show inconsistent results. The ERN or FRN has been found increased, decreased, or similar in patients with depression in comparison to healthy subjects (Chiu and Deldin, 2007, Ruchsow *et al.*, 2006, Ruchsow *et al.*, 2004, Schrijvers *et al.*, 2008, Schrijvers *et al.*, 2009, Tucker *et al.*, 2003). Several factors might underlie these discrepancies, in particular the use of antidepressants, and differences in symptom severity.

EEG source localization studies have suggested that the ERN and FRN share a common neural source: the anterior cingulate cortex (ACC; Gehring and Willoughby, 2002, Miltner *et al.*, 2003). fMRI, however, has a superior spatial resolution, making it the preferred technique to study the underlying brain regions involved. fMRI studies, measuring the Blood-Oxygen-Level-Dependent (BOLD)-response, which is an indirect measure of regional activity in the brain, have confirmed the ACC as one of the sources of the ERN and FRN (Dehaene *et al.*, 1994, Ridderinkhof *et al.*, 2004). The ERN and FRN are thought to be due to a dip in mesencephalic dopamine release, disinhibiting ACC activity (Holroyd and Coles, 2002, Nieuwenhuis *et al.*, 2002). The ACC covers a large area surrounding the anterior part of the corpus callosum (see Figure 1.1), and can be subdivided in a dorsal-cognitive part and a ventral-affective part (Bush *et al.*, 2000, Devinsky *et al.*, 1995, Mohanty *et al.*, 2007). The ACC is therefore an important hub

integrating both cognitive and affective processing, which is needed for feedback processing.

Finally, feedback processing is also reflected in heart rate. Heart rate is regulated by the autonomic nervous system (ANS). The ANS is subdivided into the sympathetic and parasympathetic nervous system. The parasympathetic branch projects to the heart via the vagal nerve. When vagal outflow increases, cardiac deceleration occurs. Cognitive and emotional processes can influence vagal outflow. Several studies have shown that negative feedback stimuli evoke a larger decelerative heart rate response than positive feedback stimuli (Crone *et al.*, 2003, Hajcak *et al.*, 2003b, Somsen *et al.*, 2000, Van der Veen *et al.*, 2004b).

Importantly, the ACC is known to exert an influence on the ANS (Critchley *et al.*, 2000, Critchley *et al.*, 2003, Devinsky *et al.*, 1995, Gianaros *et al.*, 2004). Critchley *et al.* (2000) for instance reported that reduced activity in the ACC was correlated with increased heart rate. Cardiac slowing associated with negative feedback is therefore thought to be a reflection of the same monitoring system responsible for the FRN (Crone *et al.*, 2005).

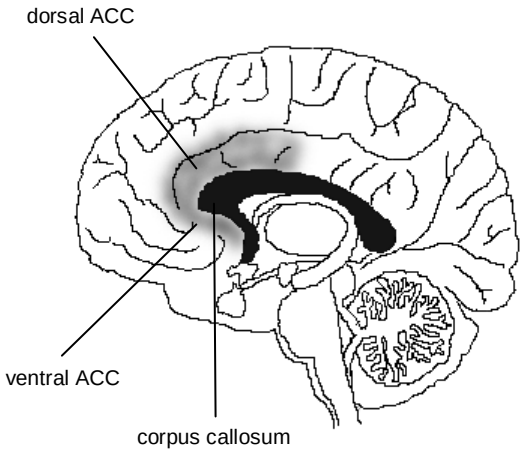

Figure 1.1 The anterior cingulate cortex (ACC) can be subdivided into two parts: the dorsal-cognitive part and the ventral-affective part

Serotonin

One of the aims of this thesis is to investigate the role of the monoamine neurotransmitter serotonin in feedback processing. Serotonin (5-HT) is associated with MDD. It is thought to play a role in appetite, sleep, thermoregulation, cardiovascular function, sexual behaviour, mood and cognition (Jacobs and Fornal, 1995). The link with depression is therefore evident. Further evidence for involvement of 5-HT in MDD comes amongst others from the notion that a substantial amount of patients responds to antidepressants targeting the 5-HT system (e.g., selective serotonin reuptake inhibitors, SSRIs).

Serotonin does not pass the blood brain barrier, which is why it is synthesized in the brain itself. The raphé nuclei, located in the midbrain, are the principal source of central 5-HT. From there 5-HT is distributed throughout the brain via serotonergic pathways. The amino acid L-tryptophan is the substrate for 5-HT, which is converted into 5-hydroxytryptophan (5-HTP) by tryptophan hydroxylase (TPH2) and subsequently converted into 5-hydroxytryptamine (5-HT, serotonin) by decarboxylase.

Acute tryptophan depletion (ATD) is a widely used method to investigate the effects of a transient decrease in central 5-HT on behaviour and brain function. By depleting the brain from its precursor tryptophan central 5-HT production is reduced. This is achieved by administration of an amino acid mixture devoid of tryptophan (Young *et al.*, 1985). ATD involves two processes: 1) due to the amino acid load protein production in the liver increases, using up body stores of tryptophan, leaving less tryptophan available to enter the brain; and 2) the large amount of amino acids compete with tryptophan for active transport through the blood-brain barrier, causing less tryptophan to enter the brain.

Another approach to investigate the influence of 5-HT on brain function is to investigate the effects of common functional polymorphisms in genes involved in 5-HT function. Two such polymorphisms are studied in this thesis, from which it is known that either the production of 5-HT is reduced (i.e., *rs1386493* in the TPH2-gene) or that the expression of the serotonin-transporter protein is reduced, which regulates the reuptake of 5-HT, and therefore regulates the availability of 5-HT in the synapse (i.e., 5-HTTLPR in the serotonin-transporter gene).

The last few years evidence has accumulated that, in addition to dopamine, serotonin may play an important role in error and feedback processing (Jocham and Ullsperger, 2009). This evidence comes from ATD studies showing direct involvement of 5-HT in ACC function (Evers *et al.*, 2010, Evers *et al.*, 2007), from studies on the relation between 5-HTTLPR and the ERN or ACC function (Althaus *et al.*, 2009, Fallgatter *et al.*, 2004, Holmes *et al.*, 2010), and indirectly from ERN/FRN studies with 5-HT-related illnesses such as depression and obsessive-compulsive disorder (e.g., Gehring *et al.*, 2000, Holmes and Pizzagalli, 2008, Johannes *et al.*, 2001b, Tucker *et al.*, 2003).

Aims of the thesis

In this thesis we investigated feedback processing in depressed, and in non-depressed individuals, by examining behavioural, electro-cortical, cardiac, and BOLD responses to positive and negative feedback using a time-estimation task. The studies revolve around three major aims.

1) Since a disturbance in 5-HT function is thought to be involved in MDD and evidence for a role of 5-HT in performance monitoring is accumulating, we aimed at investigating the link between lowered 5-HT function and aberrant feedback processing. This was indirectly measured in a study on patients with MDD, and more directly measured in a study in which we used acute tryptophan depletion to assess the influence of a transient lowered 5-HT availability on behavioural, cardiac and electro-cortical responses to feedback, and another study in which we used a genetic approach to assess the influence of lowered 5-HT function on behavioural and electro-cortical responses to feedback.

2) Another question we have tried to answer in this thesis is whether patients with MDD are more sensitive to the emotional value of feedback rather than to the information conveyed by the feedback, as suggested by Murphy *et al.* (2003). For this purpose patients performed a time-estimation task with valid and invalid feedback. The valid feedback was related to actual performance, and therefore relevant for behavioural adjustment, while the invalid feedback was random feedback, unrelated to their performance, and therefore not relevant for behavioural adjustment. Because the influence of psychotropic medication and the heterogeneity of symptoms and severity of depression is a large problem in previous studies on depression, we recruited a homogeneous group of hospitalized patients with moderate to severe MDD, who were drug-free at the time of testing.

3) Finally, because of discrepancies in previous studies regarding the role of the ACC in feedback processing, especially using time-estimation tasks, we further examined this role, with an emphasis on the possible distinct roles of the subdivisions of the ACC in the processing of the valence and validity of feedback. For this purpose we scanned healthy volunteers, and healthy volunteers with mild depressive symptoms, who are thought to be at risk of developing MDD.

Outline of the thesis

In the first two chapters we investigated feedback processing in non-depressed participants. In *chapter 2* we used a modified time-estimation task by which we were able to investigate the effect of the valence of feedback (positive or negative) as well as the effect of information value (validity of feedback) on behavioural, cardiac and electro-cortical correlates of feedback processing in non-depressed volunteers. In *chapter 3* we

used fMRI to examine the roles of the two subdivisions of the ACC in the processing of feedback valence and validity in non-depressed participants, using the same time-estimation task as described in chapter 2. In *chapter 4* we compared behavioural and electro-cortical responses to feedback valence and validity in patients with MDD with those of non-depressed participants. The patients were drug-free inpatients who were matched to a subsample of the non-depressed individuals reported on in chapter 2. The same time-estimation task was again used in *chapter 5*, in which performance and ACC-responses were compared between students who scored high on depressive symptoms and students who scored low on these symptoms. This way we were able to examine whether dysfunctions in the cortico-limbic circuit and in feedback processing were present in subclinical individuals, who might be at risk of developing MDD. In the last chapters we examined the role of serotonin in feedback processing. In *chapter 6* we examined the effect of ATD, a method to transiently lower serotonin in the brain, on behavioural, cardiac and electro-cortical responses to positive and negative feedback in a different sample of non-depressed volunteers, using a different version of the time-estimation task than reported on in the other chapters. In *chapter 7* we investigated the effects of two genetic polymorphisms influencing serotonin function (5-HTTLPR and TPH2- *rs1386493*) on behavioural and electro-cortical correlates of feedback processing. Finally in *chapter 8* a general discussion is given.

Chapter 2

Cardiac and electrophysiological responses to valid and invalid feedback in a time-estimation task

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Abstract

This study investigated the cardiac and electrophysiological response to feedback in a time-estimation task in which feedback-validity was manipulated. Participants across a wide age range had to produce 1s intervals followed by positive and negative feedback that was valid or invalid (i.e., related or unrelated to the preceding time estimate). Performance results showed that they processed the information provided by the feedback. Negative feedback was associated with a transient cardiac slowing only when feedback was valid. Correct adjustments after valid negative feedback were associated with a more pronounced cardiac slowing. Validity did not affect the feedback-related negativity (FRN), except when remedial action was taken into account. The FRN and cardiac response to feedback decreased with advancing age, but performance did not. The current pattern of findings was interpreted to suggest that the FRN and cardiac response signal 'alert' and that the cardiac response, but not the FRN, is implicated in the mechanisms invoked in remedial action.

Introduction

In a landmark study, Miltner *et al.* (1997) observed that negative feedback stimuli in a time-estimation task elicited a negative brain potential over the fronto-central regions of the scalp. The time-estimation task required participants to produce a one second interval by pressing a button following an auditory prompt. That is, the interval between prompt and button press should be as close as possible to a one-second interval. The time-window for good performance was dynamically adjusted throughout the task in such a way that the proportion of positive and negative feedback was equal and, thus, participants were assumed to expect positive and negative feedback with equal probabilities. Accordingly, a difference wave between brain potentials associated with positive vs. negative feedback should remove expectancy related variance and yield a relatively 'pure' measure of feedback processing. This measure was coined the feedback-related negativity, FRN.

The FRN is topographically and morphologically similar to a negative brain potential that is elicited when participants commit an error and which has been dubbed the error-related negativity (ERN) (Falkenstein *et al.*, 1991, Gehring *et al.*, 1993). Both the FRN and ERN have been localized to the anterior cingulate cortex (ACC) (Dehaene *et al.*, 1994, Ridderinkhof *et al.*, 2004), which is part of a network that is activated when performance outcome is worse than expected (Holroyd and Coles, 2002). It has been proposed that the FRN and ERN index a prediction error signal. When an error is committed, the mesencephalic dopamine system is thought to send a negative reinforcement learning signal to the frontal cortex that disinhibits the apical dendrites in the ACC (reinforcement learning theory; Holroyd and Coles, 2002). An alternative view is the 'conflict theory' (Carter *et al.*, 1998, Yeung *et al.*, 2004) suggesting that the ACC detects conflict during response selection which is reflected by the ERN. Within this framework, however, the FRN is difficult to interpret.

There is good evidence that regions within the ACC are implicated in the control of autonomic functioning (Critchley *et al.*, 2000, Critchley *et al.*, 2003, Devinsky *et al.*, 1995, Gianaros *et al.*, 2004). This prompted investigators to examine the cardiac concomitants of feedback and error processing. The results emerging from these studies indicated that errors and negative feedback are associated with heart rate slowing (Crone *et al.*, 2003, Groen *et al.*, 2007, Hajcak *et al.*, 2003b, Somsen *et al.*, 2000, Van der Veen *et al.*, 2004a, Van der Veen *et al.*, 2004b). The heart rate slowing is elicited under conditions that are similar to those that give rise to the ERN and FRN. Moreover, some studies examining both brain potential and heart rate data reported positive correlations between brain potential amplitudes and heart rate slowing (Groen *et al.*, 2007, but see e.g., Hajcak *et al.*, 2003b). Accordingly, several investigators interpreted the heart rate slowing associated with error and feedback processing as an autonomic index of the same mechanism that is

reflected at the cortical level by the ERN and FRN (e.g., Crone *et al.*, 2005, Groen *et al.*, 2007, Jennings and van der Molen, 2002).

The interpretation that heart rate slowing associated with negative feedback reflects a mechanism that is activated when 'outcomes are worse than expected' has been challenged by findings reported in a previous study using the Miltner *et al.* (1997) time-estimation paradigm (Van der Veen *et al.*, 2004b). In this study, participants performed the time-estimation task twice. The first time, the time-estimation task was identical to the procedures used in the Miltner *et al.* study. Participants were required to press a button with a one second delay following the prompt, and the time window for correct estimates was dynamically adjusted to ensure equal probabilities of positive and negative feedback. The performance results showed that participants processed the information provided by the feedback. That is, the probability that participants adjusted their estimates on the subsequent trial in accordance with the feedback was significantly higher than chance. As anticipated, negative feedback was associated with a slowing of heart rate relative to the cardiac response elicited by positive feedback. The second time, however, the participants performed the task under yoked-control conditions. That is, each participant received the series of events that was recorded during the first time he or she performed the task. Thus, in the yoked-control condition, the participant received positive and negative feedback, as in the experimental condition, but in contrast to the experimental condition, the feedback was unrelated to the participant's time estimates. This time, the probability of correct adjustments following feedback was basically at chance level. Heart rate, however, continued to slow following negative feedback relative to positive feedback. This finding seems to suggest that heart rate is primarily sensitive to the valence (positive vs. negative) of the feedback rather than the information provided by it (cf. Van der Veen *et al.*, 2004b).

The main goal of the present study was to re-examine the relation between feedback processing and heart rate. Valence and information value are highly correlated in virtually all paradigms used to assess feedback-related heart rate changes: positive and negative feedback are either always valid or always invalid, or it is unclear to what extent one can rely on the valence of the feedback. In the current study, however, the validity of the feedback was manipulated in an attempt at disentangling its valence and information value. Thus, the feedback provided to the participant signaled that it was valid or invalid. Valid feedback was related to actual performance and, thus, participants should use the information provided by it in order to adjust their estimates. Here, valence and information value are correlated. Invalid feedback, however, was not related to actual performance and signaled to the participants that they could ignore the information provided by it. The 'information' hypothesis would predict that heart rate would only slow after *valid* negative feedback, and not after *invalid* negative feedback. In contrast, the

'valence' hypothesis would predict that cardiac slowing would occur to both valid and invalid negative feedback.

In addition to heart rate measures, brain potentials were recorded to explore the sensitivity of the FRN to the validity manipulation. It was anticipated that, in line with the reinforcement learning theory, the FRN would be less pronounced or even absent to invalid feedback as those signals annihilate effectively the motivational significance of the feedback for performance adjustment. The same was expected for the feedback P3, a positive-going ERP component that is also thought to reflect the evaluation of feedback outcome (e.g., Wu and Zhou, 2009).

Another purpose of this study was to investigate the cardiac and electrophysiological manifestations of feedback processing vis-a-vis remedial action. Both the FRN and error-related cardiac slowing have been suggested to be related to remedial action (Hajcak *et al.*, 2003b, Van der Veen *et al.*, 2008, Van der Veen *et al.*, 2004b). In line with previous studies, increased cardiac slowing, a larger FRN response, and a larger feedback P3 were expected when valid negative feedback was followed by a correct adjustment.

Finally, the participants in the current study were recruited to serve as controls in a larger Depression study. Thus, the number of participants is much larger (n = 98) and they varied across a larger age range (22 – 76 years) than participants in most studies of performance and/or feedback monitoring. Since our focus is not on age-related changes, the factor age will be included as covariate in all relevant analyses. In addition, we will explore general age-related trends in time estimation-performance, FRN, and heart rate responses to feedback processing. This information will add to the relatively small number of studies using only two extreme groups (i.e., young adults vs. a group of elderly participants) to assess age-related changes in the psychophysiology of performance and feedback processing.

Methods

Participants

Data were obtained from 98 participants between 22 and 76 years old, who were recruited from the hospital (mainly staff) and the medical and psychology faculties by means of advertisements. Demographic characteristics are presented in Table 2.1. Level of education was average to high. Overall, younger participants were more highly educated than older participants. All participants gave written informed consent and the study was approved by the local medical ethics committee. Participants received EUR 25 for participation.

Exclusion criteria were: neurological illness, a history of major head injury, stroke or heart attack, current severe somatic illness, a history of or current psychiatric illness, a first-degree relative with depressive disorder, substance use in the past three months, use of medication which affects the central nervous system (including beta blocking agents), and pregnancy. Two participants had hypertension, for which they both took an ACE-inhibitor and one of them additionally received diuretic medication. Health was assessed by means of a self-developed questionnaire. A history of and current psychiatric illness was assessed by means of the Structured Clinical Interview for DSM-IV Axis I disorders (SCID-I).

Table 2.1 Demographic characteristics

		N	*Mean age (range)*
Total		98	51.7 (22-76)
Gender	male	25	55.3 (40-72)
	female	73	50.4 (22-76)
Level of education*	low	5	65.2 (58-73)
	average	47	54.4 (27-76)
	high	46	47.3 (22-72)

* Level of education was divided into three groups (see Van der Elst *et al.*, 2008). The levels refer to participants with only primary education (low), those with at most junior vocational training or high school (average) and those with at most senior vocational or academic training (high).

Time-estimation task

The time-estimation task was based on the paradigm developed by Miltner *et al.* (1997). Modified versions of this paradigm have been used before (Holroyd and Krigolson, 2007, Nieuwenhuis *et al.*, 2005b, Van der Veen *et al.*, 2008, Van der Veen *et al.*, 2004b, Wild-Wall *et al.*, 2009). Participants were instructed to produce a one-second interval. The stimulus sequence consisted of the following: 1) an asterisk, which functioned as a fixation stimulus and was presented for two seconds; 2) a question mark, which cued the estimation (i.e., the estimation prompt); 3) a second asterisk following the estimation, which was presented for one second; and 4) the feedback stimulus, which was presented for one second (see Figure 2.1).

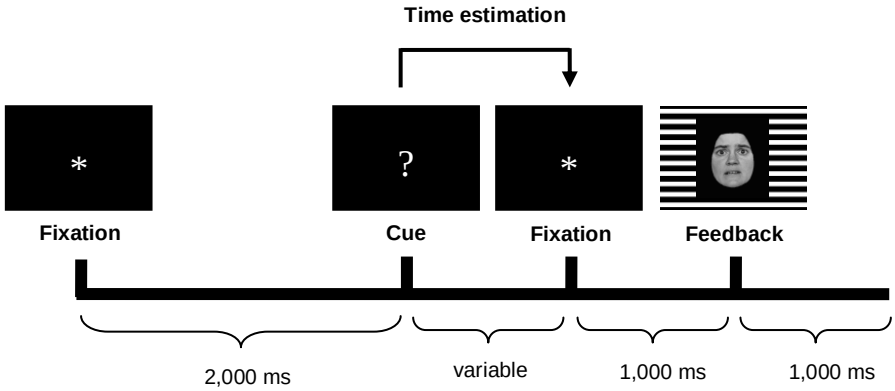

Figure 2.1 Trial sequence with an example of the feedback stimulus. Happy facial expressions indicated positive feedback, fearful expressions indicated negative feedback. The gender (male/female) of the face indicated whether the estimation was too short or too long (counterbalanced across participants). The background grid (horizontal/vertical) indicated whether feedback was valid or invalid (counterbalanced across participants). See text for further details.

Participants had to indicate the end of the one-second interval by pressing the button of the response device. Following the button press, they received performance feedback, i.e., positive feedback if their response occurred within a specified window around the target, and negative feedback if the response occurred outside the window. Unbeknownst to participants, the window was dynamically adjusted to ensure an equal amount of positive and negative feedback stimuli. After a correct estimation, the window in the subsequent trial was shortened by a fixed amount of time (20 ms). After an incorrect estimation, it was lengthened by the same amount in the subsequent trial (see also Miltner *et al.*, 1997).

Estimates were followed by feedback. The feedback consisted of face stimuli presented against a horizontal or vertical background grid. The background grid communicated the validity of the feedback stimulus to the participants (valid vs. invalid). Valid feedback was based on the participant's performance. Invalid feedback was determined randomly by the computer, with a maximum of three invalid feedback trials in a row. Participants received invalid feedback in 50% of the trials. The emotional expression of the face informed participants that their estimate was correct or incorrect (respectively, a happy vs. a fearful face). Finally, in case of incorrect estimates, the gender of the face indicated whether the estimate was too short (e.g., a male face) or too long

(e.g., a female face). The faces used in this study were from the Ekman & Friesen pictures set (Ekman and Friesen, 1978). Four versions of the task were counterbalanced across participants to correct for possible effects of the gender of the face stimuli (underestimation x male and overestimation x female, or vice versa) and background grid (valid x horizontal and invalid x vertical, or vice versa).

Procedure

Participants were seated approximately 1 m from a computer screen. After the electrodes had been placed for the EEG and ECG recordings, the participants were instructed on how to perform on the time-estimation task. Then they received 24 practice trials, each lasting about five seconds in total. Trials were presented in four blocks of 120 stimuli (about 10 minutes). Total task duration was about 40 minutes (480 stimuli). After each experimental block, participants had a short self-paced break. EEG and ECG measurements including electrode attachments, task instructions and breaks took about 1.5 hours in total.

Participants were asked to abstain from coffee and tobacco for at least two hours before the measurements.

Cardiac measures

The ECG was derived from two electrodes, one placed below the right clavicle and one below the lowest left rib. A ground electrode was placed on the sternum. ECG, EEG, and EOG were recorded using a Vitaport 3 recorder (Temec Instruments BV, Kerkrade, the Netherlands). The ECG was sampled at 512 Hz, low-pass filtered at 70 Hz, and high-pass filtered with a time constant of 0.33 s. Electrode impedance was kept below 8 kΩ.

Data were analyzed using locally developed software which was implemented in Vitascore (Temec Instruments BV, Kerkrade, the Netherlands). The R-peak occurrence times were detected with an accuracy of two milliseconds, and stored offline. The R-peak occurrence times were manually checked for artifacts and corrected when necessary. Five inter-beat intervals (IBIs) surrounding the feedback stimulus were selected for further analysis: i.e., the preceding IBI (IBI -1), the concurrent IBI (i.e., IBI 0), and three subsequent IBIs (i.e., IBIs 1, 2, and 3). IBI -1 was subtracted from the average of the other four IBIs, resulting in a mean cardiac change score for each condition.

Electrophysiological measures

The EEG was derived from five electrodes placed at Fz, Cz, Pz, C3 and C4 according to the 10-20 system (Sharbrough *et al.*, 1991). Linked mastoids were used as a reference. The electro-oculogram (EOG) was derived from four electrodes, one placed above and one below the right eye, and one each on the outer canthus of each eye. The electrode placed on the sternum was used as a ground electrode for both the ECG and EEG. The EEG was

sampled at 256 Hz, low-pass filtered at 30 Hz, and high-pass filtered with a time constant of 0.33 s. Electrode impedance was again kept below 8 kΩ.

Event-related potentials (ERPs) were locked to the onset of the feedback stimulus, and epochs were extracted between 100 ms preceding and 700 ms following feedback onset. The method of Gratton *et al.* (1983) was used to correct EEG traces for vertical EOG only[1] (criteria for blinks: EOG signal exceeding 40 microvolt within a 20 ms time interval). Epochs were manually checked for artifacts (e.g., noise) and excluded from analysis when necessary.

Each ERP was baseline-corrected by averaging the first 100 ms before feedback onset and subtracting this average from the ERP.

Statistical analyses

The percentage of correct adjustments to valid negative feedback was compared with the percentage of 'correct' adjustments to invalid feedback by means of repeated-measures analyses. Adjustments were considered 'correct' whenever a negative feedback stimulus indicating that the estimate was too short or too long was followed by, respectively, a lengthening or shortening of the time estimate on the subsequent trial. Adjustments were considered 'incorrect' when negative feedback was followed by a lengthening or shortening of the time estimate, while the feedback stimulus indicated that the time estimate was too long or too short, respectively.

The mean cardiac change scores, defined as the average of IBI 0, IBI 1, IBI 2 and IBI 3 minus IBI -1, were used for the cardiac analyses. Valence (positive vs. negative feedback) and validity (valid vs. invalid feedback) were used as within-subjects factors in a repeated-measures analysis.

To define the FRN, difference waves were created by subtracting the ERPs associated with positive feedback from the ERPs associated with negative feedback (Holroyd and Krigolson, 2007, Holroyd *et al.*, 2009). This was done separately for the valid and invalid condition. For each participant and each channel, the most negative deflection (peak amplitude) within 200 and 350 ms following feedback onset was measured. ERP data were analyzed by using channel and validity as within-subjects factors in a repeated-measures analysis. To investigate the effect of valence, i.e., whether the negative deflection was significantly different from zero, we used one-sample t-tests for each channel separately for both valid and invalid feedback.

We conducted an additional ERP analysis in which we focused on the 350 – 500 ms time window. Most likely, this window captures the feedback P3 (e.g., Mathewson *et al.*,

[1] We did not correct for horizontal EOG because all stimuli were presented in the centre of the screen, and therefore few eye movements were made.

2008, Wu and Zhou, 2009). For each of the four feedback conditions, we calculated the mean amplitude of the baseline-corrected ERP waveforms in this time window. Valence, validity and channel were used as within-subjects factors in a repeated-measures analysis.

We additionally examined cardiac and electrophysiological responses to valid negative feedback stimuli that were followed by correct adjustments and those followed by incorrect adjustments. For the cardiac data type of adjustment (correct vs. incorrect) and valence were used as within-subjects factors in a repeated-measures analysis. For the FRN, difference waves were constructed by subtracting the ERPs associated with valid positive feedback from the ERPs associated with valid negative feedback followed by correct adjustments vs. incorrect adjustments. Type of adjustment and channel were used as within-subjects factors in a repeated-measures analysis. For the feedback P3, we used the mean amplitude of the ERP waveforms within the 350-500 ms window for valid negative feedback followed by correct adjustments vs. incorrect adjustments. Type of adjustment, valence, and channel were used as within-subjects factors in a repeated-measures analysis.

We included age as a covariate in all analyses to investigate if there were effects of age on the dependent variables. Because of a large sum-of-squared error resulting from the addition of age as a covariate in the analyses, age was centred using the method of Delaney and Maxwell (1981); i.e., age minus mean age of all participants (Delaney and Maxwell, 1981, Thomas *et al.*, 2009). When necessary, the degrees of freedom were adjusted using the Huynh-Feldt correction procedure, but uncorrected degrees of freedom are reported. Partial eta squared was reported as measure for effect size in the results of repeated-measures ANCOVAs. Finally, we will report age-related trends in time-estimation and feedback processing.

Results

Due to recording problems and incomplete data, the data of four participants were excluded from analyses. One further participant dropped out of the study because of an allergic reaction to the electrode paste.

A box-plot analysis revealed eight participants who produced extreme time estimates (>1408 or <539 ms, extremes defined by a stem-and-leaf plot). These participants were therefore excluded from analyses. The EEG data of another participant showed too many artifacts; this individual was excluded. The data of the remaining 84 participants (63 female), aged between 22 and 76 years (mean = 51), were used for the analyses. With respect to gender distribution, age and level of education, the 14 participants whose data were not used were similar to the others.

Behavioural response

Participants received slightly more negative feedback (53%) than positive feedback (47%) ($t(83) = 14.8$, $p < 0.001$). Mean estimation time of the 84 participants was 1058 ±110 milliseconds (average ± inter-individual SD). Age did not correlate with mean estimation time ($r = 0.11$, $p = 0.33$) or mean intra-individual standard deviation of estimation time ($r = 0.04$, $p = 0.74$).

As expected, participants adjusted their estimations more frequently to valid negative feedback than they did to invalid negative feedback ($F(1,82) = 837.0$, $p < 0.001$, partial $\eta^2 = 0.91$). Age was not associated with this difference ($F(1,82) = 2.5$, $p = 0.115$, partial $\eta^2 = 0.03$). After valid negative feedback, correct adjustments were made in an average of 77.5% ±7.0 of the trials. This was significantly higher than chance level (50%) ($t(83) = 36.0$, $p < 0.001$). After invalid negative feedback, participants made 'correct' adjustments in only 51.8% ±5.7 of the trials, but, statistically, this was also significantly higher than chance level ($t(83) = 2.8$, $p = 0.006$). The current data pattern suggests that participants processed the validity of the feedback and undertook remedial action depending on the information provided by the valid feedback.

Cardiac response to feedback

Figure 2.2A shows the cardiac response to positive and negative feedback in the valid and the invalid conditions. It can be seen that heart rate slowed towards the presentation of the feedback stimulus that was presented during IBI 0. The cardiac slowing was followed by an acceleratory recovery that was delayed for valid negative feedback relative to valid positive feedback (main effect of valence: $F(1,82) = 9.9$, $p = 0.002$, partial $\eta^2 = 0.11$). The cardiac response did not discriminate between negative and positive feedback when the feedback was invalid (see Figure 2.2B). This was supported by a significant interaction between valence and validity ($F(1,82) = 5.6$, $p = 0.021$, partial $\eta^2 = 0.06$). The analysis also yielded an interaction between age and valence ($F(1,82) = 5.0$, $p = 0.028$, partial $\eta^2 = 0.06$): the difference in mean cardiac change between positive and negative feedback decreased with advancing age (see Figure 2.4A). To summarize, in line with previous studies, the current data showed heart rate slowing to negative feedback relative to positive feedback. Importantly, this effect of feedback was present only when feedback was valid, not when it was invalid.

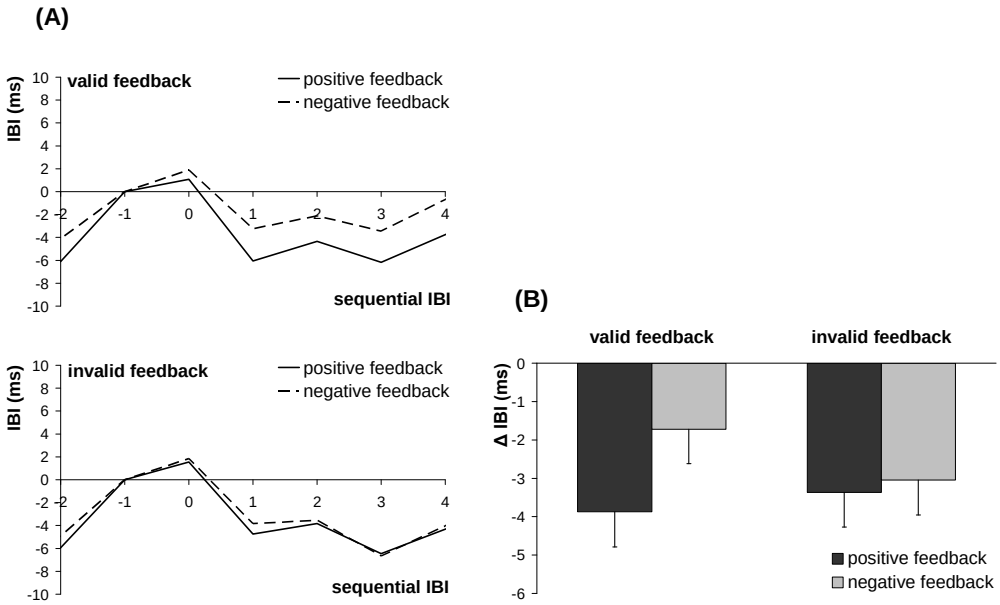

Figure 2.2 (A) Cardiac response to positive and negative feedback in the valid (upper panel) and invalid condition (bottom panel) using IBI -1 as baseline. (B) The mean cardiac change in milliseconds (average of IBI 0, IBI 1, IBI 2 and IBI 3 minus IBI -1) for the four feedback conditions with error bars (SEM).

Electrophysiological response to feedback

Figure 2.3A shows the grand-average ERPs for valid feedback, and Figure 2.3B shows the difference waves (negative - positive feedback) for the valid and invalid conditions at channels Fz, Cz and Pz. The focus of the analyses was on the data in the 200 – 350 ms window; the time interval that is typically used to assess the FRN. For this time window, the repeated-measures ANOVA with validity and channel as within-subjects factors, and age as covariate yielded a main effect of channel ($F(1,82) = 4.5$, $p = 0.003$, partial $\eta^2 = 0.05$). The maximum amplitude of the difference wave was largest at Fz (-2.1±1.5μV for valid feedback; -2.2±1.7μV for invalid feedback). To compare, the amplitudes were -2.0±1.3μV, and -2.0±1.6μV at Cz, and -1.9±1.4μV, and -1.7±1.4μV at Pz. This pattern is in line with previous studies showing larger amplitudes at fronto-central recording sites than at the parietal site. Separate one-sample t-tests confirmed that the maximum amplitudes of the difference waves were significantly different from zero, indicating an effect of valence, i.e., an FRN-response (all p's < 0.001). Furthermore, at Fz, the FRN decreased with advancing age, as reflected by an interaction between channel and age in the repeated-measures ANCOVA ($F(1,82) = 3.7$, $p = 0.008$, partial $\eta^2 = 0.04$, see Figure 2.4B).

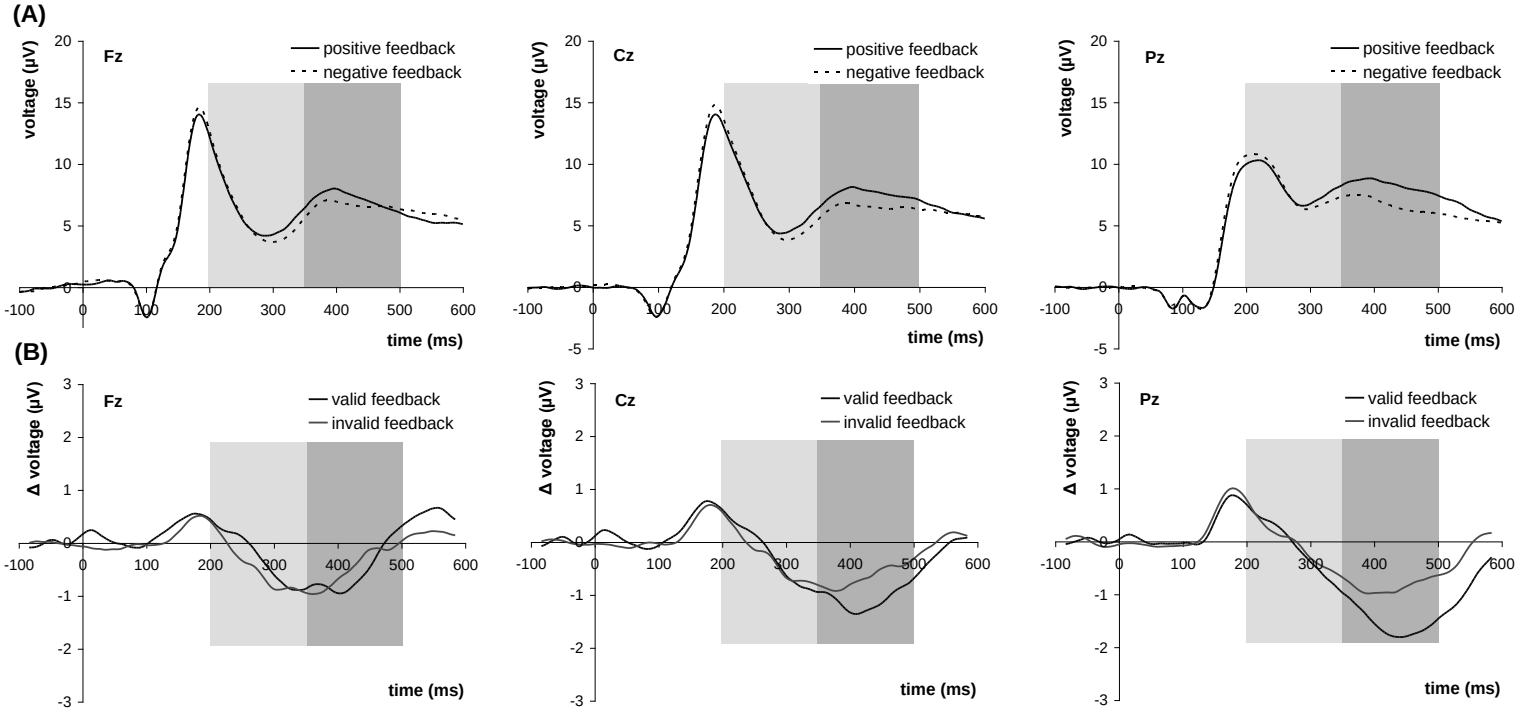

Figure 2.3 (A) Grand-average event-related brain-potentials from the frontal (Fz), central (Cz) and posterior electrode (Pz) evoked by positive and negative feedback in the valid condition only. (B) The difference waves (negative minus positive feedback) for valid and invalid feedback. The light grey area indicates the time window in which the FRN was measured, and the dark grey area indicates the time window in which the feedback P3 was measured.

No effect of task-validity was found on the FRN, but Figure 2.3B suggests that an effect of validity occurred at posterior-central sites at around 400 ms after feedback onset. We conducted a repeated-measures ANOVA with valence, validity, and channel as within-subjects factors and age as covariate on the time-window in which the feedback P3 is thought to occur (350 - 500 ms). As expected, this analysis showed that, in addition to a main effect of valence ($F(1,82) = 56.9$, $p < 0.001$, partial $\eta^2 = 0.41$), task-validity interacted with valence ($F(1,82) = 4.1$, $p = 0.045$, partial $\eta^2 = 0.05$), indicating that the difference between positive and negative feedback was larger in the valid feedback condition than in the invalid condition (post-hoc analyses on valid and invalid feedback separately both showed a main effect of valence, but the effect was stronger in the valid condition, as shown in Figure 2.3B; valid feedback: $F(1,82) = 46.9$, $p < 0.001$, partial $\eta^2 = 0.36$; invalid feedback: $F(1,82) = 22.6$, $p < 0.001$, partial $\eta^2 = 0.22$). This effect was most pronounced at posterior-central recording sites, as indicated by a three-way interaction between validity, valence and channel ($F(1,82) = 5.7$, $p = 0.001$, partial $\eta^2 = 0.07$), and post-hoc analyses on each channel separately (interaction between validity and valence: Fz: $F(1,82) = 0.2$, $p = 0.63$, partial $\eta^2 = 0.003$; Cz: $F(1,82) = 4.3$, $p = 0.041$, partial $\eta^2 = 0.05$; Pz: $F(1,82) = 13.8$, $p < 0.001$, partial $\eta^2 = 0.14$). Age interacted with validity ($F(1,82) = 6.8$, $p = 0.011$, partial $\eta^2 = 0.08$), and with both validity and channel ($F(1,82) = 3.5$, $p = 0.014$, partial $\eta^2 = 0.04$). With advancing age, the effect of validity decreased, which was most pronounced at the fronto-central recording sites, but not at Pz (interaction between age and validity in separate post-hoc tests: Fz: $F(1,82) = 11.6$, $p = 0.001$, partial $\eta^2 = 0.12$; Cz: $F(1,82) = 6.4$, $p = 0.014$, partial $\eta^2 = 0.07$; Pz: $F(1,82) = 3.0$, $p = 0.087$, partial $\eta^2 = 0.04$). Finally, feedback P3 amplitudes increased with advancing age, independent of valence, validity, and channel (main effect of age: $F(1,82) = 9.7$, $p = 0.003$, partial $\eta^2 = 0.11$). To summarize, the FRN, observed within the 200 – 350 ms window following feedback onset, was maximal at fronto-central channels but did not respond to feedback validity. The feedback P3, on the other hand, observed within the 350 - 500 ms window, was maximal at posterior-central channels, and responded to both valence and validity[2].

[2] We additionally investigated the association between the cardiac and ERP measures. The cardiac response to valid positive and negative feedback was neither correlated with the FRN measured at Fz ($r = 0.14$, $p = 0.20$), nor with the feedback P3 measured at Pz ($r = 0.08$, $p = 0.48$ for positive feedback, and $r = 0.12$, $p = 0.30$ for negative feedback). To compare the cardiac response with the FRN, a difference score was calculated.

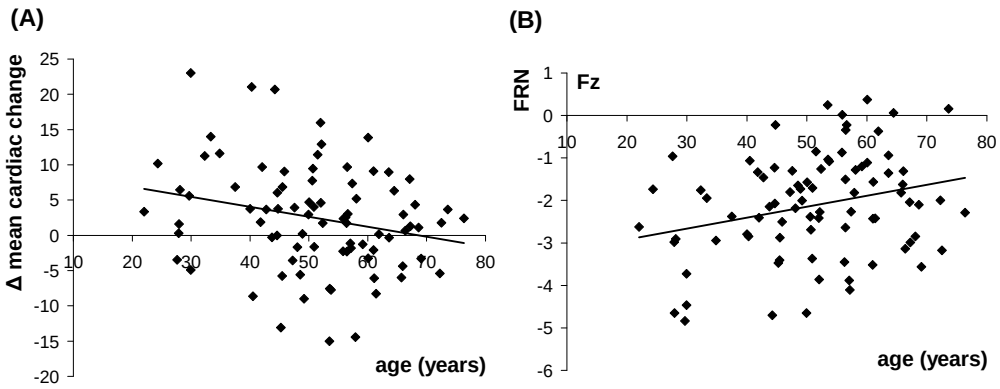

Figure 2.4 (A) Difference in mean cardiac change (valid negative + invalid negative feedback) minus (valid positive + invalid positive feedback) as a function of age. (B) The FRN measured as the peak negativity within 200 and 350 ms following feedback onset (average of valid and invalid feedback) measured at channel Fz as a function of age.

Cardiac and electrophysiological changes associated with correct versus incorrect adjustments

The cardiac response in relation to remedial action in valid negative feedback trials followed by a correct behavioural adjustment showed a more pronounced slowing than in trials followed by an incorrect adjustment ($F(1,82) = 5.5$, $p = 0.021$, partial $\eta^2 = 0.06$). Thus, acceleratory recovery was delayed for valid negative feedback followed by a correct adjustment compared to incorrect adjustment, as shown in Figure 2.5A. This finding was not altered by age.

The brain potentials for valid positive feedback, valid negative feedback associated with correct adjustments, and valid negative feedback associated with incorrect adjustments are presented in Figure 2.5B and 2.5C. In contrast to expectations, the FRN associated with correct adjustments was smaller than the FRN associated with incorrect adjustments ($F(1,82) = 48.3$, $p < 0.001$, partial $\eta^2 = 0.37$). The feedback P3, on the other hand, was larger for valid negative feedback followed by a correct adjustment than for valid negative feedback followed by an incorrect adjustment ($F(1,82) = 4.5$, $p = 0.036$, partial $\eta^2 = 0.05$).

Similar analyses were done on the invalid feedback data. The results of these data failed to show a significant relation between remedial action and cardiac slowing, the FRN, and the feedback P3. This negative result is consistent with the assumption that participants processed the validity of the feedback stimulus. To summarize, both heart rate and ERPs were associated with remedial action, albeit in opposite direction.

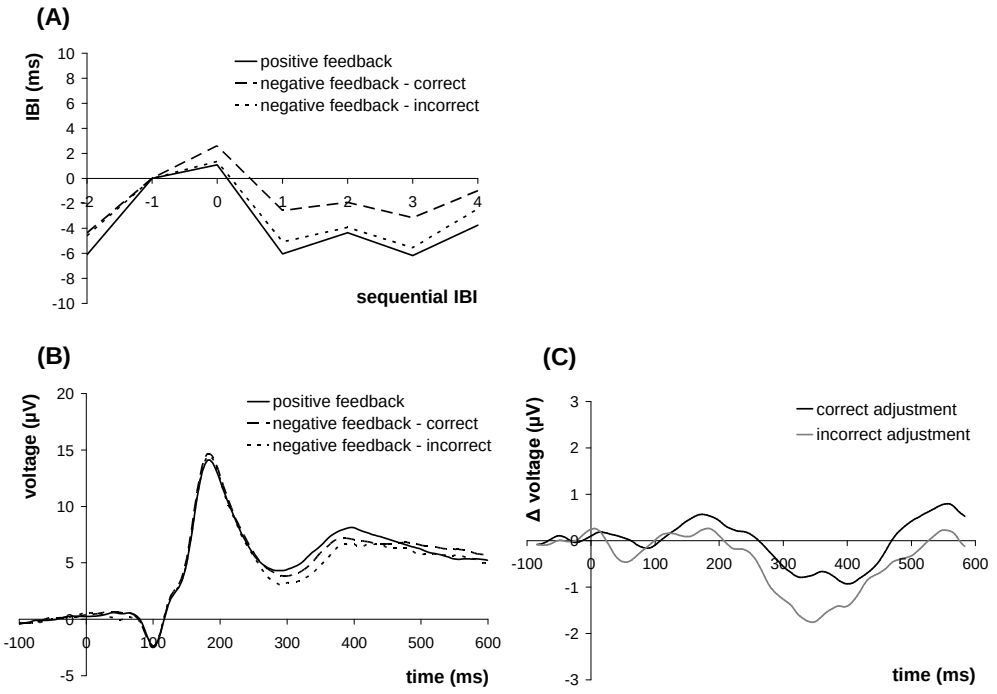

Figure 2.5 (A) Cardiac response to valid negative feedback followed by a correct adjustment, to valid negative feedback followed by an incorrect adjustment, and to valid positive feedback. (B) Grand-average electrophysiological response (measured at Fz) to valid negative feedback followed by a correct adjustment, to valid negative feedback followed by an incorrect adjustment, and to valid positive feedback. (C) The corresponding difference waves: negative feedback followed by a correct adjustment minus positive feedback; and negative feedback followed by an incorrect adjustment minus positive feedback.

Discussion

This study examined brain potential and cardiac responses to performance feedback in a time-estimation task adopted from Miltner *et al.* (1997) and revised by Van der Veen *et al.* (2004b). Miltner *et al.* required their participants to estimate 1s intervals, and a feedback stimulus informed them whether their estimate was correct or incorrect. Their results showed that participants used the feedback provided to them for guiding their estimates. That is, participants changed their estimates more following negative compared to positive feedback. Van der Veen *et al.* obtained similar findings using a slightly revised version of the Miltner *et al.* paradigm. Their participants received more specific feedback.

That is, a '■' sign indicated that the estimate was correct, a '+' indicated that the estimate was too long, and a '-' sign informed participants that their estimate was too short.

As in the Miltner *et al.* study, participants used the feedback to guide their estimates; the proportion of correct adjustments following feedback was much greater than chance (83%). The current performance results largely corroborated the results of previous reports (see also Holroyd and Krigolson, 2007, Mars *et al.*, 2004, Van der Veen *et al.*, 2008). The proportion of correct adjustments following negative feedback was 78%. The current findings extend previous results by showing that the proportion of correct adjustments depends on the validity of the feedback signal. Thus, the proportion of correct adjustments to *invalid* negative feedback was 52%, close to chance level. The latter finding is important, in that it indicates that participants processed the validity of the feedback provided to them.

Interestingly, time-estimation was not sensitive to advancing age. Previously, Wild-Wall *et al.* (2009) observed that the proportion of correct estimates was lower in older (57.5 years) compared to younger (23.7 years) participants; respectively, 42% vs. 51%. It should be noted, however, that Wild-Wall *et al.* employed a fixed time-estimation window of 200 ms whereas we used a window that was dynamically adjusted to the estimates of individual participants. The definition of a correct estimate was therefore more rigid in the Wild-Wall *et al.* study than in ours, which makes their task more sensitive to detect age-related differences. Moreover, in line with our results, the mean estimation time in the Wild-Wall *et al.* study did not discriminate between the two age groups.

The primary goal of the current study was to re-examine the feedback validity effect on the cardiac response. In a series of studies, we observed that negative feedback is consistently associated with a transient heart rate slowing (Crone *et al.*, 2003, Somsen *et al.*, 2000, Van der Veen *et al.*, 2004a, Van der Veen *et al.*, 2004b, see also Groen *et al.*, 2007, Hajcak *et al.*, 2003b, Luman *et al.*, 2007). The current findings extend our previous results in showing that the cardiac response to feedback depends crucially on the validity of this feedback. That is, valid, but not invalid feedback resulted in a transient heart rate slowing. In a previous time-estimation study, we observed transient heart rate slowing to both valid and invalid negative feedback (Van der Veen *et al.*, 2004b). The critical difference between the current and previous study is that in the current study participants were informed about feedback validity whereas in the previous study this information was implicit. Thus, there is a fair chance that participants continued to process the feedback as if it was valid, while it was not. Consistent with the results of previous studies (Van der Veen *et al.*, 2008, Van der Veen *et al.*, 2004b), the current findings showed a relation between the cardiac responses to valid but not invalid feedback and remedial action. That is, transient slowing to valid negative feedback was more pronounced for correct compared to incorrect adjustments. Finally, the difference in cardiac response to positive

and negative feedback decreased with advancing age. It has been suggested that the transient heart rate slowing to negative feedback is a vagally mediated response of a performance monitoring system (e.g., Jennings and van der Molen, 2002). Furthermore, it is known that, as people age, the heart receives less input from the parasympathetic branch (vagus nerve) of the autonomic nervous system (e.g., Ai *et al.*, 2007). Thus, the attenuated difference in cardiac response to positive and negative feedback in the elderly is most likely due to reduced parasympathetic control of the heart (De Meersman and Stein, 2007, Kaye and Esler, 2008).

The current electrophysiological data showed an FRN that was larger over fronto-central sites relative to the parietal electrode position. The amplitude of the FRN was smaller than in comparable reports (e.g., Donkers *et al.*, 2005, Holroyd and Krigolson, 2007, Van der Veen *et al.*, 2008). One possible reason for the current attenuated FRN refers to the sample of participants. The age range in the current study was from 22 to 76 years and several aging studies showed a decrease in the FRN with advancing age (e.g., Eppinger *et al.*, 2008, Mathewson *et al.*, 2008, Wild-Wall *et al.*, 2009). For example, in the Wild-Wall *et al.* time-estimation study, the amplitude of the FRN in the elderly was about half the size of the young adults' FRN. Another factor that might have contributed to the attenuated FRN refers to expectancy. The current time-estimation paradigm, using a dynamical adjustment of the target window, does not allow the building of strong expectancies. Moreover, by manipulating the validity of the feedback the possibility of building strong expectancies is reduced even further. Previously, it has been demonstrated that, in a time-estimation paradigm, expectancy exerts a strong influence on the FRN with a larger FRN when strong expectancies are being violated (Holroyd and Krigolson, 2007). Finally, the feedback stimuli in the current task were more complex than in most feedback tasks. It is, therefore, also possible that the processing of the feedback information induced more variability, which might have led to more variability in the latency of the FRN-response, and therefore to a smaller FRN. In line with this, it has been observed that the FRN is smaller when more information is provided by the feedback. Mars *et al.* (2004) observed that the FRN is smaller when the feedback conveys information not only about the correctness of the time-estimate, but also whether the estimate was too long or too short.

One of the main goals of the current study was to examine the effect of feedback validity on the FRN. The results showed that both valid and invalid feedback elicited an FRN. This finding suggests that the FRN is not sensitive to feedback validity, which does not support the reinforcement learning theory. At first glance, this finding seems at odds with previous results reported by Nieuwenhuis *et al.* (2005b). In their time-estimation study, participants received feedback indicating that their estimates were correct or incorrect (informative feedback) and, in addition, they received feedback that did not

provide information concerning their estimates (uninformative feedback). The results that emerged revealed that the brain potential associated with uninformative feedback was markedly different from those elicited by informative feedback. It should be noted, however, that the uninformative feedback in the Nieuwenhuis *et al.* study was different from the invalid feedback in the current study, in that the uninformative feedback consisted of a single question mark whereas the invalid feedback in our study communicated valence (i.e., positive or negative) in addition to the information that valence was not related to the participant's estimate. Because the amount of feedback information that needed to be processed in the current study was larger than in most tasks, it is also possible that the validity information was not completely processed in time to affect the FRN. The effect of task-validity was perhaps postponed to a later time window, i.e., that of the feedback P3. The current finding suggesting that the FRN is not sensitive to feedback validity is consistent with the results reported recently by Ohira *et al.* (2010). In that study, participants performed on two versions of a decision-task; a contingent-feedback version in which participants received information regarding the correctness of their decisions and a random-feedback version in which the information provided by the feedback was unrelated to their decisions. Consistent with the present findings, the results of Ohira *et al.* showed that the random feedback elicited a sizeable FRN. Their finding, provided that the participants learned that the random feedback was irrelevant, and our results are consistent with the hypothesis that the systems underlying the generation of the FRN are primarily sensitive to the valence communicated by the feedback rather than the information regarding the quality of performance.

It should be noted, however, that the 'valence' hypothesis of the FRN is challenged by the current observation that the FRN to valid but not invalid feedback is related to remedial action. This finding does seem to suggest that the FRN is sensitive to feedback validity. At this point, we don't have a unified explanation for the finding that (a) feedback validity does not seem to affect the FRN and (b) the valid but not invalid FRN is associated with remedial action. The weight of the latter finding is difficult to assess. First, based on the ERN and FRN literature, we anticipated that the FRN would be larger for correct than incorrect adjustments (e.g., Cohen and Ranganath, 2007, Coles *et al.*, 1995, Gehring *et al.*, 1993, Gentsch *et al.*, 2009, but see also Endrass *et al.*, 2007, Nieuwenhuis *et al.*, 2001). However, the current results showed the opposite pattern. Secondly, the typical finding in time-estimation studies is that the size of the FRN is not related to remedial action (e.g., Mars *et al.*, 2004, Miltner *et al.*, 1997). The Holroyd and Krigolson (2007) study is a notable exception, but in this time-estimation study the relation between the FRN and remedial action was mediated significantly by the expectedness of the feedback. Given the inconsistencies in the time-estimation FRN literature, we are reluctant to interpret our current finding before its replication.

In contrast to the FRN, the feedback P3 was clearly sensitive to task-validity. The feedback P3 is thought to reflect the evaluation of the significance of the feedback stimulus (e.g., Wu and Zhou, 2009), and is known to be influenced by several factors such as the emotional value of the stimulus, and the amount of attention paid to a stimulus (Nieuwenhuis *et al.*, 2005a). Invalid feedback had no motivational significance for our participants, probably leading to a smaller difference between positive and negative feedback than in the valid condition. This effect did not diminish with advancing age at the posterior recording site, which is in line with other studies showing that the locus coeruleus-norepinephrine system, which is thought to underlie the P3-response, does not decline with age the way the dopamine system underlying the FRN-response does (Mathewson *et al.*, 2008, Nieuwenhuis *et al.*, 2005a, Nieuwenhuis *et al.*, 2005b). Furthermore, in line with expectations, the feedback P3 was larger when valid negative feedback was followed by a correct adjustment than when it was followed by an incorrect adjustment. Although this implies that more attention was being paid to feedback that was used for performance adjustment, we are reluctant to interpret this finding as prove that the feedback P3 is related to remedial action, because it appears to be an effect that is strongly influenced by the association between the FRN and remedial action (see also Figure 2.5B).

In conclusion, the current study yielded straightforward heart rate findings using a modified time-estimation paradigm. Heart rate slowed to negative feedback but only for valid feedback. This transient delay in heart beat timing was more pronounced when the feedback was followed by correct adjustments but, again, only when the feedback was valid. This pattern of findings is inconsistent with our previous proposal suggesting that heart rate is primarily sensitive to the valence of the feedback rather than to the information provided by it (Van der Veen *et al.*, 2004b). The current findings indicate that the mechanisms underlying the cardiac response to feedback are influenced by the processing of both the validity of the feedback and by the specific information regarding the time estimate (correct vs. too long or too short). In this regard, the transient heart rate slowing to negative valid feedback can be conceptualized in terms of the cardiac manifestation of a prediction error signaling that performance is falling short of expectations and guiding the specific actions required for remedial actions. There is good evidence that the feedback-related heart rate slowing is mediated by the parasympathetic nervous system (e.g., Dywan *et al.*, 2008, Hajcak *et al.*, 2003b, 2004), which is under top-down control of a prefrontal-striatal network, most notably the rostral portion of the ACC (Ohira *et al.*, 2010).

The results regarding the FRN were less clear. The results showed that the FRN was not directly influenced by the validity of the feedback. Together with the findings of several other time-estimation studies (e.g., Mars *et al.*, 2004, Miltner *et al.*, 1997), this

finding seems to suggest that the FRN observed using this paradigm reflects an error prediction signal that acts as an 'alert' (e.g., Mars *et al.*, 2004). That is, it signals the need for action but it is ignorant of the specific actions that are needed (but see Holroyd and Krigolson, 2007). This interpretation is challenged by our observation of an association between the FRN and remedial action to valid feedback. However, future studies should determine its robustness before crediting weight to this atypical finding; the FRN was smaller not larger when followed by correct adjustments.

The results regarding the feedback P3, were, on the other hand, in line with the cardiac results; both measures were sensitive to task-validity. This is possibly due to a common neural substrate. Several brain regions have been found involved in the generation of the feedback P3, one of them being the ACC (e.g., De Pascalis *et al.*, 2010). It is therefore feasible that the part of the ACC that influences autonomic activity also contributes to the feedback P3. The FRN, on the other hand, which is thought to be a reflection of the same mechanism responsible for the cardiac slowing in response to feedback, is not sensitive to validity, and not similarly related to remedial action. The temporal difference of the measures might underlie this difference: the FRN is a fast response in comparison to the slower cardiac effect. It is also possible that the neural substrates of the FRN are different from those underlying the feedback P3 and the regulation of heart rate. We should note that the amount of cardiac slowing was unrelated to both the size of the FRN and the size of the feedback P3 (Footnote 2). Other studies have also reported a dissociation between the FRN/ERN and cardiac slowing (Hajcak *et al.*, 2003b, Van der Veen *et al.*, 2004a). In addition, in a previous time-estimation study we found that tryptophan depletion, a method to transiently lower central serotonin levels, also affected the two measures differently (Van der Veen *et al.*, 2008). This supports the idea that different neural substrates contribute to the different measures.

Finally, we examined age-related change in the cardiac and electrophysiological responses to feedback. The current results indicated that time-estimation performance did not change with advancing age. The FRN, on the other hand, decreased with advancing age. This finding is consistent with the results reported by Wild-Wall *et al.* (2009) and studies using other FRN paradigms (e.g., Eppinger *et al.*, 2008, Nieuwenhuis *et al.*, 2002, Pietschmann *et al.*, 2008). Similarly, the difference in cardiac response to positive and negative feedback decreased with advancing age. Thus, with regard to cognitive aging, the current findings point to dissociation between behavioural and electrophysiological indices of feedback monitoring. This dissociation has been noted previously (e.g., Falkenstein *et al.*, 2001) and several interpretations have been proposed to account for it (see for a review Pietschmann *et al.*, 2011). The current findings seem to be explained best

by assuming that the FRN and cardiac response to feedback both signal an 'alert', but only the cardiac response is implicated in the mechanisms invoked for remedial action.

Acknowledgements

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Chapter 3

The anterior cingulate cortex responds differently to the validity and valence of feedback in a time-estimation task

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Abstract

This study examined the role of the medial frontal cortex in the processing of valence and validity of performance feedback using a time-estimation paradigm. Participants had to produce 1s intervals followed by positive and negative feedback that could be valid or invalid (i.e., related or unrelated to task performance). Performance results showed that participants used the validity information to adjust their time estimations to negative feedback. The rostral cingulate zone (RCZ) was more active after valid feedback than after invalid feedback, but was insensitive to the valence of the feedback. The rostral anterior cingulate cortex (rACC), posterior cingulate and right superior frontal gyrus, however, appeared to be primarily sensitive to the valence of the feedback; being more active after positive feedback. The results are discussed along the lines of the ACC's cognitive and affective subdivisions and their structural and functional connections.

Introduction

Performance monitoring, i.e., the continuous assessment of ongoing actions and their consequences, is an important prerequisite for goal-directed behaviour. When a person detects an error or receives external negative feedback, subsequent behaviour should be adjusted. After errors, a negative-going event-related brain potential occurs, the so-called error-related negativity (ERN; e.g., Falkenstein *et al.*, 1991, Gehring *et al.*, 1993). A similar component occurs after negative performance feedback, called the feedback-related negativity (FRN; e.g., Miltner *et al.*, 1997). Source localization studies have suggested that the ERN and FRN share a common neural source, the anterior cingulate cortex (ACC) (Dehaene *et al.*, 1994, Holroyd *et al.*, 1998, Miltner *et al.*, 1997). Many imaging studies confirmed this finding (Carter *et al.*, 1998, Holroyd *et al.*, 2004, Kiehl *et al.*, 2000, Ullsperger and von Cramon, 2003).

The ACC covers quite a large area surrounding the frontal part of the corpus callosum. Within this area, subdivisions with specific functions have been defined. The dorsal/caudal part is also known as the cognitive part, while the rostral/ventral part is known as the emotional part (e.g., Bush *et al.*, 2000, Mohanty *et al.*, 2007). The rostral cingulate zone (RCZ), an area in the medial frontal cortex more or less equivalent to the dorsal/caudal part of the ACC, has been rather consistently found active in response to unfavorable outcomes such as response errors, pre-response conflict, decision uncertainty, and negative feedback (Ridderinkhof *et al.*, 2004). One of the leading theories suggests that the RCZ is activated when a negative deviation from expectancy occurs, i.e., when an outcome is worse than expected (reinforcement learning theory, Holroyd and Coles, 2002). According to this theory, after such a deviation, there is a dip in dopamine release in the striatum. The resulting lowered activation leads through its inhibitory projections to the RCZ to a disinhibition of the RCZ. The resulting increase in RCZ activation is thought to signal the need for behavioural adjustment to other brain regions involved in action selection.

In line with the reinforcement learning theory, fMRI studies on feedback processing using probabilistic learning tasks have rather consistently shown that the RCZ, or dorsal part of the ACC, is more active in response to negative feedback than to positive feedback (e.g., Holroyd *et al.*, 2004, Jocham *et al.*, 2009, Van den Bos *et al.*, 2009). A different pattern of results emerged from studies using a time-estimation task, another paradigm that has been employed frequently to assess feedback processing (e.g., Holroyd and Krigolson, 2007, Miltner *et al.*, 1997, Nieuwenhuis *et al.*, 2005b). In a recent study, Van der Veen *et al.* (2011) found the RCZ, unexpectedly, more active in response to positive feedback than to negative feedback. In earlier studies, however, the RCZ was not found to play a role in the processing of feedback valence: Van Veen *et al.* (2004) and Nieuwenhuis *et al.* (2005b) found no difference in BOLD response to positive and negative feedback. On the basis of their fMRI and EEG source localization results, Nieuwenhuis *et al.* (2005b)

suggested that not the RCZ, but the rACC was one of the underlying neural substrates of the FRN in time-estimation paradigms, together with the right superior frontal gyrus and the posterior cingulate. Importantly, these areas were more active in response to positive feedback than to negative feedback.

It should be noted, however, that the time-estimation paradigm differs considerably from probabilistic learning tasks. In probabilistic learning tasks, participants have to learn stimulus-response mappings based on probabilistic feedback. Thus, during the initial stages of the task all feedback is potentially relevant but when participants master the mapping rule feedback is superfluous. The situation is quite different in a time-estimation task. Here participants are required to press a button following a cue when they feel that the required interval elapsed. They receive positive feedback when their button press happened to fall within a pre-specified window around the end of the interval and negative feedback when their response fell outside the window. Importantly, the pre-specified window is adjusted from trial-to-trial by using a dynamic tracking algorithm. The window for the upcoming trial is shortened following a correct estimate and lengthened following an incorrect estimate. Thus, in principle, equal estimates might attract different feedback depending on the particular setting of the window across trials. Consequently, in time-estimation tasks it remains difficult to build up strong expectations that might be confirmed or disconfirmed by feedback. In other words, feedback validity is an important issue in time-estimation tasks.

Only a few studies examined the validity issue in time-estimation tasks using fMRI (Nieuwenhuis *et al.*, 2005b, Tsukamoto *et al.*, 2006). Nieuwenhuis *et al.* (2005b) included uninformative feedback in their design to function as a control condition for cognitive processes that already occurred before feedback onset. In their study the informative feedback stimuli were '+' for correct estimations, '–' for incorrect estimations, and '?' for uninformative feedback. The uninformative feedback therefore communicated no valence, which was reflected in the electro-cortical results: uninformative feedback showed a markedly different ERP than the informative feedback. However, when they specifically focused on the RCZ in their fMRI counterpart of the study, this brain region appeared to be just as active in response to uninformative feedback as it was to informative positive and negative feedback. A time-estimation study by Tsukamoto *et al.* (2006) also revealed no difference in brain activity between true (valid) and random (invalid) feedback, even though the participants knew beforehand that the feedback they would receive was invalid in the random feedback condition. These results suggest that the RCZ does not distinguish between invalid and valid feedback, which is surprising, given the difference in importance of the two types of feedback for future performance.

The unclarity that emerged from the above studies regarding the role of the RCZ in the processing of the valence and validity of feedback stimuli in time-estimation paradigms was the main reason for designing the present study. We aimed at increasing

our understanding of this role, and were specifically interested in the possible distinct roles of the RCZ and rACC in the processing of the valence of feedback and the validity or relevance of feedback. A clear separation of validity vs. valence is important because it allows us to decide whether the FRN (i.e., ACC activation) is an obligatory response to negative evaluation, or a response of a system promoting performance adjustment. In the current study, we therefore manipulated the feedback in an attempt to disentangle its valence and information value. Thus, the feedback provided to the participant signaled that the valence information was valid or invalid. Valid feedback was related to actual performance, and participants should therefore use the valence information provided by it in order to adjust their estimates. Invalid feedback, however, was not related to actual performance and signaled to the participants that they could ignore the valence information provided by it.

In examining the feedback effects we focused on the ACC. More specifically, it was anticipated that the RCZ is sensitive to both the validity and valence of the feedback. This hypothesis is based upon the notion that the RCZ is implicated in cognitive control (Bush *et al.*, 2000, Mohanty *et al.*, 2007) and that both validity and valence have to be processed for ensuing control operations. In addition, it was expected that feedback valence but not validity would activate the rACC. This hypothesis is based upon findings indicating that this brain region is involved in emotion processing (Bush *et al.*, 2000, Mohanty *et al.*, 2007). Finally, in line with previous findings reported by Van der Veen *et al.* (2011), we expected to observe more pronounced activations to positive feedback than to negative feedback. If these hypotheses are proven wrong, this will have implications not only for the current theories about the roles of the RCZ and rACC in feedback processing, but also for the cognition-emotion subdivision hypothesis of the ACC.

The current study differs from most feedback studies in that the participants varied along a considerable age range (19-69 years). The older participants served as controls in a companion study on depression. Although the focus of the current study is not on aging we will report significant effects of age if they would surface.

Materials and methods

Participants

Thirty healthy volunteers, 22 female, aged between 19 and 69 (mean = 45; SD = 15), participated in this study. Three participants were left-handed, but all participants used their right hand to respond. Fourteen of them participated in a psychophysiological study (EEG and heart rate) first, reported elsewhere (Mies *et al.*, 2011b), in which they performed the same time-estimation task. Participants were recruited by means of advertisements. The study was approved by the local medical ethics committee and all participants gave written informed consent. Participants received EUR 25 for participation.

Exclusion criteria were: neurological illness, severe somatic illness, psychiatric illness, substance abuse, use of medication which affects the central nervous system, pregnancy, and any contra-indication for having an MRI-scan. Health was assessed by means of a self-developed questionnaire and contra-indications for MRI were assessed by means of a standard questionnaire from the department of Radiology.

Time-estimation task

The time-estimation task used in the present study was the same as reported earlier (Mies *et al.*, 2011b). Participants were instructed to produce 1 s intervals. Each trial started with the presentation of an asterisk ("*") in the centre of a black screen for 2 s. This asterisk was followed by the cue for estimation: a question mark ("?"), which was replaced with another asterisk (1 s) after the estimation. This second asterisk was followed by the feedback stimulus (1 s).

Participants had to indicate the end of the one-second interval by pressing the button of a response device. Following the button press, they received performance feedback, i.e., positive feedback if their response occurred within a specified window around the target (900-1100 ms), and negative feedback if the response occurred outside the window. Unbeknownst to participants, the window was dynamically adjusted to ensure an equal amount of positive and negative feedback stimuli. After a correct estimation, the window on the subsequent trial was shortened by a fixed amount of time (20 ms). After an incorrect estimation, it was lengthened by the same amount on the subsequent trial (see also Miltner *et al.*, 1997).

Estimates were followed by feedback. The feedback consisted of face stimuli presented against a horizontal or vertical background grid. The background grid communicated the validity of the feedback stimulus to the participants (e.g., horizontal grid for valid feedback and vertical grid for invalid feedback). Valid feedback was based on the participant's performance. Invalid feedback was determined randomly by the computer, with a maximum of three invalid feedback trials in a row. Participants received invalid feedback in 50% of the trials. The emotional expression of the face informed participants that their estimate was correct or incorrect (respectively, a happy vs. a fearful face). Finally, in case of incorrect estimates, the gender of the face indicated whether the estimate was too short (e.g., a male face) or too long (e.g., a female face). The faces used in this study were from the Ekman and Friesen pictures set (Ekman and Friesen, 1978).

Procedure

Participants were asked to abstain from coffee and tobacco for at least two hours before scanning. Participants were given task instructions and they completed 36 practice trials of the time-estimation task on a computer outside the scanner. When participants were inside the scanner, the visual stimuli were projected on a screen at the end of the scanner

bed, which could be viewed by the participant through a small mirror mounted on the head coil. Participants responded by pressing the button of a response device with their right index finger. Inside the scanner participants again performed some practice trials (maximum of 36 trials), after which the first session started consisting of 120 trials (10 minutes). A structural scan was obtained after the first session, followed by a second session of the task which again lasted 10 minutes. Participants performed 240 trials inside the scanner. Total time spent in the scanner was about 35 minutes.

Magnetic Resonance Imaging data acquisition

Blood-oxygen-level-dependent (BOLD) fMRI data were acquired on a 3T GE Healthcare (Milwaukee, WI) scanner. For the functional scans a single-shot gradient echo echo-planar imaging (EPI) sequence was used. The T2*-weighted images were acquired in 26 axial slices (thickness = 3.5 mm, interslice gap = 0.5 mm) with a repetition time (TR) of 2000 ms, echo time (TE) of 30 ms, field of view (FOV) of 220 mm, and voxels of 1.72 x 1.72 x 3.50 mm. The interval between trials was about 5 seconds. In order to give participants enough time to complete all trials, 310 volumes (8060 functional images) were obtained in each session of 120 trials. In addition, five dummy scans were made before the task started in order to obtain a steady-state magnetization.

For anatomical reference, a 3D high-resolution inversion recovery fast spoiled gradient recalled echo T1-weighted sequence was used, which covered the whole brain. 192 slices were acquired with a slice thickness of 1.6 mm and 0.8 mm overlap, FOV of 250 mm, and voxels of 0.49 x 0.49 x 0.80 mm.

For preprocessing and processing of the fMRI data SPM5 (Statistical Parametric Mapping, Wellcome Trust Centre for Neuroimaging, University College London, UK) and MATLAB6 (The MathWorks, Inc.) was used. Preprocessing of the structural data included manual reorienting, segmentation using the Montreal Neurological Institute T1 templates for grey matter, white matter, and CSF, and normalization. Preprocessing of the functional data included manual reorienting, slice time correction, realignment using the middle slice as a reference, and unwarping, co-registration (functional images were co-registered to the grey matter structural image derived from segmentation), normalization, and smoothing using a Gaussian kernel of 8 mm full width at half maximum, and a high-pass filter of 128 seconds for temporal smoothing. In three cases the co-registration and normalization outcomes were unsatisfactory. In those cases, we co-registered the functional images to the unsegmented structural image.

Statistical analysis

Performance data were analyzed using SPSS 16.0 by comparing the percentage of correct adjustments after valid negative feedback with the percentage of 'correct' adjustments after invalid negative feedback by means of repeated-measures ANCOVA. Age was added

as a covariate, and was centred using the method of Delaney and Maxwell (1981); i.e., age minus mean age of all participants (Delaney and Maxwell, 1981, Thomas *et al.*, 2009).

For the fMRI analyses, first a model was made in which the preprocessed fMRI data were coupled to the vectors of feedback onset of each condition (valid positive feedback, valid negative feedback, invalid positive feedback, and invalid negative feedback) in both task sessions. Then two different t-contrasts were computed: positive – negative feedback (main effect of valence), and valid – invalid feedback (main effect of validity). On average, in these analyses approximately 57 ±6 valid positive, 63 ±6 valid negative, 60 invalid positive, and 60 invalid negative feedback stimuli were included. The individual contrast images were used in a second-level analysis.

First, whole-brain analyses were performed on the two contrasts using two separate one-sample t-tests with age as covariate, for which we used a height threshold of $p < 0.001$. Significant voxels and clusters are reported as significant if $p < 0.05$ corrected with the family-wise error (FWE) approach. MRIcron and the AAL atlas were used to label the significant clusters and voxels.

Subsequently, two main region-of-interest (ROI) analyses and five additional ROI analyses were performed using MarsBaR 0.41 (MARSeille Boîte À Région d'Intérêt; Brett *et al.*, 2002). First of all, the RCZ (±8 30 32; coordinates adopted from Mars *et al.*, 2005, and implemented in the AAL map of MarsBaR), and the rACC (0 40 -2; coordinates adopted from Nieuwenhuis *et al.*, 2005b) were defined as ROIs. In line with the results of Nieuwenhuis *et al.* (2005b) and Van Veen *et al.* (2004), the posterior cingulate (-1 -30 33) and a part of the superior frontal gyrus (SFG; ±20 39 43), coordinates for these areas adopted from Nieuwenhuis *et al.* (2005b), were defined as additional ROIs to test the hypothesis that these areas are, similar to the rACC, more likely to be involved in valence processing during time-estimation tasks than the RCZ. Furthermore, the pre-supplementary motor area (pre-SMA, ±8 10 55; coordinates adopted from Mars *et al.*, 2005), an area in close proximity of the RCZ, was defined as ROI because this area has been found to be involved in performance monitoring and in motor planning (e.g., Rushworth *et al.*, 2004), and was therefore expected to show similar results as the RCZ. Finally, the nucleus accumbens (NAcc, ±10 12 -2; coordinates adopted from Knutson *et al.*, 2008) and the amygdala (AAL map of MarsBaR, Tzourio-Mazoyer *et al.*, 2002) were defined because these areas are known for their involvement in basic emotion and reward processing. These two latter areas were expected to be specifically sensitive to the valence of the feedback, irrespective of its validity.

Beta-values were extracted from the fMRI data for each feedback condition separately. For each ROI, the extracted beta-values of each participant were exported to SPSS, and subsequently analyzed using valence (positive or negative feedback), feedback-

validity (valid or invalid feedback) and hemisphere (left or right)[1] as within-subjects factors in repeated-measures ANCOVA. Age was added as a covariate.

Only statistically significant effects ($p < 0.05$) and marginal effects of interest ($0.05 \leq p < 0.10$) are reported.

Results

One of the 30 participants was excluded from analysis because this participant did not ignore the invalid feedback (76% adjustments of the type indicated by the feedback, which is high above chance level of 50%, and more than 3 SD from the mean).

Behavioural results

Mean estimation time of the remaining 29 participants was 1051 ms ±116 (average ±SD). On average, participants received only slightly more negative feedback (122 ±6 times) than positive feedback (116 ±6 times), which indicates that the task manipulation performed as expected. Participants made in 81.9% ±8.6 of the valid feedback trials an adjustment of the type indicated by the feedback, which was well above chance level (50%, $t(28) = 19.9$, $p < 0.001$), and significantly higher than the 52.2% ±5.9 adjustments made of the type indicated by the invalid feedback ($F(1,27) = 249.5$, $p < 0.001$). As expected, the amount of adjustments after invalid feedback was about equal to chance level ($t(28) = 2.0$, $p = 0.057$).

With advancing age fewer correct adjustments were made, as indicated by a main effect of age ($F(1,27) = 11.1$, $p = 0.002$). No other significant effects of age were found.

Thirteen of the 29 participants had already participated in a psychophysiological study measuring EEG and ECG while performing the same time-estimation task (Mies *et al.*, 2011b; 480 stimuli in total). Those participants were therefore well trained and might have performed better. This was, however, not the case: the proportion of adjustments to both valid and invalid feedback did not differ between the two groups ($F(1,27) = 3.2$, $p = 0.086$).

FMRI results

Whole brain analysis

The activated brain areas for the two contrasts (main effect of valence and main effect of validity) are summarized in Table 3.1. There were no brain areas more active in response to valid feedback than to invalid feedback. Invalid feedback, however, elicited more

[1] Hemisphere was left out as a within-subject factor in the analyses of the rACC and posterior cingulate, because the coordinates of these regions were on the midline.

activation in the mid-cingulate than valid feedback. Importantly, there were no brain areas that were more activated by negative feedback than by positive feedback. Positive feedback, on the other hand, resulted in more activation than negative feedback, especially in the inferior parietal lobule, but also in the rostral anterior cingulate, middle frontal gyrus and the putamen. No effects of the covariate age were found.

Table 3.1 Whole brain analyses for the contrasts positive – negative feedback and invalid – valid feedback (with age taken into account)

Area	*L/R*	*BA*	*Cluster size*	*Z*	*MNI coordinates* *x* *y* *z*
positive feedback > negative feedback					
Inferior parietal lobule	L	40	15276	5.13	-52 -46 42
Supramarginal gyrus	R	40	[a]	4.84	54 -38 36
Inferior parietal lobule	L	39	[a]	4.70	-48 -58 48
Anterior cingulate	R	32/9	[a]	4.68	8 42 18
Anterior cingulate	R	32	[a]	4.63	12 44 18
Precuneus	L		[a]	4.56	-18 -50 36
Middle frontal gyrus	L	8	1297	5.02	-22 24 48
Middle frontal gyrus	L		[b]	4.91	-28 22 42
Putamen	L	48	1890	4.64	-30 8 0
Inferior temporal gyrus	L	37	425	4.54*	-56 -58 -8
Postcentral gyrus	L		251	4.15*	-34 -16 42
Rolandic operculum	R	48	268	4.14*	58 4 12
invalid feedback > valid feedback					
Mid cingulate	R		373	3.77*	10 -38 44

Brain regions were labeled using the AAL atlas and MRIcron

[a,b]local maximum (on voxel level) within cluster a and b, respectively ($p < 0.05$, FWE-corrected)

*significant at cluster level only ($p < 0.05$, corrected)

ROI analyses

The ROI analyses were focused on two regions within the ACC: the RCZ and the rACC. Interestingly, the RCZ was more active in response to valid feedback than in response to invalid feedback ($F(1,27) = 7.3$, $p = 0.012$) (see Figure 3.1). This effect was independent of valence, and it was strongest in the right hemisphere, as indicated by an interaction between hemisphere and validity ($F(1,27) = 9.3$, $p = 0.005$). There was also an effect of age. Age interacted with valence, validity and hemisphere ($F(1,27) = 4.7$, $p = 0.039$). With advancing age RCZ-activity slightly decreased in response to feedback. Because the RCZ

was sensitive to the validity of the feedback, we investigated whether performance had an influence on this effect. We defined performance as the ability to distinguish valid feedback from invalid feedback at the behavioural level. For this purpose we calculated for each participant the extent to which their number of correct adjustments after negative feedback deviated from chance level (50%) in both the valid and invalid feedback condition and calculated a difference score. We added this performance measure as a covariate in the analysis, and found a marginal effect of performance on the interaction between validity and hemisphere ($F(1,27) = 4.2$, $p = 0.051$). As expected, the effect of validity in the RCZ was more pronounced with better performance.

The rACC was, as expected, sensitive to the valence of the feedback; it was more active in response to positive feedback as compared to negative feedback ($F(1,27) = 8.6$, $p = 0.007$) (see Figure 3.2). The interaction between valence and validity failed to reach an acceptable significance level ($F(1,27) = 3.1$, $p = 0.089$); the difference between positive and negative feedback was slightly larger in the valid condition than in the invalid condition. When the above-defined performance measure was taken into account, we found that this latter effect increased with better task performance, ($F(1,27) = 9.1$, $p = 0.005$). Age had no effects.

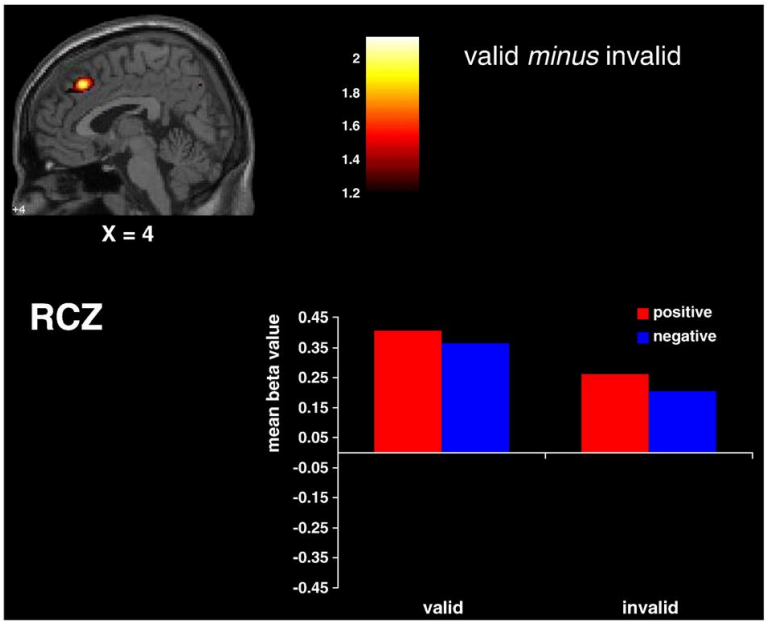

Figure 3.1 Activation map for the predefined rostral cingulate zone (RCZ) in the contrast valid minus invalid feedback in the sagittal plane (at Talairach coordinate x = 4), combined with a bar graph showing the beta values for the four predictors averaged across the right and left hemisphere.

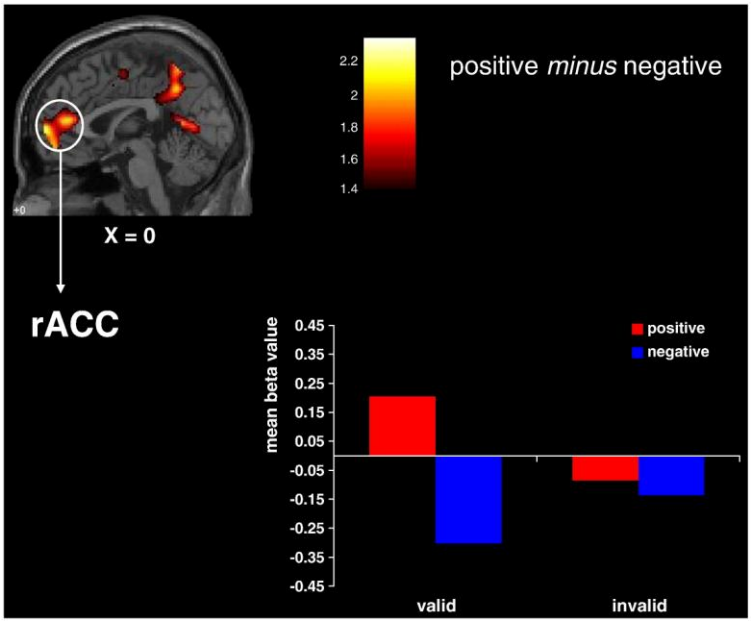

Figure 3.2 Activation map for the predefined rostral anterior cingulate (rACC) in the contrast positive minus negative feedback in the sagittal plane (at Talairach coordinate x = 0), combined with a bar graph showing the beta values for the four predictors.

Other ROIs were the pre-SMA, known for its involvement in action selection, the superior frontal gyrus and posterior cingulate, selected on the basis of the results by Nieuwenhuis *et al.* (2005b), and the nucleus accumbens and amygdala, known for their involvement in reward and emotion processing. The activation patterns of these ROIs are shown in Figure 3.3. For the pre-SMA we found no effects. The posterior cingulate, however, was more active in response to positive feedback than to negative feedback ($F(1,27) = 11.0$, $p = 0.003$). As expected, no effects of task-validity were found in this area, neither were there any effects of age.

The SFG was also more active after positive feedback than after negative feedback ($F(1,27) = 19.9$, $p < 0.001$). Validity interacted with this valence effect ($F(1,27) = 7.3$, $p = 0.012$); follow-up analyses showed that the valence effect was only present when the feedback was valid ($F(1,27) = 23.9$, $p < 0.001$). Task performance, as defined above, interacted with valence and validity ($F(1,27) = 4.3$, $p = 0.047$), indicating that the effect of validity increased with better performance. We also found effects of hemisphere. The right SFG was more active than the left SFG ($F(1,27) = 8.5$, $p = 0.007$). In addition, there was an interaction between hemisphere and valence ($F(1,27) = 9.6$, $p = 0.004$), between hemisphere and validity ($F(1,27) = 4.6$, $p = 0.042$), and a three-way interaction between

hemisphere, valence and validity ($F(1,27) = 10.4$, $p = 0.003$). Follow-up analyses on the right and left hemisphere separately revealed that the *right* SFG was sensitive to valence only ($F(1,27) = 15.7$, $p < 0.001$), while the *left* SFG was sensitive to validity as well, as indicated by an interaction between valence and validity ($F(1,27) = 9.7$, $p = 0.004$). Finally, age also interacted with hemisphere ($F(1,27) = 4.7$, $p = 0.040$); the left SFG was more active with advancing age.

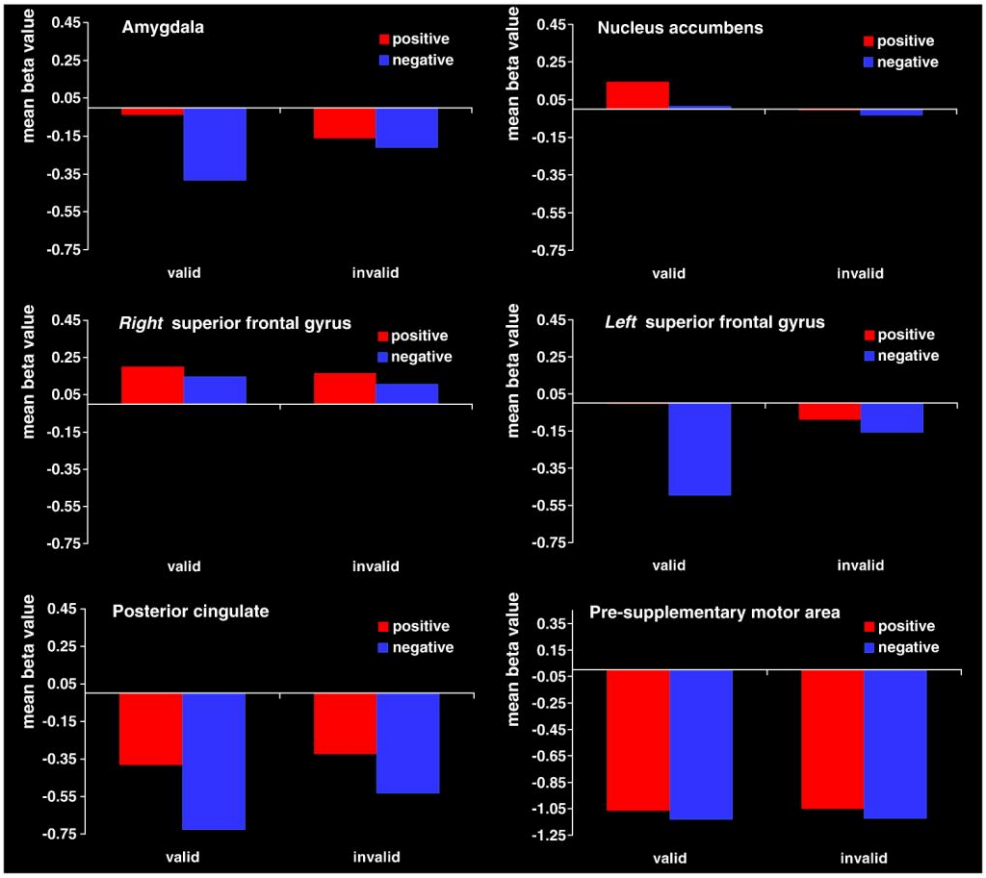

Figure 3.3 Bar graphs of the five additionally predefined ROIs (amygdala, nucleus accumbens, superior frontal gyrus, posterior cingulate, and pre-supplementary motor area) showing the beta values for the four predictors averaged across the right and left hemisphere, or for both hemispheres separately (superior frontal gyrus).

We finally investigated the responses of the NAcc and amygdala to feedback. The NAcc was, as expected, more active in response to positive feedback than to negative feedback ($F(1,27) = 5.5$, $p = 0.027$). Importantly, it was also more active in response to valid feedback than to invalid feedback ($F(1,27) = 7.2$, $p = 0.012$), but valence did not interact with validity. Age and performance did not influence the NAcc results.

The amygdala was more active after positive feedback than after negative feedback ($F(1,27) = 8.9$, $p = 0.006$). This was, however, only the case after valid feedback, as reflected by a marginal interaction between valence and validity ($F(1,27) = 3.9$, $p = 0.058$), and follow-up analyses on valid and invalid feedback separately (main effect of valence in valid feedback condition: $F(1,27) = 10.6$, $p = 0.003$). These results were also unaffected by age and performance.

Discussion

This study investigated the neural basis of the processing of feedback-valence and feedback-validity in a time-estimation task. Participants had to produce 1-second intervals, followed by positive and negative feedback that was either valid or invalid. At the behavioural level, participants made a clear distinction between valid and invalid feedback, reflected by the high amount of behavioural adjustments after valid negative feedback (82%) and an adjustment rate at about chance level after invalid negative feedback (52%). This is in line with a previous psychophysiological study in which we used the same task (78% vs. 52%; Mies *et al.*, 2011b), and indicates that participants performed according to task instructions.

We expected that the RCZ, equivalent to the dorsal part of the ACC, would be primarily sensitive to the validity of the feedback, representing the cognitive part of the ACC, while the rostral part of the ACC would be primarily sensitive to the valence of the feedback, representing the emotional part of the ACC. Our results confirmed this hypothesis.

The RCZ was, as expected, more active in response to valid feedback than in response to invalid feedback. Moreover, the larger this difference in activity, the better a participant distinguished between these two types of feedback, as indicated by the amount of behavioural adjustments. These results indicate that the RCZ evaluates the relevance of feedback, which might exert its influence on task-performance. Our results seem to contradict other studies in which feedback-validity was manipulated in a time-estimation paradigm and in which no effect of validity in the RCZ could be found (Nieuwenhuis *et al.*, 2005b, Tsukamoto *et al.*, 2006). It should be noted that these studies differed in a number of aspects from the current study. Nieuwenhuis *et al.* (2005b) included uninformative feedback instead of invalid feedback, and Tsukamoto *et al.* (2006) manipulated feedback-validity between trial blocks instead of within. The lack of finding

an effect in the RCZ in these two studies might be due to their methods of analysis. Both studies did not specify this specific region as a region of interest. Nieuwenhuis *et al.* (2005b) focused on a slightly different part of the dorsal ACC partly overlapping with the pre-SMA, and found no difference in BOLD signal for informative and uninformative feedback in that area. Note that we also found no differences in activity in the pre-SMA. Our finding that the RCZ was more active in response to valid than to invalid feedback is in line with the reinforcement learning theory (Holroyd and Coles, 2002); the RCZ seems to evaluate whether feedback is relevant or not, and this appears to be reflected in the person's performance. Our results are also in line with a study that manipulated validity of the feedback stimulus using a very different paradigm comparing a learning condition (valid feedback) with a non-learning condition (invalid feedback) (Volz *et al.*, 2005). It should be noted, however, that they only found a validity effect of feedback when this feedback was positive.

The RCZ was insensitive to the valence of the feedback, that is, it appears to be activated to an equal extent by positive and negative feedback. These results are in line with previous reports by Nieuwenhuis *et al.* (2005b) and Van Veen *et al.* (2004), who also found no effect of feedback-valence in the RCZ using a time-estimation task. In a recent study, however, Van der Veen *et al.* (2011) observed that the RCZ did respond to the valence of feedback (i.e., more activation to positive feedback relative to negative feedback). The discrepancy between the present study and the study by Van der Veen *et al.* is probably related to different versions of the time-estimation paradigm. In the Van der Veen *et al.* study, negative feedback did not inform participants about whether their estimate was too long or too short leaving them uncertain about the adjustment needed on the subsequent trial. In contrast, positive feedback resolved any adjustment uncertainty. Thus, negative and positive feedback in the Van der Veen *et al.* study differed in terms of uncertainty resolution. In the current study, however, negative and positive feedback were equal in this regard; that is, the information provided by the feedback, either negative (estimate is too long or too short) or positive (estimate is correct) should provide sufficient information to the participant for adjusting performance on the subsequent trial if needed. Thus, in the Van der Veen *et al.* study, the uncertainty associated with negative feedback might have elicited the RCZ response.

In line with the dorsal-cognitive and rostral-affective subdivision of the ACC, the rACC was expected to be primarily sensitive to the valence of the feedback. This was indeed what we found. Task-validity, however, did have an influence on the rACC when performance was taken into account. On the basis of their connectivity findings, Mohanty *et al.* (2007) were reluctant to interpret the differential engagement of the subdivisions of the ACC in cognitive and emotional processing in their study as a double dissociation. Our results are in line with their conclusion that the dorsal-cognitive and rostral-affective

distinction of the ACC is not a strict anatomical and functional division, but rather a continuum (Mohanty *et al.*, 2007).

In addition to the rACC, both Van Veen *et al.* (2004) and Nieuwenhuis *et al.* (2005b) found the posterior cingulate more active in response to positive feedback than to negative feedback. The current ROI analysis confirmed that this region is primarily sensitive to the valence of feedback. Our results therefore support the idea that the rACC and posterior cingulate play a more important role in the processing of the valence of feedback than the RCZ does. This fits with the anatomical and functional connections of these areas. The posterior cingulate has connections to reward-processing areas such as the striatum, and has been found to play a role in reward processing in macaques (McCoy *et al.*, 2003, Pearson *et al.*, 2009).

Two other regions involved in reward processing are the nucleus accumbens and the amygdala. The amygdala was more active in response to valid positive feedback than to valid negative feedback, while this difference was absent in the invalid condition. The NAcc was also sensitive to both the valence and the validity of the feedback, although no interaction between valence and validity was found. Both areas are therefore not sensitive to the valence of the feedback per se, as we initially hypothesized. The amygdala and the ACC are part of the same neural circuit involved in emotion processing and decision making. The regions are not only structurally, but also functionally connected. From primate research it is known that the amygdala and ventral striatum project to the rACC (e.g., Kunishio and Haber, 1994, Paus, 2001). The rACC has many reciprocal connections with the caudal/dorsal part of the ACC (cACC), which is more or less equivalent to the RCZ, and the cACC in its turn is thought to project back to the amygdala forming a feedback loop (Pezawas *et al.*, 2005). The effect of task-validity in the amygdala and NAcc might therefore be a result from efferent projections from the RCZ.

For the pre-SMA we found no effects, consistent with the study by Van der Veen *et al.* (2011). They did find stronger activation in the pre-SMA when negative feedback was followed by a correct adjustment compared with when it was followed by an incorrect adjustment, confirming its role in action selection. Inherent to the design used in the current study, we had insufficient power to replicate the results of Van der Veen *et al.*, because we had fewer valid feedback stimuli and our participants made too few incorrect adjustments. On average only 12 (±6) valid negative feedback stimuli followed by an incorrect adjustment were available to evaluate.

Finally, because of the large age range of the participants, age was taken into account in the behavioural and fMRI analyses. In contrast to our previous study (Mies *et al.*, 2011b), but in line with other studies on feedback processing (e.g., Wild-Wall *et al.*, 2009), the proportion of correct adjustments decreased with advancing age. This was independent of the validity of the feedback, which suggests that the ability to distinguish between valid and invalid feedback does not decrease with advancing age. Age did not

have major effects on the fMRI results. We just completed another study with a more restricted age range (18-32 years) and replicated the findings reported in the current manuscript (Mies *et al.*, submitted), which further confirms that age is not an important factor in explaining our current findings.

In conclusion, our results show that the RCZ is primarily involved in the processing of feedback-validity, while the rACC is primarily involved in the processing of feedback-valence. This is in line with the cognition-emotion subdivision hypothesis of the ACC. To our knowledge, this is the first demonstration of such a dissociation using a feedback task. Our findings suggest that a subdivision of the ACC is necessary to better understand how various aspects of feedback stimuli, and possibly also of errors, are processed and how these aspects are integrated in order to optimize future behaviour.

Acknowledgement

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Chapter 4

Drug-free patients with major depression show an increased electrophysiological response to valid and invalid feedback

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Abstract

Background: Depressed patients are biased in their response to negative information. They have been found to show a maladaptive behavioural and aberrant electrophysiological response to negative feedback. The aim of this study was to investigate the behavioural and electrophysiological response to feedback-validity in drug-free depressed patients.

Methods: Fifteen drug-free inpatients with unipolar major depression and 30 demographically matched controls performed a time-estimation task in which they received valid and invalid (i.e., related and unrelated to performance) positive and negative feedback. The number of behavioural adjustments to the feedback and the feedback-related negativity (FRN) were measured.

Results: Patients made fewer correct adjustments after valid negative feedback than controls, and their FRNs were larger. Both patients and controls did not adjust their time estimates following invalid negative feedback.

Conclusions: The FRN-results suggest that depressed drug-free inpatients have an atypical rostral anterior cingulate response to feedback, which is independent of feedback-validity. Their behavioural response to invalid negative feedback, however, is not impaired. This study confirms the idea that the behavioural responses of depressed individuals to negative feedback are context-dependent.

Introduction

According to Beck's cognitive theory of depression (Beck, 1979), persons with unipolar major depressive disorder (MDD) have dysfunctional attitudes and assumptions that lead to a depressed mood. For instance, they suffer from a 'negativity bias', that is, they focus more on negative information than on positive information. This negativity bias is reflected in the way depressed patients interpret social situations, which may lead to social dysfunction. It is therefore interesting to investigate how people with MDD process external feedback. It has been found that errors and negative feedback disrupt their subsequent performance (Beats *et al.*, 1996, Douglas *et al.*, 2009, Elliott *et al.*, 1997, Elliott *et al.*, 1996, Fladung *et al.*, 2010, Steffens *et al.*, 2001). This maladaptive response to negative feedback has been suggested to be a key deficit linking the negative affect and the cognitive impairments associated with depression (Elliott *et al.*, 1997).

Patients' maladaptive response to negative feedback can be interpreted in two ways: depressed individuals are either *hyper*sensitive or *hypo*sensitive to feedback in comparison with non-depressed individuals (Eshel and Roiser, 2010). In the case of hypersensitivity, perceived failure due to negative feedback might lead to more negative thoughts, which interfere with subsequent performance. This is in line with Beck's cognitive theory of depression (Beck, 1979). In the case of hyposensitivity, patients simply do not use the negative feedback to adjust behaviour, possibly because they are less motivated to obtain positive feedback than others (e.g., Elliott *et al.*, 1997, Eshel and Roiser, 2010).

Attempts have been made to understand the neural mechanisms underlying the maladaptive response to negative feedback in depression (Ruchsow *et al.*, 2006, Ruchsow *et al.*, 2004, Santesso *et al.*, 2008, Steele *et al.*, 2007, Taylor Tavares *et al.*, 2008, Tucker *et al.*, 2003). The feedback-related negativity (FRN) can be used as an electrophysiological index for responses to feedback. The FRN is a negative component in the event-related brain potential (ERP) occurring around 200-350 ms after feedback onset (Miltner *et al.*, 1997). The few studies that have investigated the FRN in depressed individuals reported increased FRN-amplitudes in patients compared to controls (Santesso *et al.*, 2008, Tucker *et al.*, 2003).

In these ERP studies, the neural responses to negative feedback differed between patients and controls. No differences in behavioural responses were, however, found, which is surprising given the increasing number of performance studies reporting maladaptive behavioural responses. An abnormal behavioural response to negative feedback, however, appears not to be a default reaction, but to depend on the type of feedback. In a study by Murphy *et al.* (2003) patients responded normally to accurate negative feedback in a spatial working memory task, but they responded differently to misleading negative feedback in a probabilistic reversal learning task. The patients with MDD were more likely to switch their behaviour after misleading negative feedback than

the healthy controls. This effect was recently replicated by Taylor Tavares *et al.* (2008). Murphy and colleagues suggested that negative feedback that is more affective in nature might disrupt performance, while negative feedback that is more informational might not.

This interesting finding demands further examination. It suggests that the information value or validity of feedback, and the valence of feedback (positive or negative) are differently processed by MDD patients than by healthy persons. The goal of the current study was, therefore, to investigate the behavioural response of patients to valid and invalid feedback, using a classical paradigm to investigate feedback processing, that is, a time-estimation task. Valid positive and negative feedback was related to actual performance, and invalid feedback was unrelated, random positive and negative feedback. In each trial the feedback stimulus signaled to participants whether the valence of the feedback was valid or not. If patients are indeed unable to ignore misleading negative feedback because of its affective value (Murphy *et al.*, 2003), they will make more unnecessary adjustments after invalid negative feedback than non-depressed controls. In addition, we investigated the FRN-response. In line with previous studies (Santesso *et al.*, 2008, Tucker *et al.*, 2003), we expected increased FRN-responses in patients after both valid and invalid feedback. Importantly, in contrast to these previous FRN studies, the patients included were depressed (not in remission) and drug-free at the time of testing.

Methods

Participants

Data were obtained from 15 patients with MDD and 30 demographically matched non-depressed control participants (see Table 4.1 for demographic characteristics). Patients were inpatients at the Depression Unit of the Department of Psychiatry at the Erasmus MC, University Medical Centre Rotterdam. After admission it is routine practice to discontinue all psychotropic drugs. During the drug-free period, the diagnosis of unipolar major depression was confirmed with a semi-structured clinical interview (SCID-I) and the severity of depression was assessed with the Hamilton Rating Scale for Depression (17-item HRSD, (Hamilton, 1960). All patients suffered from depression with melancholic features and none of them suffered from depression with psychotic features. Excluded were patients with schizophrenia, schizoaffective disorder, bipolar disorder, organic brain syndrome, a clinically relevant somatic illness, and patients who were pregnant. Patients were excluded when they scored below 18 on the HRSD. Furthermore, patients were excluded when they used medication affecting the central nervous system, including beta-blocking agents, or received ECT treatment. On average they were drug-free for 9.3 days (SD=4.5) prior to the experiment, with a minimum of 4 days. Two patients were medication-naïve. The others had, prior to the drug-free period, received benzodiazepines

(n=10), tricyclic antidepressants (TCAs; n=5), selective serotonin reuptake inhibitors (SSRIs; n=4), lithium (n=4), antipsychotics (n=5), duloxetine (n=1), or a combination of these drugs. None of them had used fluoxetine during the past month. Two other patients had received ECT during a previous depressive episode (8 and 14 years ago). Thirteen patients suffered from recurrent depression, and for 6 patients the index depressive episode lasted for over a year.

Non-depressed controls were recruited by means of advertisements throughout the hospital and the medical and psychology faculties. All control participants were found on interview (SCID-I) to have no past history of, or evidence for current psychiatric disorder. Similar to the patients, the controls were excluded when they used medication affecting the central nervous system, suffered from a clinically relevant somatic illness, or were pregnant. Non-depressed volunteers with a first-degree relative with depressive disorder were also excluded from participation. All participants gave written informed consent and the study was approved by the local medical ethics committee. The non-depressed participants received EUR 25 for participation.

Table 4.1 Demographic characteristics

	Patients	*Controls*
N (female)	15 (7)	29 (14)
Mean age (range)	51.8 (35-62)	52.1 (34-72)
Level of education (low/average/high)*	3 / 7 / 5	2 / 20 / 7

* Level of education was divided into three groups (see Van der Elst *et al.*, 2008): low refers to participants with only primary education, average refers to those with at most junior vocational training or high school, and high refers to those with at most senior vocational or academic training.

Time-estimation task

The time-estimation task was a modified version (Mies *et al.*, 2011b) of the original paradigm developed by Miltner *et al.* (1997). Participants had to produce a one-second interval by pressing the button of a response device. Each trial started with the presentation of an asterisk ("*") in the centre of a black screen for 2 s. This asterisk was followed by the cue for estimation: a question mark ("?"), which was replaced with another asterisk (1 s) after the button press. This second asterisk was followed by the feedback stimulus (1 s) (see Figure 2.1). If the response fell within a certain window around the target of one second, a *happy* male or female face was presented (positive feedback). If the estimation did not fall within this window, a *fearful* face was presented

(negative feedback), indicating that the produced interval was either too long (e.g., a *male* fearful face) or too short (e.g., a *female* fearful face) (2x4 male and 2x4 female pictures were selected from Ekman and Friesen (1978) with 100% fearful and happy expressions). Unbeknownst to the participants, the window was dynamically adjusted to ensure an equal amount of positive and negative feedback stimuli (see Miltner *et al.*, 1997). The face stimuli were presented against a horizontal or vertical background grid. This background grid informed participants about the validity of the feedback. Valid feedback was based on the participant's performance, while invalid feedback was determined randomly by the computer. Participants received invalid feedback in 50% of the trials. Four versions of the task were counterbalanced across participants to correct for possible effects of the gender of the face stimuli and background grid.

Procedure

Prior to participation, both patients and controls had to fill out a self-developed questionnaire to assess health, including questions about medication use in the past three months, possible brain injury due to concussion, and psychiatric illness in first-degree relatives.

The patients were presented with the diagnostic interview and several standardized neuropsychological tasks, and took the EEG-measurements on two separate, usually consecutive days. For control participants all assessments were on the same day. After the electrodes were attached for the EEG recordings, participants were instructed on how to perform on the time-estimation task and given 24 practice trials. Each trial lasted about five seconds in total. Stimuli were presented in four blocks of 120 stimuli. Task duration, therefore, was 40 minutes in total. Between the four blocks, participants took self-paced breaks. Participants were asked to restrain from coffee and tobacco at least two hours before the EEG-measurements.

Electrophysiological measures

The EEG was derived from five electrodes placed at Fz, Cz, Pz, C3 and C4 according to the 10-20 system (e.g., Sharbrough *et al.*, 1991). Linked mastoids were used as a reference. Electro-oculogram (EOG) was derived from two electrodes placed above and below the right eye, and one each on the outer canthi of the eyes. A ground electrode was placed at the sternum. EEG and EOG were recorded using a Vitaport 3 recorder (Temec Instruments BV, Kerkrade, the Netherlands). The EEG was sampled at 256 Hz, low-pass filtered at 30 Hz, and high-pass filtered with a time constant of 0.33 s. Electrode impedance was kept below 8 kΩ.

Data were analyzed using locally developed software which was implemented in Vitascore (Temec Instruments BV, Kerkrade, the Netherlands). Event-related potentials (ERPs) were locked to the onset of the feedback stimulus, and epochs were extracted

between 100 ms preceding and 700 ms following feedback onset. The method of Gratton *et al.* (1983) was used to correct EEG traces for vertical EOG only (criteria for blinks: EOG signal exceeding 40 microvolt within a 20 ms time interval). Epochs were manually checked for artifacts and excluded from analysis when necessary.

Each ERP was baseline-corrected by averaging the first 100 ms before feedback onset and subtracting this average from the ERP.

Statistical analyses

Patients and controls were compared on the amount of correct adjustments they made after valid and invalid negative feedback by means of an ANOVA. Adjustments were considered 'correct' whenever a negative feedback stimulus indicating that the estimate was too short or too long was followed by, respectively, a lengthening or shortening of the time estimate on the subsequent trial. Adjustments were considered 'incorrect' when negative feedback was followed by a lengthening or shortening of the estimate, while the feedback stimulus indicated that the estimate was too long or too short, respectively.

To define the FRN, difference waves were created by subtracting the ERPs associated with positive feedback from the ERPs associated with negative feedback. This was done separately for valid and invalid feedback. For each participant and each channel, the most negative peak of this difference wave within 200 and 350 ms after feedback onset was measured, which is the time window in which the FRN is usually found (e.g., Nieuwenhuis *et al.*, 2005b). ERP data were analyzed by using channel (Fz, Cz, Pz, C3 and C4) and validity (valid vs. invalid) as within-subjects factors and group (depressed vs. non-depressed) as between-subjects factor.

When necessary, degrees of freedom were corrected using the method of Huyn-Feldt. Corrected p-values, but uncorrected degrees of freedom are reported.

Results

Patients had a mean HRSD-score of 23.9 ±3.1 (range 18-28). There were 3 patients who did not complete the total amount of 480 trials (4x10 minutes) due to increasing fatigue, restlessness, or anxiety, but we had sufficient data to include them in the analyses (at least three time-estimation blocks). In one of the patients, the Fz channel showed too many artifacts, and this patient could therefore not be included in the ERP analyses, but was included in the behavioural analyses.

A box-plot analysis revealed that one of the control participants was an extreme outlier; this person had a mean estimation time of 539 ms, and was therefore excluded from all analyses. No outliers were found within the MDD group.

Behavioural results

Despite the dynamic time window, participants received slightly more negative feedback (54%) than positive feedback (46%). The percentage of negative feedback received by patients differed slightly from that received by controls (55% versus 53%; $t(42) = 2.29$, $p = 0.027$). Mean estimation time did not differ between the patients and controls (1163 ±259 vs. 1085 ±118 ms, respectively; $t(42) = 1.1$, $p = 0.289$), nor did the variation in time estimates, as indicated by the standard deviation (373 vs. 294 ms, respectively; $t(42) = 1.78$, $p = 0.083$).

Both patients and controls adjusted their estimates more often after valid negative feedback than after invalid negative feedback ($F(1,42) = 224.5$, $p < 0.001$). The interaction between validity and group did not reach significance ($F(1,42) = 2.8$, $p = 0.104$), but there was a main effect of group ($F(1,42) = 7.0$, $p = 0.012$), indicating that patients had lower adjustment rates than controls. Patients adjusted their estimates in 50.6 ±3.4% of the invalid negative feedback trials, and controls in 52.1 ±6.2% of the trials ($t(42) = 1.04$, $p = 0.305$), which is both close to chance level (50%). After *valid* negative feedback, however, the proportion of correct adjustments was slightly lower in patients than in controls (70.5 ±8.5% vs. 76.9 ±7.3%; $t(42) = 2.64$, $p = 0.012$) (see Figure 4.1).

In order to assess whether this performance difference was due to neuropsychological dysfunction, we investigated whether patients differed from controls on two neuropsychological tests assessing sustained attention (Continuous Performance Task, CPT, e.g., Van den Bosch *et al.*, 1996), and working memory (Digit Span, Wechsler, 1997), and if so, whether these test scores correlated with performance on the time-estimation task. Patients performed slightly worse on the CPT than controls (Mann-Whitney U test: $Z = 1.9$, $p = 0.058$), but did not differ in performance on the Digit Span ($Z = 1.2$, $p = 0.24$). Subsequent analyses revealed no significant correlations between CPT score and percentage adjustments within the patient group (Spearman $r = 0.29$, $p = 0.32$; $r = 0.18$, $p = 0.35$ for valid and invalid feedback respectively). Within the control group CPT scores also did not correlate with performance[1].

We additionally investigated whether severity of depression influenced adjustment rates of patients by means of two regression analyses (enter method) including HRSD-score and age as predictors. Age was added because it also might affect performance. We found no influence of severity of depression ($R = 0.12$, $F(2,12) = 0.09$, $p = 0.92$, and $R = 0.11$, $F(2,12) = 0.07$, $p = 0.93$, for valid and invalid feedback respectively).

[1] Non-parametric tests were used because the CPT and Digit Span scores were not normally distributed.

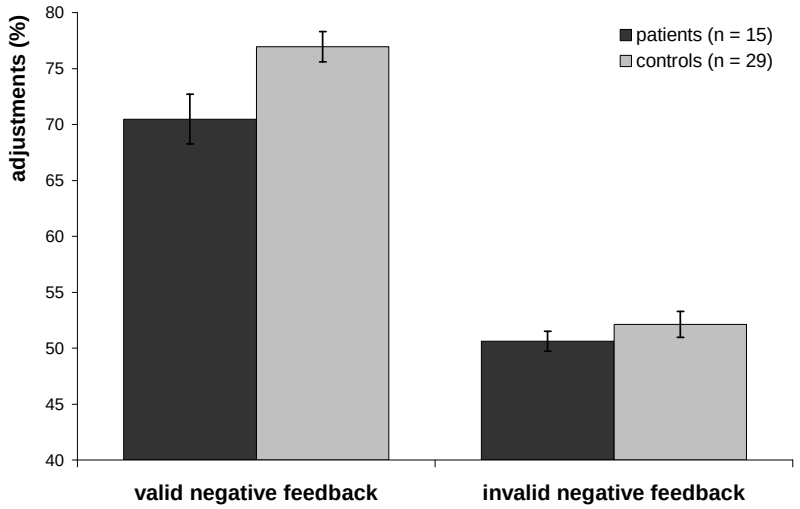

Figure 4.1 Mean percentage of correct adjustments after valid negative feedback and mean percentage of adjustments in accordance with the valence of the invalid negative feedback for patients and controls (with SEM).

Electrophysiological results

Figure 4.2 shows the grand-average ERPs and difference waves at channel Fz, Cz, and Pz for patients and controls. Patients had significantly larger FRNs than controls ($F(1,41)$ = 7.94, p = 0.007; -3.8 ±2.7μV vs. -1.8 ±1.4μV at Fz after valid feedback for patients vs. controls). The FRN was significantly different from zero in both groups (all p's < 0.001). We also found a main effect of channel ($F(4,164)$ = 4.64, p = 0.003) indicating that the FRN was largest at fronto-central electrode sites (-2.4 ±2.1, -2.3 ±1.9, -2.3 ±1.9, and -2.4 ±1.8μV, for valid and invalid feedback at Fz and Cz respectively, and, to compare, these values were -2.1 ±1.5μV and -2.0 ±1.6μV at Pz). We additionally found a three-way interaction between validity, channel and group ($F(4,164)$ = 2.65, p = 0.046). Patients showed a slightly, but not significantly, larger response to valid feedback than to invalid feedback at Fz, whereas control participants showed this effect at Pz (both p's > 0.1 in follow-up analyses for the patients and controls separately).

To evaluate whether the increased FRN in patients was primarily caused by a difference in response to negative feedback or to positive feedback, we additionally calculated the peak negativity at Fz between 200 and 350 ms in the baseline-corrected ERPs associated with positive and negative feedback separately. A repeated-measures analysis with valence and validity as within-subjects factors and group as between-subjects factor revealed a main effect of valence ($F(1,41)$ = 12.9, p = 0.001) and a marginal

interaction between valence and group (F(1,41) = 3.4, p = 0.074). Subsequent t-tests comparing patients and controls on these peak values revealed no significant effects for either positive or negative feedback. This suggests that the patients' differential FRN response was caused by a combination of differential responses to both negative and positive feedback.

We did another analysis for a later time window corresponding to the feedback P3, a positive-going ERP component that is thought to reflect the evaluation of feedback outcome (e.g., Mathewson *et al.*, 2008, Wu and Zhou, 2009). For this purpose we calculated the mean amplitude of the baseline-corrected ERP waveforms between 350 and 500 ms after feedback onset for each of the four feedback conditions. Valence, validity and channel were used as within-subjects factors, and group as between-subjects factor in a repeated-measures analysis.

Results were similar to the FRN findings. We found a main effect of valence (F(1,41) = 26.1, p < 0.001), a main effect of channel (F(4,164) = 10.0, p < 0.001), and an interaction between valence and channel (F(4,164) = 11.2, p < 0.001). As can be seen in Figure 4.2, this indicates that participants had larger P3 amplitudes for positive feedback than for negative feedback, and this effect was larger at posterior-central recording sites. Importantly, there was a marginally significant interaction between valence and group (F(1,41) = 4.0, p = 0.053). Patients had a slightly larger difference between positive and negative feedback in this time window than controls. This was not specifically due to either a larger response to positive feedback or a smaller response to negative feedback, as follow-up tests for positive and negative feedback, separately, showed no significant group differences (all p's > 0.1).

We also investigated whether severity of depression had an influence on the FRN by means of two regression analyses (enter method), including the FRN at Fz for valid and invalid feedback as dependent variables, and HRSD-score and age as predictors. Age was added in this analysis because it has been found to affect FRN-size (Eppinger *et al.*, 2008, Mies *et al.*, 2011b, Wild-Wall *et al.*, 2009). Severity of depression appeared to have no influence on the FRN in response to invalid feedback (R = 0.3, F(2,11) = 0.56, p = 0.59). Although the analysis on valid feedback did not reach significance either, the FRN-response appeared to decrease with symptom severity (R = 0.54, F(2,11) = 2.22, p = 0.16, HRSD: standardized beta = 0.53, t(11) = 1.94, p = 0.079; see Figure 4.3). This latter result is most likely due to a lack of power (power = 0.45, Cohen 1988).

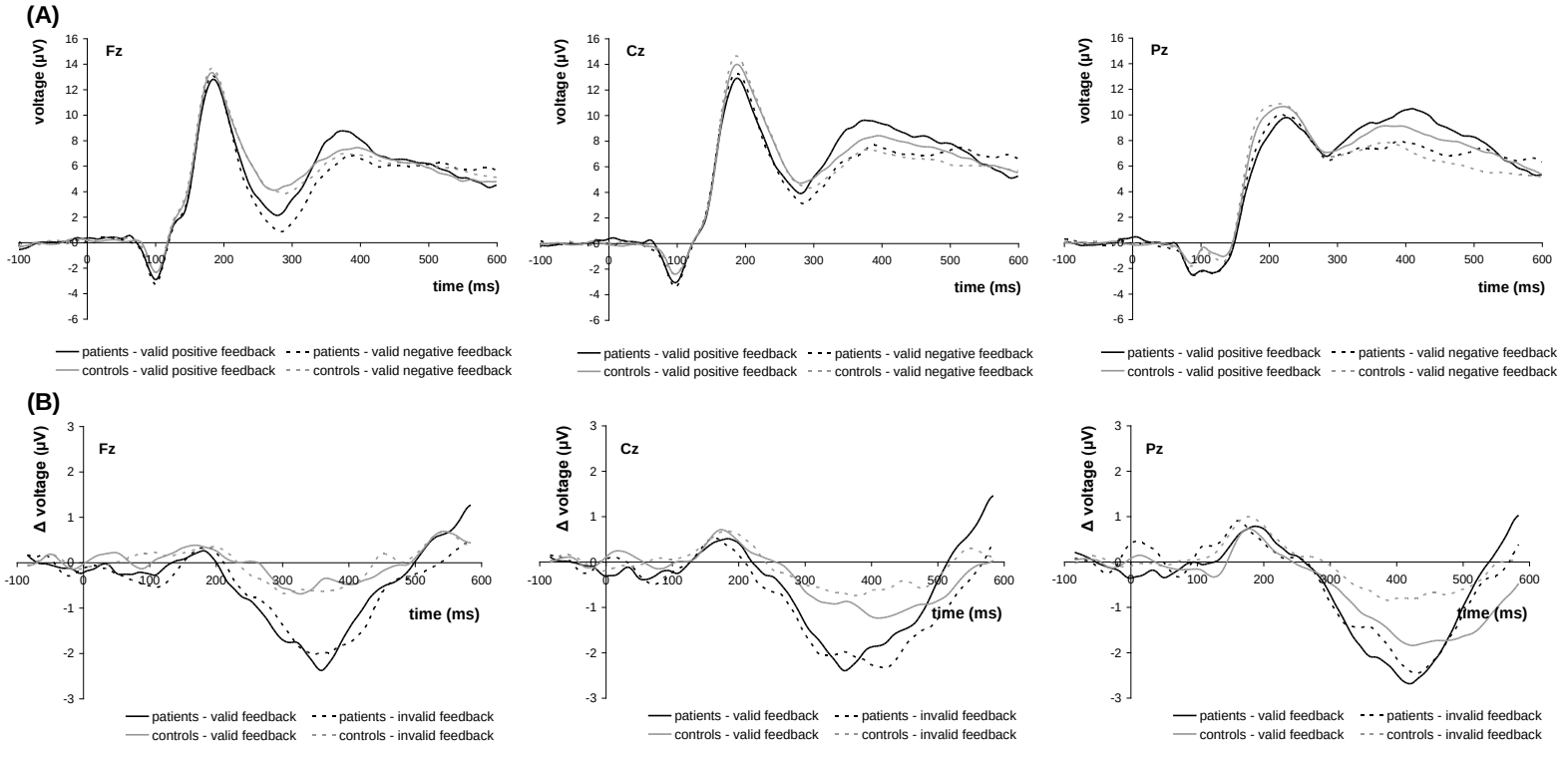

Figure 4.2 (A) Grand-average event-related brain-potentials from the midline electrodes Fz, Cz and Pz evoked by positive and negative feedback in the valid condition only for patients and controls. (B) The corresponding difference waves (negative minus positive feedback) for valid and invalid feedback for patients and controls.

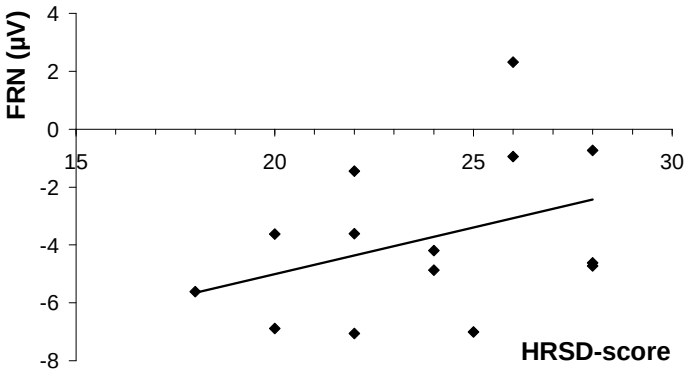

Figure 4.3 FRN-size of patients at electrode position Fz for valid feedback as a function of HRSD-score (severity of depression).

Discussion

The goal of our study was to investigate behavioural and electrophysiological responses to valid and invalid feedback in patients with MDD. A major strength of this study is that we included patients with a relatively high HRSD-score who were drug-free at the time of testing. The behavioural results show that patients were just as capable as non-depressed controls to ignore invalid negative feedback. When feedback was valid, however, they made fewer correct adjustments than controls. The electrophysiological results show, in line with expectations, that patients had larger FRNs than controls, independent of the validity of the feedback.

In line with most studies (Beats *et al.*, 1996, Douglas *et al.*, 2009, Elliott *et al.*, 1997, Elliott *et al.*, 1996, Fladung *et al.*, 2010, Steffens *et al.*, 2001), patients tended to make fewer correct adjustments after valid negative feedback than controls. In contrast with previous reports, however, patients did not adjust their behaviour after invalid negative feedback. This difference with the studies by Murphy *et al.* (2003) and Taylor Tavares *et al.* (2008), in which patients changed their behaviour after misleading negative feedback, is most likely due to paradigmatic differences. In our time-estimation paradigm, feedback-validity was explicitly communicated to patients. In the probabilistic reversal learning paradigm used in the previous reports, patients might have experienced uncertainty about the validity of the feedback, because negative feedback sometimes indicated a rule reversal. Therefore, the misleading negative feedback was ambiguous, and might have induced negative emotion, in line with the negativity bias from which patients are known to suffer.

The electrophysiological results show that patients had larger FRN-responses than controls, which is in line with other studies (Santesso *et al.*, 2008, Tucker *et al.*, 2003), and this effect extended into the feedback P3. This larger FRN was due to the combined effect of a somewhat larger response to negative feedback and a smaller response to positive feedback. To our knowledge, we are the first to show this effect in drug-free inpatients. The larger FRN was not limited to valid feedback signals. Invalid feedback also led to larger FRNs. Importantly, patients did not differ from controls in this regard: both groups showed a similar-sized FRN to valid and invalid feedback, but in patients the FRN was larger in both conditions.

The FRN is thought to be generated in the anterior cingulate cortex (ACC). Imaging studies have shown structural alterations, i.e., reductions in gray matter (Koolschijn *et al.*, 2009, van Tol *et al.*, in press), and functional alterations in this brain region in patients with MDD. The dorsal ACC (dACC), known for its involvement in cognitive control (Bush *et al.*, 2000), has been found hypoactive in depression, while the rostral/ventral ACC (rACC), known for its involvement in emotion processing, has rather consistently been found hyperactive (Davidson *et al.*, 2002, Mayberg, 1997, 2003, Pizzagalli, 2011). The present FRN and behavioural results are in line with a previous fMRI study in which we used the same task (Mies *et al.*, 2011a). In this latter study we found the rACC primarily sensitive to valence, while the dACC was sensitive to validity. In time-estimation tasks, the rACC is likely to be the generator of the FRN (Nieuwenhuis *et al.*, 2005b). Atypical valence processing in this region might account for the increased FRN-responses found in the MDD group. Since the rACC is less sensitive to the validity of the feedback, this might explain why the FRN-response did not distinguish between valid and invalid feedback. The validity of the feedback is, however, also being evaluated, as both patients and controls adjusted their performance after valid, but not after invalid negative feedback. This evaluation probably involves the dACC, a possibility that is consistent with a recent study by Van der Veen *et al.* (2011), showing that activity in the dACC was related to behavioural adjustments in a time-estimation task. Since the dACC is mainly active in response to valid feedback (Mies *et al.*, 2011a), impaired performance after valid negative feedback in our study and those of others might be related to a decreased dACC-response in patients.

In line with previous studies, our results show that MDD patients have increased FRN-responses, probably due to atypical rACC activity. Studies on a similar component, the error-related negativity (ERN), however, show inconsistent results. The ERN occurs after a self-detected error, and is in many aspects similar to the FRN. They are considered to be reflections of the same general performance monitoring system, and are both manifestations of activity in the ACC. The ERN is, however, thought to be generated in the dACC (Ridderinkhof *et al.*, 2004). Several studies reported *larger* ERNs in depressed or otherwise affectively distressed individuals compared to controls (Chiu and Deldin, 2007, Hajcak *et al.*, 2003a, 2004, Holmes and Pizzagalli, 2008, 2010, Johannes *et al.*, 2001b, Luu

et al., 2000). Others, however, have found equivalent or *smaller* ERNs (Compton *et al.*, 2008, Olvet *et al.*, 2010b, Ruchsow *et al.*, 2006, Ruchsow *et al.*, 2004, Schrijvers *et al.*, 2008, Schrijvers *et al.*, 2009). Discrepant results could perhaps be caused by different contributions from the dorsal and rostral ACC, since error and feedback processing encompass both cognitive and affective processing. Besides, the FRN appears to be the sum of several components from different sources (Foti *et al.*, 2010). It is, therefore, possible that both ACC subdivisions play a role in the generation of the ERN and FRN, but, depending on the specific paradigm used, which may be more affective or more informational, the rostral or dorsal part may prevail.

In contrast to our present study, virtually all patients in the abovementioned ERN studies used medication. The use of different types of medication might play an important role in the discrepancies between studies. The patients included in the studies by Schrijvers *et al.* (2008, 2009) are the most comparable to our patients with respect to symptom severity, but they found no difference in ERN-response between patients and controls. Half of the patients in the Schrijvers *et al.* (2008) study, however, used benzodiazepines and the authors showed that this subgroup had attenuated ERN-responses compared to controls. This attenuating effect of benzodiazepines has been found in healthy volunteers as well (De Bruijn *et al.*, 2004, Johannes *et al.*, 2001a), and has been explained by GABAergic pathways directly inhibiting ACC-activity (De Bruijn *et al.*, 2004). The serotonin system is also thought to play a role in performance monitoring and feedback processing (Evers *et al.*, 2005, Fallgatter *et al.*, 2004, Jocham and Ullsperger, 2009, Van der Veen *et al.*, 2010, Van der Veen *et al.*, 2008), however, SSRIs do not seem to affect ERN size (De Bruijn *et al.*, 2006, Stern *et al.*, 2010). Norepinephrine reuptake inhibitors, on the other hand, appear to *increase* the ERN (Jocham and Ullsperger, 2009, Riba *et al.*, 2005). These neurotransmitter systems at the source of antidepressant action might therefore lead to differences in ERN and FRN responses via their influence on the subdivisions of the ACC.

Before closing, it should be noted that this study has some limitations. Although we only included patients who were drug-free, there might have been long-lasting effects of previous medication on the brain, such as altered number and sensitivity of receptors, and perhaps some withdrawal effects. Co-morbidity such as an anxiety disorder might also have had an influence on the response to feedback, since most patients suffered to some degree from co-morbid anxiety. In addition, we failed to observe a significant relationship between severity of depression and FRN-size. This is probably due to a lack of statistical power. Alternatively, this failure could be due to the selection of a homogeneous group of patients with rather severe depression. Our patients are therefore difficult to compare with the less severely depressed patients of Tucker *et al.* (2003), who found increased FRN-responses in moderately depressed, but not in more severely depressed patients. It

should be noted, however, that our results, although statistically not significant, do point in the same direction.

In conclusion, we showed that patients with MDD did not change their behaviour in response to invalid negative feedback. When feedback was valid, however, patients performed slightly worse than controls. These results support the idea postulated by Murphy *et al.* (2003) that depressed patients' responses to negative feedback are context-dependent. In addition, patients had larger FRN-responses than controls, irrespective of the information communicated by the feedback (valid or invalid). This implies that drug-free inpatients have an atypical rACC response to feedback, which is independent of context. If this atypical response can be normalized, with the use of antidepressants and/or cognitive therapy, perhaps the patients' sensitivity to a relapse decreases. Future studies should therefore focus on the effects of chronic medication use and cognitive therapy on feedback processing in homogeneous groups of depressed patients.

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Chapter 5

Neurophysiological correlates of depressed mood and anhedonia on feedback processing

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Abstract

Disturbances in feedback processing and a dysregulation of the cortico-limbic circuit have been frequently observed in depression. There is some evidence that these disturbances might already be present in people at risk for depression, perhaps predisposing an individual to depression. This study, therefore, investigated the roles of the different subdivisions of the anterior cingulate cortex (ACC) in feedback processing, comparing undergraduates with mild depressive symptoms to those without. In addition, the two main symptoms of depression were examined separately: depressed mood and anhedonia. Participants had to perform a time-estimation task in which they received positive and negative feedback that was either valid or invalid (i.e., feedback related vs. unrelated to actual performance). No difference in performance was found between the two groups, nor was performance related to depressed mood or anhedonia. The subgenual ACC, however, was more active in response to feedback in undergraduates with higher levels of depressed mood and anhedonia. The rostral cingulate zone (RCZ), corresponding to the dorsal part of the ACC, was less active in response to feedback in the more anhedonic individuals. Interestingly, the differential response of the RCZ to the validity of the feedback was decreased in individuals with higher levels of depressed mood. This study therefore shows differential neurophysiological correlates of depressed mood and anhedonia on feedback processing. These different cortico-limbic responses might render individuals vulnerable to depression.

Introduction

Major depressive disorder (MDD) is a serious mental illness, characterized by at least one of two core symptoms: depressed mood and anhedonia (i.e., the loss of pleasure). MDD affects both affective and cognitive functioning. One of the deficits in MDD in which cognition and affect both play a role is impaired feedback processing. Behavioural studies have shown that depressed individuals are hypersensitive to negative feedback. When they make an error or receive negative feedback on their performance, their subsequent performance deteriorates (e.g., Beats *et al.*, 1996, Elliott *et al.*, 1997, Steffens *et al.*, 2001). More recent studies have shown that this maladaptive response to negative feedback is context-dependent (Mies *et al.*, in press, Murphy *et al.*, 2003, Taylor Tavares *et al.*, 2008). In a study by Murphy *et al.* (2003) patients responded normally to accurate negative feedback in a spatial working memory task, but they responded differently to misleading negative feedback in a probabilistic reversal learning task. The MDD patients were more likely to switch their behaviour after misleading negative feedback than the healthy controls. The authors suggested that negative feedback that is more affective in nature might disrupt performance, while negative feedback that is more informational might not. This suggests that the information value or validity of feedback, and the valence of feedback (positive or negative) are differently processed by MDD patients than by healthy persons. In a previous study we examined this by using a time-estimation task with valid (informative) and invalid (uninformative) positive and negative feedback. We found that MDD patients adjusted their behaviour less often than healthy controls when feedback was valid, but they were equally able to ignore invalid negative feedback (Mies *et al.*, in press).

In addition to these aberrant behavioural responses, depressed patients have been found to show an increased electrophysiological response to negative feedback, reflected by the feedback-related negativity (FRN; Mies *et al.*, in press, Santesso *et al.*, 2008, Tucker *et al.*, 2003), an event-related brain potential (ERP) component that occurs after receiving negative feedback (Miltner *et al.*, 1997). In contrast to behavioural responses, this increased electrophysiological response appears to be independent of context (Mies *et al.*, in press).

The FRN is presumed to be generated in the anterior cingulate cortex (ACC; Ridderinkhof *et al.*, 2004). The ACC can be divided in two subdivisions: a dorsal part (dACC) and a ventral part, which can be further subdivided into a rostral (rACC) and subgenual part (sgACC). Early theories suggested that the dorsal part is primarily involved in cognitive processing, while the ventral part is more involved in emotion processing (Bush *et al.*, 2000). Recent observations, however, seem to challenge this model and point in the direction of both subdivisions being involved in negative emotion processing (Etkin *et al.*, 2011, Shackman *et al.*, 2011).

A part of the dACC, the rostral cingulate zone (RCZ), has received a lot of attention in the literature on error and feedback processing, since it has repeatedly been found more active during errors, conflict and negative feedback than during correct responses and positive feedback (Ridderinkhof *et al.*, 2004). There is, however, accumulating evidence that the rACC plays an important role in feedback processing as well (Mies *et al.*, 2011a, Nieuwenhuis *et al.*, 2005b, Van Veen *et al.*, 2004).

In depressed individuals, brain regions primarily involved in emotion processing, such as the amygdala and sgACC, have been found *hyper*active, while the regions thought to be mainly involved in cognitive control, such as the dACC, have been found *hypo*active (Davidson *et al.*, 2002, Mayberg, 1997, 2003, Pizzagalli, 2011). It is therefore thought that the top-down control of these 'cognitive' areas over the 'affective' areas is disturbed in depression (e.g., Taylor Tavares *et al.*, 2008). This dysregulation appears to persist in fully recovered patients (Hooley *et al.*, 2009), which may make them vulnerable to a relapse. It is, however, possible that this dysregulation is not a *result* of the depression, but *predisposes* an individual to develop a mood disorder such as MDD. In this light it is interesting to investigate healthy persons at risk of developing depression.

Differences in the cortico-limbic circuit in healthy individuals at risk for depression, such as those with a family history of depression, subclinical depression-related symptoms or personality traits, have not been thoroughly investigated yet. Most effects have been found for the ventromedial prefrontal cortex or sgACC. This area has been found more active in healthy or subclinical subjects with high neuroticism scores (Haas *et al.*, 2007), higher self-ratings of negative affect (Zald *et al.*, 2002), and higher levels of trait anhedonia (Harvey *et al.*, 2007), either in rest, or in response to emotional or conflicting stimuli. Daughters of mothers with recurrent MDD have been found to show increased dACC activity in response to negative feedback, and decreased activity in this region in response to positive feedback as compared to controls (Gotlib *et al.*, 2010).

In the present study, we aimed to identify a dysregulation in the cortico-limbic circuit in response to feedback in subclinically depressed subjects, by comparing undergraduates who displayed mild depressive symptoms (a score of at least 10 on the Beck Depression Inventory, BDI) with those without any symptoms. For this purpose, a time-estimation paradigm was used with two important dimensions of feedback: valence (positive vs. negative feedback) and validity (valid vs. invalid feedback, i.e., feedback that is informative and therefore relevant for behavioural adjustments vs. uninformative or irrelevant feedback). In a previous study, this paradigm showed different roles of the rACC and RCZ in feedback processing. The RCZ was primarily sensitive to the validity of the feedback, and the rACC to the valence of the feedback (Mies *et al.*, 2011a). In the current study we expected to find differences in neural responses to feedback between the high-BDI and low-BDI group. Parallel to the findings in depression, we expected decreased activity in the RCZ and increased activity in the rACC, sgACC, and amygdala in the

individuals with mild depressive symptoms compared to those without symptoms. We further expected that the effect of validity in the RCZ would be smaller or absent in the individuals with mild depressive symptoms because of diminished cognitive control, while the effect of valence in the rACC would be larger. This might, in addition, lead to different interactions between valence and validity within these brain regions. At the behavioural level, we expected, on the basis of our results in depressed inpatients (Mies *et al.*, in press), that the mildly depressed individuals would make fewer correct adjustments after valid negative feedback than controls.

In addition to examining the influence of the broad range of depressive symptoms, as measured with the BDI, we separately investigated the effects of the two core symptoms of depression on feedback processing, that is, depressed mood and anhedonia. In most studies these symptoms are not separated, although it is known that depressed mood is associated with increased negative affect, while anhedonia is associated with decreased positive affect (Pizzagalli *et al.*, 2005, Snaith, 1993), and that positive and negative affect are two independent constructs (Watson *et al.*, 1988). Anhedonia has been associated with a blunting of behavioural and neural responses to the valence of stimuli (Dowd and Barch, 2010, Steele *et al.*, 2007). Therefore, we hypothesized that anhedonia would cause a blunted neural response to the valence of the feedback in the rACC, i.e., a smaller difference between responses to positive and negative feedback. Depressed mood has been associated with a negativity bias, i.e, the tendency to interpret ambiguous information in a negative way (e.g., Bouhuys *et al.*, 1995). This might lead to a negative interpretation of both valid negative feedback and invalid feedback, bringing these two closer together. We therefore expected that depressed mood would lead to a blunted neural response to the validity of the feedback in the RCZ, i.e., a smaller difference between responses to valid and invalid feedback.

Materials and methods

Participants

Participants were recruited by means of advertisements on college-wide electronic bulletin boards of the Erasmus University and the Erasmus MC – University Medical Centre Rotterdam. The study was approved by the ethics committee of the Erasmus MC and all participants gave written informed consent. Participants received EUR 25 for participation.

Respondents were asked to fill out the Dutch translation of the Beck Depression Inventory (BDI; Beck *et al.*, 1961, Bouman *et al.*, 1985) assessing depression severity, and a short questionnaire assessing eligibility for participation in an MRI study. Unbeknownst to the respondents, only those who had a low score on the BDI (<3) and those who had a high score (≥10) were invited to participate and were further screened for eligibility. These cutoffs were chosen because a score of 10 on the BDI is generally accepted as indicative of

mild depressive symptoms (e.g., Bouman *et al.*, 1985), and because we wanted to create two extreme groups for the main depression analyses, making sure that depressive symptoms were virtually absent in the low-BDI group. Because there was a delay of a couple of weeks between online screening and the first visit (the actual time of testing), the score on the BDI could change. Respondents who were asked to participate, but scored between 3 and 9 on the BDI during the time of testing, were not included in the main analyses on depression, but were included in the analyses on the core symptoms of depression.

Exclusion criteria were: neurological illness, severe somatic illness, psychiatric illness other than depression, current treatment for any psychiatric illness (including depression), substance abuse, use of medication which affects the central nervous system, pregnancy, and any contra-indication for having an MRI-scan. Health was assessed by means of a self-developed questionnaire and contra-indications for MRI were assessed by means of a standard questionnaire from the department of Radiology.

Eventually, 42 healthy volunteers, 26 female, aged between 18 and 32 (M=23, SD=3.5), participated in this study.

Questionnaires

We used the BDI to assess depressive symptoms in general. To further specify the core symptoms of depression, we used the Dutch version of the shortened Profile of Mood States (POMS; McNair *et al.*, 1971, Wald and Mellenbergh, 1990) to assess depressed mood, and the Dutch version of the Snaith-Hamilton Pleasure Scale (SHAPS; Franken *et al.*, 2007, Snaith *et al.*, 1995) to assess anhedonia.

The BDI consists of 21 items, each including four statements (ranging from 0 to 3), assessing several symptoms of depression experienced in the last week. High scores indicate more depressive symptoms. The visual analogue version of the shortened POMS consists of 32 bipolar adjectives to assess current mood. For each pair of adjectives, scores range from 0 to 100, based on how many millimeters from the left participants made a mark on the line. This version of the POMS measures five dimensions: depression, anger, fatigue, tension, and vigor. The dimension 'depression' was used as a measure for depressed mood. Finally, the SHAPS consists of 14 items to be answered on a 1-4 scale. Higher total scores indicate higher levels of anhedonia.

Other questionnaires participants had to fill out were the Dutch versions of the Eysenck Personality Questionnaire (EPQ-RSS; Eysenck and Eysenck, 1975, Sanderman *et al.*, 1991), State and Trait Anxiety Inventory (STAI; Spielberger *et al.*, 1970, Van der Ploeg *et al.*, 1980), and Behavioural Inhibition System and Behavioural Approach System scales (BIS/BAS; Carver and White, 1994, Franken *et al.*, 2005). These questionnaires were used to validate the distinctness of our selected groups on the basis of the BDI.

Time-estimation task

The time-estimation task used in the present study was the same as reported earlier (Mies *et al.*, 2011a, Mies *et al.*, 2011b). Participants were instructed to produce 1 s intervals. Each trial started with the presentation of an asterisk ("*") in the centre of a black screen for 2 s. This asterisk was followed by the cue for estimation: a question mark ("?"), which was replaced with another asterisk (1 s) after the estimation. This second asterisk was followed by the feedback stimulus (1 s).

Participants had to indicate the end of the one-second interval by pressing the button of a response device. Following the button press, they received performance feedback, i.e., positive feedback if their response occurred within a specified window around the target (900-1100 ms), and negative feedback if the response occurred outside the window. Unbeknownst to participants, the window was dynamically adjusted to ensure an equal amount of positive and negative feedback stimuli (see Miltner *et al.*, 1997).

Estimates were followed by feedback. The feedback consisted of face stimuli presented against a horizontal or vertical background grid. The background grid communicated the validity of the feedback stimulus to the participants (valid vs. invalid). Valid feedback was based on the participant's performance. Invalid feedback was determined randomly by the computer, with a maximum of three invalid feedback trials in a row. Participants received invalid feedback in 50% of the trials. The emotional expression of the face informed participants that their estimate was correct or incorrect (respectively, a happy vs. a fearful face). Finally, in case of incorrect estimates, the gender of the face indicated whether the estimate was too short (e.g., a male face) or too long (e.g., a female face). The faces used in this study were from the Ekman & Friesen pictures set (Ekman and Friesen, 1978).

Procedure

Participants were seen twice. The first time, participants were asked to fill out the questionnaires (BDI, SHAPS, EPQ-RSS, STAI, BIS/BAS), and they practiced the two tasks they had to perform in the scanner. The first task participants had to perform was the time-estimation task as described above. Participants were given task instructions and they completed 36 practice trials of the time-estimation task on a computer outside the scanner. The other task was an unrelated task, which is not described in this paper. Filling out these questionnaires and practicing the time-estimation task took about 35 minutes.

Within 4 days of this first meeting (in most instances the next day), participants were scanned. They were asked to abstain from coffee and tobacco for at least two hours before scanning. Participants first had to fill out the POMS, and were again given task instructions before entering the scanner. When participants were inside the scanner, the visual stimuli were projected on a screen at the end of the scanner bed, which could be

viewed by the participant through a small mirror mounted on the head coil. During the time-estimation task participants responded by pressing the button of a response device with their right index finger. Inside the scanner participants performed several practice trials (maximum of 36 trials), after which the first session started, consisting of 120 trials (10 minutes). After a short break, a second session of the task started which again lasted 10 minutes. Participants performed 240 trials of the time-estimation task inside the scanner. After these two time-estimation sessions a structural scan was obtained, which lasted about 5 minutes.

Magnetic Resonance Imaging data acquisition

Blood-oxygen-level-dependent (BOLD) fMRI data were acquired on a 3T GE Healthcare (Milwaukee, WI) scanner. For the functional scans a single-shot gradient echo echo-planar imaging (EPI) sequence was used. The T2*-weighted images were acquired in 26 axial slices (thickness = 3.5 mm, interslice gap = 0.5 mm) with a repetition time (TR) of 2000 ms, echo time (TE) of 30 ms, field of view (FOV) of 220 mm, and voxels of 1.72 x 1.72 x 3.50 mm. The interval between trials was about 5 seconds. In each session of 120 trials 310 volumes (8060 functional images) were obtained. In addition, five dummy scans were made before the task started in order to obtain a steady-state magnetization.

For anatomical reference, a 3D high-resolution inversion recovery fast spoiled gradient recalled echo T1-weighted sequence was used, which covered the whole brain. 192 slices were acquired with an effective slice thickness of 0.8 mm, FOV of 250 mm, and voxels of 0.49 x 0.49 x 0.80 mm.

For preprocessing and processing of the fMRI data SPM5 (Statistical Parametric Mapping, Wellcome Trust Centre for Neuroimaging, University College London, UK) was used. Preprocessing of the structural data included manual reorienting, segmentation using the Montreal Neurological Institute T1 templates for gray matter, white matter, and CSF, and normalization. Preprocessing of the functional data included manual reorienting, slice time correction, realignment using the middle slice as a reference, and unwarping, co-registration (functional images were co-registered to the gray matter structural image derived from segmentation), normalization, and smoothing using a Gaussian kernel of 8 mm full width at half maximum, and a high-pass filter of 128 seconds for temporal smoothing.

Statistical analyses

Performance data were analyzed by comparing the percentage of correct adjustments after valid negative feedback with the percentage of 'correct' adjustments after invalid negative feedback by means of repeated-measures ANOVAs with 1) BDI-group as

between-subjects factor, 2) POMS-depression scores as covariate, and 3) SHAPS scores as covariate[1].

For the fMRI analyses, a model was made in which the preprocessed fMRI data were coupled to the vectors of feedback onset of each condition (valid positive feedback, valid negative feedback, invalid positive feedback, and invalid negative feedback) in both task sessions. Then two t-contrasts were computed: positive – negative feedback (main effect of valence), and valid – invalid feedback (main effect of validity). The individual contrast images resulting from these contrasts were used in a second-level analysis.

First, whole-brain analyses were performed on the two contrasts: 1) independent of group (one-sample t-test); 2) comparing the high-BDI group with the low-BDI group (two-sample t-test); and 3) adding the POMS-depression score and the SHAPS-anhedonia score separately as covariates in the first analysis. Significant voxels and clusters are reported as significant if $p < 0.05$ corrected with the family-wise error (FWE) approach. The AAL atlas was used to label the significant clusters and voxels.

Of main interest were, however, the region-of-interest (ROI) analyses. Four ROI analyses were performed using MarsBaR 0.41 (Brett *et al.*, 2002). The amygdala (AAL map of MarsBaR, Tzourio-Mazoyer *et al.*, 2002), the RCZ (8 mm sphere around ±8 30 32; coordinates adopted from Mars *et al.*, 2005 and implemented in the AAL map of MarsBaR), the rACC (8 mm sphere around 0 40 -2; coordinates adopted from Nieuwenhuis *et al.*, 2005b), and the subgenual ACC (sgACC, 8 mm sphere around 1 32 -6; coordinates adopted from Matthews *et al.*, 2009) were defined as ROIs. Figure 5.1 illustrates the different subdivisions of the ACC examined.

For the ROI analyses, beta-values were extracted from the fMRI data for each feedback condition separately. For each ROI, the extracted beta-values of each participant were exported to SPSS, and subsequently analyzed using valence (positive or negative feedback), feedback-validity (valid or invalid feedback) and hemisphere (left or right)[2] as within-subjects factors in repeated-measures ANOVAs. Not only main effects, but also interactions between valence, validity and depressive symptoms were therefore examined. In the BDI-depression analyses BDI-group was added as between-subjects factor. The examination of the influence of depressed mood and anhedonia was done by adding the POMS-depression scores and the SHAPS-anhedonia scores separately as covariates in the ROI analyses.

Only statistically significant effects ($p < 0.05$) and marginal effects of interest ($0.05 \leq p < 0.10$) are reported.

[1] The POMS-depression and SHAPS scores were both normally distributed, and were therefore centred by the method of Delaney and Maxwell (1981): the mean of all participants was subtracted from the absolute score.

[2] Hemisphere was left out as a within-subject factor in the analyses of the rACC and sgACC, because the coordinates of these regions were on the midline.

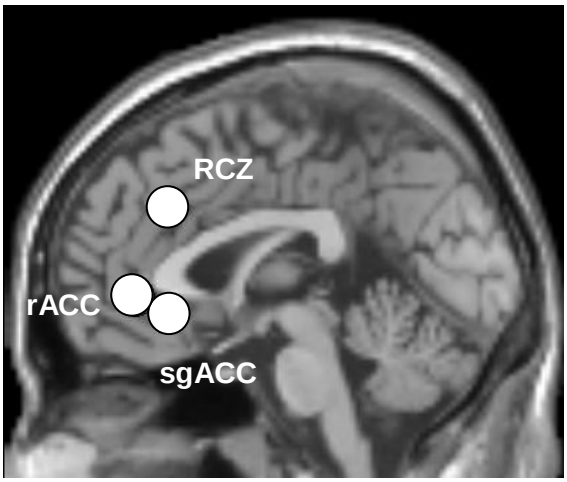

Figure 5.1 Indication of the anatomical location of the three subdivisions of the anterior cingulate cortex: the rostral cingulate zone (RCZ), rostral anterior cingulate (rACC), and subgenual anterior cingulate (sgACC), displayed on the SPM5 canonical single subject T1 image.

Results

The 42 participants included in the study consisted of 23 students with a high BDI-score (≥10) and 19 students with a low BDI-score (<3) at initial screening. At the time of testing (first visit: questionnaires), two students had dropped from a high score to a low score, and were therefore excluded from the BDI-depression analyses. Another 7 participants were excluded from the BDI-depression analyses because they had a score between 3 and 9 on the BDI at the time of testing. Furthermore, one student, in the high-BDI group, did not perform according to task instructions, and was therefore excluded from all analyses. Therefore, 32 participants were included in the BDI-depression analyses and 41 in all other analyses.

The high-BDI group (*N*=15; 11 females) had a mean BDI score of 14.9 ±4.4 (range 10-26), and the low-BDI group (*N*=17; 11 females) had a mean score of 0.8 ±0.8. Table 5.1 shows the scores of the high-BDI and low-BDI group on all self-report questionnaires. BDI-scores correlated positively with POMS-depression scores (r = 0.60, $p < 0.001$) and SHAPS-anhedonia scores (r = 0.52, p = 0.001). POMS-depression scores ranged from 8 to 457 (M=187, SD=96), and SHAPS scores ranged from 14-34 (M=22, SD=5).

Table 5.1 Scores on all self-report questionnaires for individuals scoring high and low on the BDI

		Depressive symptoms (BDI score)		
		low (n=17)	*high (n=15)*	*p-value*
BDI	Depression	0.8 (±0.8)	14.9 (±4.4)	<0.001
EPQ	Neuroticism	2.0 (±2.2)	8.1 (±2.9)	<0.001
	Psychoticism	2.5 (±1.8)	2.5 (±1.6)	1.00
	Extroversion	8.9 (±2.8)	8.6 (±3.4)	0.76
	Lie	4.2 (±2.8)	5.2 (±2.5)	0.32
STAI	Anxiety (trait)[a]	27.4 (±4.1)	50.0 (±8.3)	<0.001
	Anxiety (state)	26.9 (±4.6)	41.6 (±10.5)	<0.001
SHAPS	Anhedonia	19.8 (±4.9)	24.8 (±4.8)	<0.01
BIS/BAS	Inhibition (BIS)	18.8 (±2.6)	23.6 (±3.2)	<0.001
	Approach (BAS)	42.5 (±4.5)	38.2 (±4.7)	<0.05
POMS	Depression	130 (±60)	252 (±109)	<0.01
	Anger	116 (±50)	165 (±58)	<0.05
	Fatigue	153 (±90)	273 (±85)	<0.01
	Vigor	383 (±72)	310 (±63)	<0.01
	Tension	150 (±77)	179 (±90)	0.33

[a]data of two participants were incomplete for this questionnaire (one in each group)

Behavioural results

The behavioural responses to valid and invalid negative feedback did not differ between participants with high BDI scores and those with low scores. The two groups had equal mean time estimates (1056 ±120 ms in the low-BDI group vs. 1044 ±52 ms in the high-BDI group, $t(30) = 0.36$, $p = 0.72$), and more importantly, both groups adjusted their behaviour more often in response to valid negative feedback than in response to invalid negative feedback (84 ±9% vs. 51 ±6% in the low-BDI group; 86 ±7% vs. 54 ±7% in the high-BDI group; main effect of validity: $F(1,30) = 311.5$, $p < 0.001$; no interaction between validity and group: $F(1,30) = 0.0$, $p = 1.0$; no main effect of group: $F(1,30) = 2.0$, $p = 0.17$). Depressed mood, measured with the POMS, and anhedonia, measured with the SHAPS, did not influence the behavioural results either.

Whole brain analyses

The results of the whole brain analyses on the total group of participants are shown in Table 5.2 and 5.3. At the FWE-corrected threshold of $p < 0.05$, all contrasts revealed significant activation patterns, except negative feedback minus positive feedback. Importantly, the whole brain analyses did not reveal any significantly different activation patterns for participants with high or low BDI scores. Depressed mood and anhedonia had no influence either.

Table 5.2 Whole brain analyses including all participants (*N*=41) for the contrast positive feedback – negative feedback

Area	L/R	BA	Cluster size	Z	MNI coordinates x y z
positive feedback > negative feedback					
Insula/Putamen	L		830	6.92	-26 10 -12
Putamen	L		[a]	6.58	-26 -4 4
Putamen	L		[a]	6.56	-22 -6 16
Orbital medial frontal gyrus	R	10/11	584	6.82	4 54 -8
Medial frontal gyrus/Anterior cingulate	L	10	[b]	6.76	-8 48 2
Orbital medial frontal gyrus	L	11	[b]	6.54	-6 56 -10
Putamen	R		671	6.72	24 -8 12
Putamen	R		[c]	6.66	28 12 -10
Putamen	R		[c]	6.59	24 6 0
Putamen	R		[c]	6.40	26 -2 8
Precuneus/Posterior cingulate	L	23/31	153	6.46	-4 -56 24
Precuneus/Posterior cingulate	R	23	[d]	6.10	6 -50 26
Inferior occipital gyrus	R	18	31	6.37	28 -92 -2
Superior frontal gyrus	L	32	79	6.23	-16 36 42
Superior frontal gyrus	L	9	[e]	6.21	-22 26 40
Paracentral lobule	L		17	6.23	-14 -28 54

The AAL atlas and MRIcron were used to label the significant clusters and voxels. In some cases the nearest gray matter is shown. XJview was used to further specify these brain regions when necessary. [a,b,c,d,e]local maximum within cluster a, b, c, d, and e, respectively ($p < 0.0001$, FWE-corrected). To simplify only the significant activations at the more conservative FWE-corrected threshold of $p < 0.0001$ are shown.

Table 5.3 Whole brain analyses including all participants (*N*=41) for the contrasts valid – invalid feedback, and invalid – valid feedback

Area	L/R	BA	Cluster size	Z	MNI coordinates x y z
valid feedback > invalid feedback					
Insula	L	47	341	4.62	-30 18 0
Orbital inferior frontal gyrus	R		338	4.60	32 24 -6
Precentral gyrus	L	6	333	4.58	-54 4 18
Inferior parietal gyrus	L	40	740	4.56*	-46 -46 42
Middle frontal gyrus	R	46	296	4.56*	46 48 6
Inferior parietal gyrus	R	40	562	4.46*	52 -40 54
Caudate	R	25	549	4.37*	10 18 0
Mid cingulate	R	32	338	4.17*	4 26 40
Caudate	L	25	460	3.98*	-8 16 -2
invalid feedback > valid feedback					
Middle temporal gyrus	R	39	1212	6.39	52 -62 20
Middle frontal gyrus	L		1511	5.76	-26 26 34
Medial superior frontal gyrus	L	10	[a]	4.93	-6 56 18
Calcarine sulcus	L	17	3947	5.06	-14 -62 16
Superior parietal gyrus	R	5	[b]	4.95	18 -50 60
Precuneus	L		[b]	4.74	-8 -46 46
Superior temporal gyrus	R	42	2686	4.78	54 -30 18
Superior temporal gyrus	R	42	[c]	4.72	56 -28 14
Middle temporal gyrus	L	39	1046	4.78	-48 -68 22
Middle frontal gyrus	R	9	675	4.57*	30 30 36
Lingual gyrus	R	30	450	4.38*	10 -52 8
Superior temporal gyrus	L	41	1032	4.32*	-50 -32 20

The AAL atlas and MRIcron were used to label the significant clusters and voxels. In some cases the nearest gray matter is shown. XJview was used to further specify these brain regions when necessary.
[a,b,c]local maximum within cluster a, b, and c, respectively ($p < 0.05$, FWE-corrected)
*significant at cluster level only ($p < 0.05$, corrected)

Region-of-interest analyses

General task effects

First we investigated the effects of the task on the group as a whole. In line with our previous study, we found the RCZ more active in response to valid feedback than in response to invalid feedback ($F(1,40) = 8.1$, $p = 0.007$). This effect was strongest for the right hemisphere ($F(1,40) = 11.2$, $p = 0.002$). Hemisphere also interacted with valence ($F(1,40) = 10.0$, $p = 0.003$), and there was a marginal interaction between validity, valence, and hemisphere ($F(1,40) = 4.0$, $p = 0.054$). Post-hoc tests showed that the left hemisphere was more active in response to positive feedback than in response to negative feedback ($F(1,40) = 5.4$, $p = 0.026$), but the right hemisphere was not.

The rACC was more active in response to positive feedback than in response to negative feedback ($F(1,40) = 30.4$, $p < 0.001$).

The amygdala was also more activated by positive feedback than by negative feedback ($F(1,40) = 37.8$, $p < 0.001$), and this effect was larger in the valid condition than in the invalid condition ($F(1,40) = 25.2$, $p < 0.001$). The left hemisphere showed a larger difference between positive and negative feedback ($F(1,40) = 13.5$, $p = 0.001$), and this effect was strongest in the valid feedback condition ($F(1,40) = 5.1$, $p = 0.029$).

Finally, the sgACC did not respond differently to the different types of feedback.

Effects of depressive symptoms (BDI)

The first purpose of this study was to examine the effects of depressive symptoms, as measured with the BDI, on the four predefined ROIs in relation to these task effects. We found a three-way interaction between validity, valence and group ($F(1,30) = 4.3$, $p = 0.047$), indicating that the high-BDI group had a larger RCZ response to positive feedback than to negative feedback in the valid condition only ($t(14) = 2.2$, $p = 0.045$), while the low-BDI group did not show this difference.

Both the rACC response and the sgACC response to the different types of feedback did not differ between the two groups. In the amygdala, however, we found two marginal effects. Activity in the amygdala was slightly, but not significantly, increased in the high-BDI group, independent of task condition ($F(1,30) = 3.3$, $p = 0.081$), and the left hemisphere appeared more active than the right hemisphere in this group, while this was not the case in the low-BDI group ($F(1,30) = 3.3$, $p = 0.079$).

Effects of depressed mood (POMS)

The second purpose was to examine the separate influences of depressed mood and anhedonia on feedback processing. Depressed mood interacted with the effect of validity in the RCZ ($F(1,39) = 4.5$, $p = 0.041$). As expected, individuals with higher levels of depressed mood showed less difference between valid and invalid feedback (Figure 5.2A). No effects of depressed mood were found on activity in the rACC, or amygdala, but there was a main effect of depressed mood on the sgACC ($F(1,39) = 4.4$, $p = 0.043$), indicating that the sgACC was more active in response to feedback in participants with higher levels of depressed mood (Figure 5.2B).

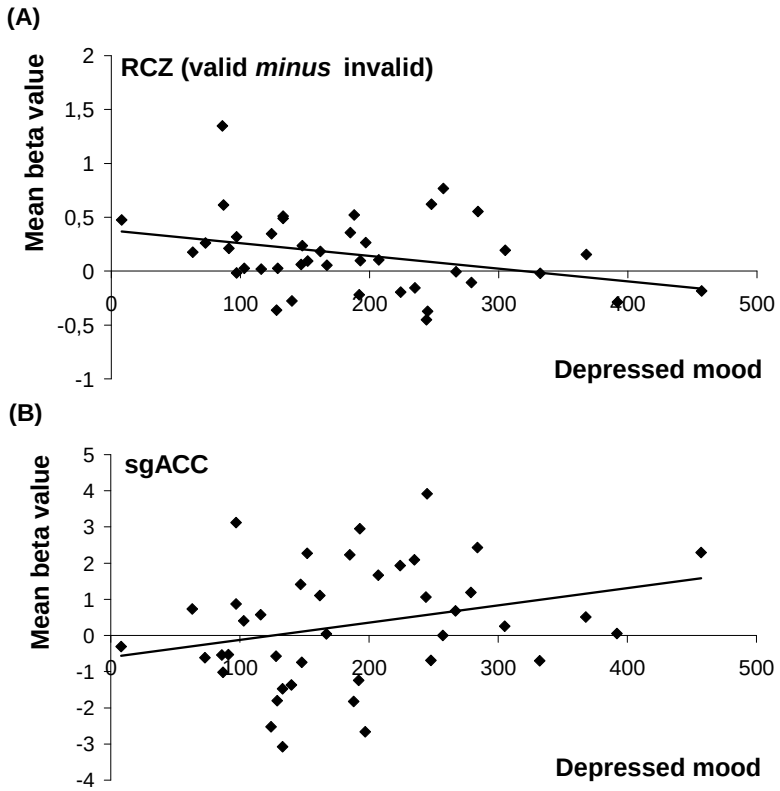

Figure 5.2 (A) Scatter plot of mean beta values representing the difference in activity in the rostral cingulate zone (averaged across both hemispheres) between valid and invalid feedback as a function of depressed mood (measured with the POMS). (B) Scatter plot of mean beta values representing activity in the subgenual anterior cingulate as a function of depressed mood.

Effects of anhedonia

In the RCZ we found a main effect of anhedonia ($F(1,39) = 7.7$, $p = 0.009$). Higher levels of anhedonia were associated with decreased activity in the RCZ, independent of task condition (Figure 5.3A). Furthermore, we found a marginal interaction between hemisphere and anhedonia ($F(1,39) = 3.6$, $p = 0.064$); in the more hedonic individuals, the right RCZ was slightly more active in response to feedback than the left RCZ. Also in the sgACC a main effect of anhedonia was found. In this area higher levels of anhedonia were associated with increased activity, independent of task condition ($F(1,39) = 8.0$, $p = 0.007$; Figure 5.3B). In the rACC we found a marginal interaction between anhedonia and valence: more hedonic individuals showed a slightly larger difference between positive and negative feedback ($F(1,39) = 3.7$, $p = 0.062$). The level of anhedonia had no effect on the amygdala response.

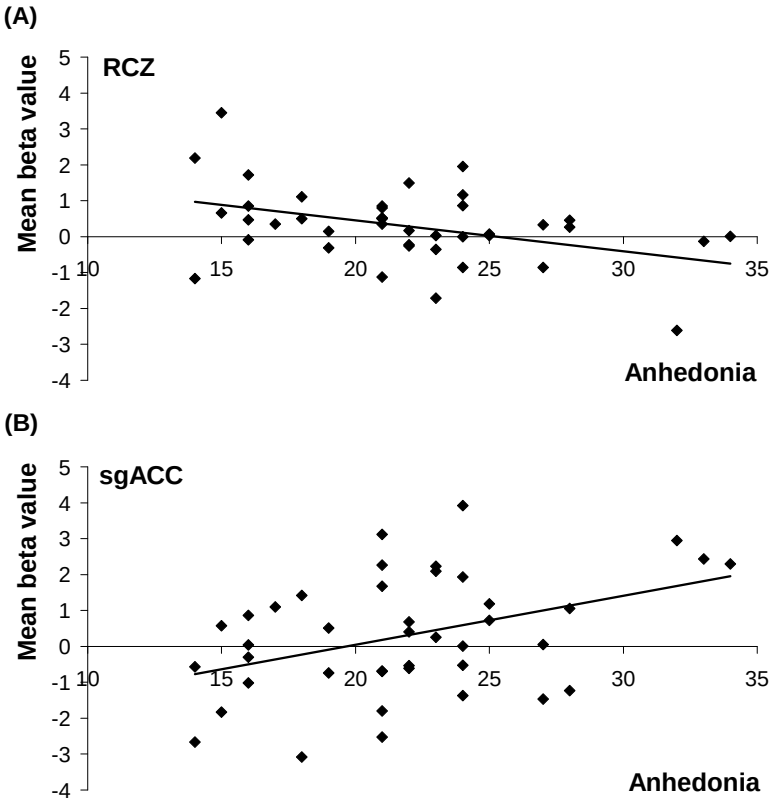

Figure 5.3 Scatter plots of mean beta values representing activity in (A) the rostral cingulate zone (averaged across both hemispheres), and (B) the subgenual anterior cingulate as a function of anhedonia (measured with the SHAPS).

Discussion

The current study investigated the behavioural and neural responses to valid and invalid positive and negative feedback in students with mild depressive symptoms compared to those without depressive symptoms. The two core symptoms of depression, depressed mood and anhedonia, were specifically examined as well. General symptoms of depression, depressed mood and anhedonia did not influence the behavioural responses to feedback. At the neural level, however, depressed mood decreased the differential response of the RCZ to valid and invalid feedback. In addition, independent of the type of feedback, activity in the sgACC was positively correlated with both depressed mood and anhedonia, while activity in the RCZ was negatively correlated with anhedonia only.

The general task effects are in line with our previous studies (Mies *et al.*, 2011a, Mies *et al.*, 2011b). At the behavioural level, participants performed according to task instructions: they adjusted their behaviour more often in response to valid negative feedback than in response to invalid negative feedback. At the neural level, we again found the RCZ sensitive to the validity of the feedback and the rACC sensitive to the valence of the feedback. The sgACC was neither sensitive to the valence, nor to the validity of the feedback, which implies that it does not play a major role in feedback processing.

The core symptoms of depression 'depressed mood' and 'anhedonia' differently affected activity in the RCZ in response to feedback. Depressed mood decreased the sensitivity of the RCZ to the validity of feedback. This finding suggests that depressed mood decreases the evaluation of the relevance of the feedback, possibly due to decreased cognitive control. We expected that this blunted response to feedback-validity would have been present in the high-BDI group as well. Our results, however, show that this effect is specific for the core symptom depressed mood. Other measures related to depression such as neuroticism, anxiety, BIS, and BAS did not contribute to this effect, except fatigue, which marginally interacted with the validity of feedback as well (data not shown). Although the blunted response to feedback-validity was not expressed at the behavioural level, it should be noted that the clinically depressed subjects in our previous ERP study did show slightly impaired performance after valid negative feedback during the same task (Mies *et al.*, in press), which may have been due to their depressed mood.

While depressed mood influenced the evaluation of valid vs. invalid feedback in the RCZ, anhedonia influenced the overall RCZ-activity in response to feedback. More anhedonic individuals had decreased RCZ-activity. This effect was not mirrored by other depression-related measures (data not shown). This decreased RCZ-activity implies less cognitive control or less attention being paid to the stimuli (e.g., Bush *et al.*, 2000), which appears in line with the lack of interest or motivation associated with anhedonia.

Depressed mood and anhedonia were both associated with increased sgACC activity in response to feedback, independent of the type of feedback. We should note

that this effect was not specific for these two measures associated with depression; increased levels of neuroticism and trait anxiety were also associated with increased sgACC activity (data not shown). The strongest association was, however, found for anhedonia. A hyperactive sgACC has been rather consistently found in clinically depressed patients (Davidson *et al.*, 2002, Mayberg, 1997, 2003, Pizzagalli, 2011), in healthy persons with high levels of neuroticism or negative affect (Haas *et al.*, 2007, Zald *et al.*, 2002), and in healthy persons subjected to negative mood induction (Berna *et al.*, 2010, Mayberg *et al.*, 1999). Since the sgACC showed no difference in response to the four types of feedback, we argued that this region probably does not play a major role in feedback processing. The sgACC is, on the other hand, thought to be involved in general emotion processing (e.g., Harvey *et al.*, 2007). The emotional content of the feedback stimuli (i.e., facial expressions), independent of its valence, might therefore explain this hyperactivity in students with high levels of depressed mood and anhedonia.

We further expected that anhedonia would decrease the effect of valence in the rACC. This expected blunted response to positive and negative feedback only marginally reached significance. Steele *et al.* (2007) found that a blunted behavioural response to positive and negative feedback correlated significantly with self-report anhedonia in both depressed patients and healthy controls. Perhaps we were not able to detect this behavioural effect because of differences in paradigm design. Steele *et al.* (2007) examined increases and decreases in reaction times after both positive and negative feedback in a gambling task (i.e., quantity of adjustment), while we examined correct and incorrect adjustments after negative feedback (i.e., quality of adjustment).

The main purpose of our study was to investigate the response to feedback in individuals with mild depressive symptoms in general, as indicated by a high score on the BDI, compared to those without depressive symptoms. In contrast to the specific symptoms depressed mood and anhedonia, no robust differences in neural responses to feedback were found on the basis of the BDI. The only significant difference between the two groups was found in the RCZ. The students with mild depressive symptoms had a larger response to valid positive feedback than to valid negative feedback. This effect was not present in the low-BDI group. In addition, the amygdala of the mildly depressed was slightly more active in response to feedback. This latter finding is in line with previous studies investigating feedback processing or emotion processing in depression (Davey *et al.*, 2011, Sheline *et al.*, 2001, Taylor Tavares *et al.*, 2008). The first finding, however, is more difficult to interpret. The RCZ in students with more depressive symptoms appears to be sensitive to both the validity and the valence of the feedback. A more liberal view of the dorsal-cognitive/ventral-affective subdivision hypothesis of the ACC is therefore necessary. As indicated by Mohanty *et al.* (2007), for instance, this hypothesis should not be interpreted as a strict division, but rather as a continuum. Recent studies, however, have cast doubt on this dichotomization, in part because there are studies reporting the

processing of negative emotion, fear and pain in the dorsal part of the ACC, including the RCZ (Etkin *et al.*, 2011, Shackman *et al.*, 2011). An 'adaptive control hypothesis' has been postulated, which suggests that the ACC, in particular the RCZ, uses information with a negative value (punishment, pain) to bias responding when the most adaptive course of action is uncertain, and therefore integrates emotion, pain and cognitive control (Shackman *et al.*, 2011). The current findings can further extend this hypothesis, without discarding the segregation hypothesis. Our findings suggest that the RCZ is primarily involved in the evaluation of the relevance of emotional information, rather than being involved in emotional processing per se. The ventral part of the ACC, on the other hand, does not appear to exert this cognitive evaluative function, which leads to this area being more clearly involved in emotion processing, and possibly control of emotional expression (Etkin *et al.*, 2011), at least in time-estimation tasks. Our results, therefore, imply that there is at least some segregation in functions of the dorsal and ventral parts of the ACC. Our findings, furthermore, suggest that the presence of (specific) depressive symptoms modulates the role of the RCZ in emotion processing and cognitive control.

In conclusion, our findings suggest that specific symptoms of depression are more informative in relation to atypical feedback processing than depressive symptoms in general. Although feedback processing was not disturbed at the behavioural level in participants with higher levels of depressed mood and anhedonia, these measures differentially affected ACC activity during feedback processing. Depressed mood was associated with a decreased evaluation of the validity of feedback in the RCZ, while anhedonia was associated with a decreased response of the RCZ to feedback in general. Both symptoms, however, contributed to the often-reported hyperactive sgACC in depression. Our results imply that increasing levels of depressed mood and anhedonia involve changes in cortico-limbic responses to feedback, especially in the ACC. These atypical ACC responses might render subjects vulnerable to depression.

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Chapter 6

Acute tryptophan depletion in healthy males attenuates phasic cardiac slowing but does not affect electro-cortical response to negative feedback

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Abstract

Rationale: Recent studies have shown that serotonin might be involved in performance monitoring, although the results have been inconclusive. Inconsistent results might be related to the type of pharmacological manipulation and the used behavioural and physiological measures. *Objectives*: The present study aimed at further specifying the role of serotonin in performance monitoring. *Methods*: The effect of serotonin on performance monitoring was studied by using acute tryptophan depletion (ATD), a well-known method to transiently lower central serotonin levels. Twenty healthy male volunteers performed a time-estimation task and their event-related brain potential (ERP), behavioural and cardiac responses to feedback stimuli were measured. Furthermore, subjective mood and amino acid levels were determined. *Results*: As expected, ATD did not affect mood and lowered tryptophan levels. ATD attenuated cardiac slowing to negative feedback, but did not affect responses to positive feedback, ERPs and performance measures. *Conclusions*: The data point in the direction of a dissociation between cardiac and electro-cortical responses. Cardiac responses appear to be more sensitive to changes in serotonin metabolism, and appear to reflect different aspects of the feedback stimulus. The phasic cardiac response appears to be an important measure that provides additional information about the impact of feedback stimuli and serotonergic functioning.

Introduction

Flexibility of behaviour is essential for adaptation to a constantly changing environment. This flexibility depends on performance monitoring which relates to the ability to monitor the successfulness of ongoing behaviour and relate this to internal goals. Performance can be monitored by using internal or external signals, that is by continuously monitoring one's own behaviour for errors or processing external feedback cues. Both types of performance monitoring have been related to the same underlying system that is involved in reinforcement learning (Holroyd and Coles, 2002). Both the detection of errors and the processing of negative feedback are accompanied by activation in a part of the dorsomedial prefrontal cortex that has been referred to as the rostral cingulate zone (Ridderinkhof *et al.*, 2004). This activation is reflected in a negative deflection in the event-related brain potential (ERP) that peaks around 50-100 ms after error commission (e.g., Falkenstein *et al.*, 2000) and around 300 ms after onset of the negative feedback stimulus (Miltner *et al.*, 1997). For errors this deflection has been called the error-related negativity (ERN; Gehring *et al.*, 1993) or error negativity (Ne; Falkenstein *et al.*, 1991) and for negative feedback it has been called the feedback-related negativity (FRN).

Performance monitoring is not only reflected in changes in central nervous system measures, but also in autonomic measures. Errors (Hajcak *et al.*, 2003b), negative feedback (Crone *et al.*, 2005, Crone *et al.*, 2004, Crone *et al.*, 2003, Van der Veen *et al.*, 2004a, Van der Veen *et al.*, 2004b) and incongruent stimuli that require additional monitoring (Jennings *et al.*, 2002) are all accompanied by cardiac deceleration. Based on their data, Crone *et al.* (2005) suggested that cardiac slowing following negative feedback reflects the informative value of the feedback stimulus as is used to adjust performance on the subsequent trial. This is more or less in line with current theories of the electro-cortical reflections of errors and negative feedback. Moreover, Groen *et al.* (2007) found a correlation between feedback-related heart rate response and electro-cortical response in children performing a probabilistic learning task. They state that feedback-related heart rate deceleration is a reflection of the same error monitoring system responsible for the ERN, in line with earlier suggestions made by Somsen *et al.* (2000) and Crone *et al.* (2003). However, there is some evidence that cardiac measures of performance monitoring reflect different aspects of negative feedback than electro-cortical measures (Van der Veen *et al.*, 2004a), and possibly reflect the affective properties of the feedback stimulus (Van der Veen *et al.*, 2004b).

Various neurotransmitter systems are thought to be involved in performance monitoring. The dopamine system is thought to play a central role. Dopamine appears to be especially important when positive feedback carries an affective value and can be seen as a reward (e.g., Wise, 2004). Furthermore, the dopamine system appears to be essential when errors or feedback stimuli signal an outcome that is worse than expected (Holroyd and Coles, 2002). Direct evidence for a role of dopamine comes from studies that directly

manipulated dopamine levels. It has been shown that the indirect dopamine agonist amphetamine enhances the ERN (De Bruijn *et al.*, 2004) whereas the dopaminergic antagonists olanzapine and haloperdidol reduce the ERN (De Bruijn *et al.*, 2006, Zirnheld *et al.*, 2004).

Recently some studies have reported that serotonin (5-HT) might also play a role in performance monitoring. A number of studies have shown that patients suffering from unipolar depression, which is often associated with a lowered 5-HT metabolism (e.g., Asberg *et al.*, 1976, Maes and Meltzer, 1995), show an altered behavioural or brain response to negative feedback stimuli (e.g., Elliott *et al.*, 1998, Murphy *et al.*, 2003, Tucker *et al.*, 2003). However, the reported effects are far from conclusive due to inconsistencies in factors like the chosen population (depressed or remitted patients), severity of depression and use of medication (especially SSRIs). Patients suffering from obsessive-compulsive disorder or anxiety, disorders which are thought to be associated with a lowered 5-HT metabolism as well (e.g., Micallef and Blin, 2001) show larger ERNs than healthy controls (Gehring *et al.*, 2000, Hajcak *et al.*, 2003a, Hajcak and Simons, 2002, Johannes *et al.*, 2001b). More evidence, however, came from a study showing that individuals with genetically determined lower 5-HT metabolism have larger ERN amplitudes (Fallgatter *et al.*, 2004). In this study it was reported that carriers of the short allele variant of a functional length variation in the transcriptional control region of the 5-HT transporter (5-HTT) gene showed higher amplitude of the ERN compared with carriers of two long alleles. The short alleles are associated with lower 5-HT reuptake and lower 5-HT function (Hariri and Holmes, 2006). Additional evidence for a role of 5-HT in performance monitoring came from studies in which central 5-HT levels were manipulated. In a number of studies we have used acute tryptophan depletion (ATD), a well established method to lower central 5-HT levels, to study the role of 5-HT in performance monitoring. In a first functional magnetic imaging (fMRI) study we found that ATD *increased* the brain response in the dorsomedial prefrontal cortex (dmPFC) to negative feedback signaling a change in behaviour in a probabilistic reversal learning task (Evers *et al.*, 2005). In a second study, however, we found that ATD *lowered* the brain response in the dmPFC to negative feedback signaling false alarms or omissions in a Go-NoGo task (Evers *et al.*, 2006b). These contradictory findings have been related to the different meanings of feedback stimuli in theses two studies. In the first study only feedback stimuli that were followed by correct behavioural adjustments elicited an increased brain response. In the second study no similar behavioural adjustments could be made due to different types of errors, namely false alarms or omissions. In some contrast to these fMRI studies are the findings of two ERP studies examining the effects of a transient *increase* in 5-HT metabolism (De Bruijn *et al.*, 2004, De Bruijn *et al.*, 2006). In the first study (De Bruijn *et al.*, 2004) no effect could be found of the antidepressant Mirtazapine on either the amplitude of the brain response to errors or the performance

directly following error commission. It could be argued, that the drug also works on the noradrenergic and histaminergic system and that these systems potentially could have contributed to the lack of effects. However, in a second study De Bruijn *et al.* (2006) also reported no effect of the selective serotonin reuptake inhibitor Paroxetine.

The reported effects of changes in serotonin levels on various measures of brain activation are inconsistent and the effects on cardiac measures are unknown. These findings combined with the possible dissociation between electro-cortical and cardiac reflections of performance monitoring (Van der Veen *et al.*, 2004a, Van der Veen *et al.*, 2004b) led to the design of the present study. We examined the effect of ATD on both ERP and cardiac measures of performance monitoring in a time-estimation task. Based on the possible stronger sensitivity of the cardiac response to affective properties of the feedback stimulus it was expected that ATD would affect cardiac slowing following negative feedback, but the direction of effects was hard to predict. Based on the multiple null effects with respect to the electro-cortical reflections of performance monitoring it was hypothesized that ATD would not affect the FRN.

Methods

Participants

Twenty male volunteers (mean ± standard deviation = 23 ± 4 years) participated in the experiment. All participants gave written informed consent and the study was approved by the local medical ethics committee. General health and personal and family history of psychiatric disorders was evaluated with an extensive questionnaire which was checked by a medical doctor. Participants with a history of psychiatric disorders or with first-degree relatives with an affective disorder were excluded from the study. Participants were paid EUR 75 for their voluntary participation.

Stimuli

Participants performed the time-estimation task that was developed by Miltner *et al.* (1997) and extended by Van der Veen *et al.* (2004b). The stimulus sequence consisted of an asterisk which functioned as a fixation stimulus and was presented for 2 seconds, a question mark which functioned as the cue to start the estimation, a second asterisk which was presented for 1 second, and the feedback stimulus which was presented for 1 second. Participants were instructed to estimate a 1-second interval starting at the onset of the question mark. If the estimation was correct a positive feedback stimulus in the form of an exclamation mark ("!") was presented. If they underestimated the interval a minus sign was presented ("-"), and if they overestimated the interval a plus sign was presented ("+"). From a previous study (Van der Veen *et al.*, 2004b) it is known that these symbols are capable of eliciting a different heart rate response to negative feedback

compared with positive feedback, irrespective of information value. In order to get similar proportions of negative and positive feedback a dynamic tracking mechanism was used. When subjects started, estimations that fell within an interval of 900 ms – 1100 ms were counted as correct. After a correct response, however, this interval was shortened with 20 ms, and after an incorrect response this interval was lengthened with 20 ms. A total of 240 stimuli were presented in two blocks of 120 stimuli each, which means that on average the participants performed two 10 minute blocks of the time-estimation task.

Procedure

Participants were tested in a placebo-controlled double-blind crossover design. All participants were tested on two separate days, with minimally 7 days in between. On one of these days participants had to ingest a balanced amino acid (AA) drink (containing tryptophan: TRP+), and on the other day they ingested the tryptophan depleted AA drink (TRP-). The order of the testing days was balanced across participants. On a testing day participants arrived between 8.00 and 13.00 hr. Participants first completed the questionnaires and a blood sample was drawn. Directly after that participants ingested the AA drink and could relax for 5 hr. In this 5 hr period participants could eat food that was low or free of protein and could drink decaffeinated coffee, tea or soft drinks. After the 5 hr period participants completed the same questionnaires again, a second blood sample was drawn and the psychophysiological measurements started.

Amino acid mixture

The specific amount of the different components of the two AA drinks was based on the proportion described by Young *et al.* (1985). The balanced mixture (78 g) consisted of 4.1 g L-alanine, 3.7 g L-arginine, 2.0 g L-cysteine, 2.4 g L-glycine, 2.4 g L-histidine, 6 g L-isoleucine, 10.1 g L-leucine, 6.7 g L-lysine monohydrochloride, 3.0 g L-methionine, 4.3g L-phenylalanine, 9.2 g L-proline, 5.2 g L-serine, 4.3 g L-threonine, 5.2 g L-tyrosine, 6.7 g L-valine and 3.0 g L-tryptophan. In the tryptophan depleted mixture (75 g) the same quantities of amino acids were present as in the balanced mixture, but now without the L-tryptophan. In order to make the AA mixture drinkable, 200 ml of tap water was added.

Biochemical measures

Blood samples (10 ml) were taken before drinking the amino-acid mixture and before the psychophysiological measurements, about 5 hours after administration, to determine plasma amino acid levels. Within 30 minutes blood was centrifuged at 4 degrees Celsius, at 4000 rpm for 10 minutes. Subsequently, an aliquot of 100 ml plasma was mixed with 4mg sulphasalicyl acid and frozen at –80°C until analysis (Van Eijk *et al.*, 1994). Analysis of plasma amino acid concentrations was carried out using high-performance liquid chromatography (Van Eijk *et al.*, 1993). For present purposes only the concentration of

free tryptophan and the ratio between tryptophan and other Large Neutral Amino Acids (LNAAs) are reported. The first provides an index of the successfulness of the depletion procedure, and the latter an indication of the level of tryptophan intake of the central nervous system (Fernstrom and Fernstrom, 1995).

Subjective measures

Mood was assessed with a visual analogue version of the shortened Profile of Mood States Scale (POMS; McNair *et al.*, 1971). The questionnaire consists of 32 bipolar sets of adjectives, which measure five mood dimensions (anger, depression, fatigue, tension and vigor) which were scored on a 10-point scale. Together with a questionnaire considering side-effects, these questionnaires were administered before the ingestion of the amino acid drink and directly preceding the scanning session.

Electro-cortical measures

The electroencephalogram (EEG) was derived from electrodes placed at F3, Fz, F4, C3, Cz, C4, and Pz which were placed according to the 10-20 system (e.g., Sharbrough *et al.*, 1991) and referenced to linked mastoids. Vertical electro-oculogram (EOG) was derived from an electrode placed below the right eye. EEG and EOG were sampled at 500 Hz and electrode impedance was kept below 8 kΩ. Data was analyzed with the EEGLAB software package (Delorme and Makeig, 2004). ERPs were locked to the onset of the feedback stimulus and epochs were extracted between 100 ms preceding feedback onset and 700 ms following feedback onset. For all epochs a baseline between 100 ms before onset and time of onset was computed and subtracted from each ERP. Independent component analysis was used to remove EOG artifacts (Makeig *et al.*, 2004). For all participants and all conditions ERPs were averaged with respect to feedback. Following previous research in which feedback-related negativity (FRN) was studied in a comparable time-estimation task (Nieuwenhuis *et al.*, 2005b), FRN amplitude was computed as the peak negativity of the difference waveform (difference between ERP to positive and negative feedback) at electrode Cz (where FRN is maximal) in a window 200 – 350 ms following feedback onset.

Cardiac measures

The ECG was derived from pre-cordial leads and was sampled at 1000 Hz. R-peak occurrence times were detected online with an accuracy of 1 ms. The R-peak occurrence times were checked for artifacts and corrected when necessary. Four inter-beat intervals (IBIs) surrounding the feedback stimulus were selected for further analysis; i.e., the preceding IBI (IBI -1), the concurrent IBI (i.e., IBI 0) and two IBIs following the feedback stimulus (i.e., IBIs 1 and 2). These IBIs were referenced to the IBI occurring four IBIs before feedback stimulus onset (IBI -4).

Statistical analysis

The results were statistically evaluated with a repeated-measures univariate analysis of variance. Whenever this was appropriate, the degrees of freedom were adjusted using the Hyunh-Feldt correction procedure. Biochemical and mood measures were tested in a design with time (t_0 vs. t_5) and treatment (TRP- vs. TRP+) as within-subjects factors. Performance measures were tested in a design with treatment as within-subjects factor. Cardiac measures were tested using treatment, sequential IBI (4 levels; IBI -1, IBI 0, IBI 1 and IBI 2) and valence (negative vs. positive feedback) as within-subjects factors. ERP measures were tested with a t-test comparing the two treatments.

Results

Order of treatment (TRP- vs. TRP+) did not affect any measures and was therefore left out from the analyses. Twenty participants were included and all participants had complete biochemical data. For various technical reasons complete POMS data is available for 18 participants, complete performance data for 19 participants, complete cardiac data for 13 participants and complete ERP data is available for 18 participants. Missing data were caused by lost files, incomplete forms and bad signals.

Biochemical measures

Statistical evaluation of the data showed that plasma tryptophan levels were affected by time ($F(1,18) = 5.1$, $p < 0.05$), and that time and treatment interacted ($F(1,18) = 12.1$, $p < 0.005$). Order did not affect tryptophan levels, nor did it interact with the other factors. Follow-up analyses show that, as expected, tryptophan levels were higher 5 hours after the ingestion of the TRP+ mixture (t_0 = 50.8 ±2.7 µMol/l vs. t_5 = 101.5 ±8.1 µMol/l; 99,8% increase; mean ±SEM) and lower after ingestion of the TRP- mixture (t_0 = 51.0 ±1.5 µMol/l vs. t_5 = 15.1 ±1.6 µMol/l; 70% decrease). The ratio between plasma tryptophan and other large neutral amino acids (Tryptophan/∑LNAA) followed about the same pattern of results. Ratio was affected by time ($F(1,18) = 9.2$, $p < 0.01$), treatment ($F(1,18) = 5.0$, $p < 0.05$), and time and treatment interacted ($F(1,18) = 19.3$, $p < 0.0005$). Order did not affect Tryptophan/∑LNAA ratio and did not interact with time and treatment. Follow-up analyses showed that ratios increased after ingestion of the TRP+ mixture (t_0 = 0.10 ±0.004 vs. t_5 = 0.13 ±0.008; 27% increase) and decreased after ingestion of the TRP- mixture (t_0 = 0.10 ±0.003 vs. t_5 = 0.02 ±0.003; 79% decrease). Both the plasma levels and Tryptophan/∑LNAA ratios showed that our manipulation of tryptophan levels was successful. To summarize, both the plasma levels and Tryptophan/∑LNAA ratios showed that the ATD manipulation was successful.

Mood

As expected for healthy male volunteers without a personal or family history of affective disorder (e.g., Evers *et al.*, 2005, Evers *et al.*, 2006b), ATD did not affect subjective mood. Baseline ratings of the scores of the five subscales of the POMS did not significantly differ between the TRP+ and TRP- sessions. A repeated-measures General Linear Model (GLM) analysis with time (baseline vs. 5 h after ingestion) and treatment (TRP+ vs. TRP-) as within-subjects factors did not reveal any significant effects on the scores of the five subscales of the POMS (see Table 6.1).

Table 6.1 Mean and standard error of mean scores for the 5 subscales of the POMS at T_0 and T_5 in the TRP+ and TRP- condition

		Depression	Anger	Fatigue	Vigor	Tension
TRP+	T_0	295(27)	254(19)	239(16)	212(12)	223(19)
	T_5	317(28)	264(22)	250(18)	213(17)	230(20)
TRP-	T_0	293(25)	258(18)	252(15)	210(13)	241(16)
	T_5	292(22)	253(19)	242(18)	211(16)	212(17)

Performance

On average the participants performed the task as expected with a mean percentage of negative feedback of 53% in both the TRP+ and the TRP- condition. The percentage correct adjustments after negative feedback in the TRP+ and TRP- condition was tested with a paired-samples t-test. This test did not reveal significant differences between the TRP+ and TRP- condition (t = 1.4, p = 0.17). On average participants made 83% (Standard Error of the Mean, SEM = 1.1) correct adjustments in the TRP+ condition as compared to 82% (SEM = 1.2) in the TRP- condition.

Cardiac responses

Cardiac responses to feedback, which are shown in Figure 6.1, were firstly tested in a design with sequential IBI, valence and ATD as within-subjects factors. This analysis revealed a significant main effect of sequential IBI, $F(1,12) = 11.6$, $p < 0.005$, indicating that the feedback stimulus significantly affected the cardiac response. The three-way interaction between sequential IBI, valence and ATD was marginally significant ($F(3,36) = 2.3$, $p < 0.1$). No main effect of valence or interaction effect between valence and sequential IBI was found[1]. Due to our special interest in negative feedback, combined with

[1] In addition to these analyses, negative feedback followed by a correct adjustment was compared with negative feedback followed by an incorrect adjustment. This analysis revealed a stronger HR deceleration on negative

this marginally significant result we decided to perform the analysis for negative and positive feedback separately. For positive feedback, this analysis only revealed the expected effect of sequential IBI ($F(1,12) = 9.3$, $p < 0.005$). For negative feedback we also found the expected effect of sequential IBI ($F(1,12) = 11.6$, $p < 0.005$), but we also found an interaction between sequential IBI and condition ($F(1,12) = 4.7$, $p < 0.05$). Follow-up analyses in which we computed the average deceleration of IBI0, IBI1 and IBI2 as compared to IBI-1 showed that cardiac deceleration was stronger in the TRP+ condition (mean ±SEM, 23 ms ±6 ms) as compared to the TRP- condition (12 ms ±3 ms) ($t = 2.3$, $p < 0.05$).

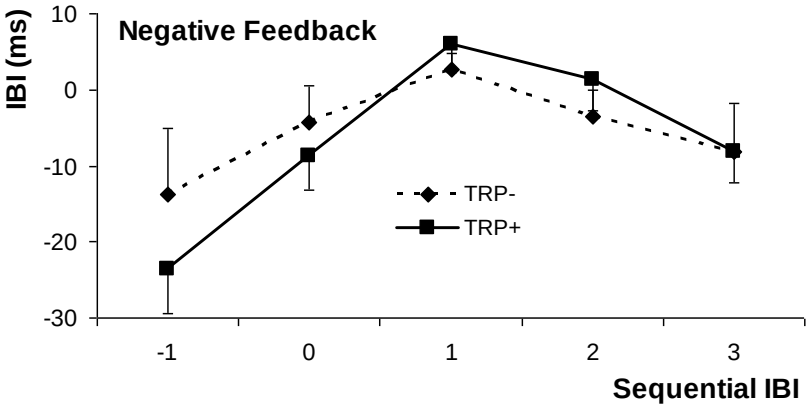

Figure 6.1 Cardiac response to negative feedback in the balanced (TRP+) and the tryptophan-depleted (TRP-) condition.

ERPs

ERPs derived from Cz are presented in Figure 6.2. In this figure can be seen that negative feedback elicited the expected negative deflection, which was maximal around 300 ms after feedback onset. The difference between positive and negative feedback was highly consistent over participants, and when defined as the maximal difference between 200 and 350 ms after feedback onset, it differed significantly from zero in both the TRP+ ($t = 7.1$, $p < 0.0005$), and the TRP- condition ($t = 8.1$, $p < 0.0005$). Further statistical evaluation of the FRN amplitude did not reveal a difference between the TRP+ and TRP- conditions ($t = 0.95$, $p = 0.35$).

feedback stimuli which were followed by correct adjustments. This has also been reported in several other studies (Van der Veen *et al.* 2004b; Crone *et al.* 2003). ATD did not interact with this effect.

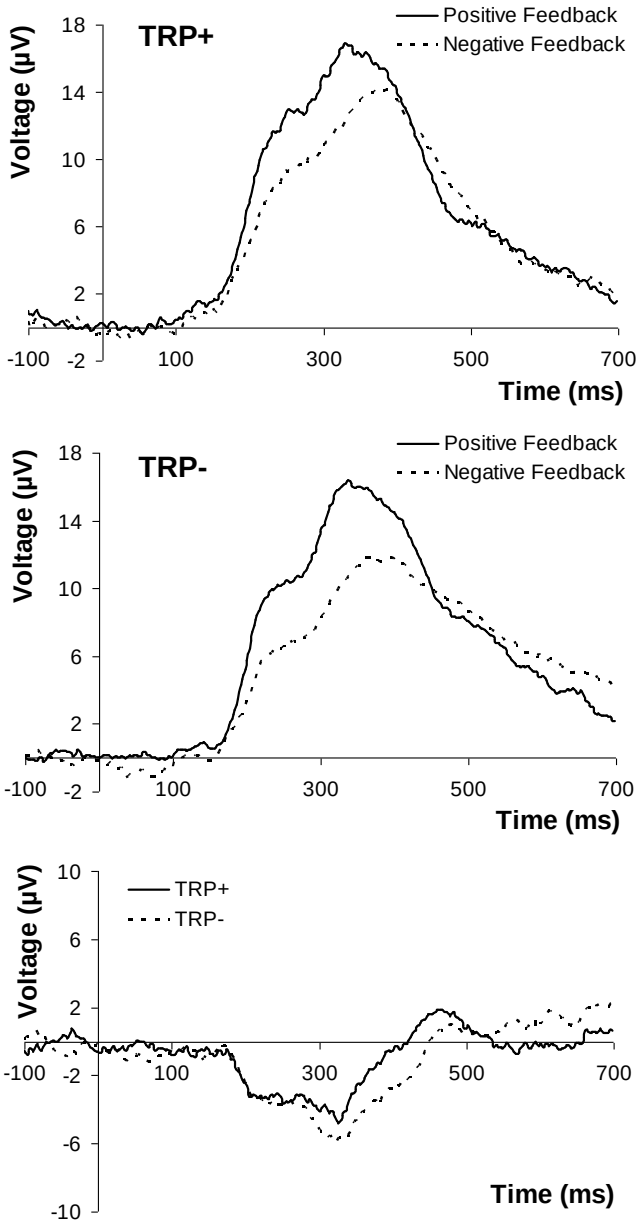

Figure 6.2 Event-related brain potentials evoked by negative and positive feedback in the balanced (TRP+, top panel) and the tryptophan-depleted (TRP-, middle panel) condition. The difference waves between positive feedback and negative feedback in the TRP+ and TRP- condition are presented in the bottom panel.

Due to the fact that statistical evaluation of the cardiac responses did reveal an effect of ATD, we performed some additional analyses to make sure that the present results were not related to the chosen analysis strategy and participant selection. First, we selected all participants with complete cardiac data (n=13) and performed the same t-test on FRN amplitude as described above. This test did not reveal a significant effect of ATD (t = 0.6, p = 0.5). Secondly, we defined FRN differently to focus solely on the negative deflection elicited by negative feedback, without taking the response to positive feedback into account. We defined FRN as the mean value in the ERP to negative feedback between 200 and 350 ms after feedback onset. We tested this FRN measure for both the larger sample (n=18) and the smaller sample (n=13), and these tests did not reveal significant differences (t = 1.0, p = 0.4, and t = 1.3, p = 0.2, respectively)[2].

Discussion

In the present study the effects of transiently lowering 5-HT levels by means of ATD in healthy male participants on electro-cortical and cardiac concomitants of performance monitoring were studied. Participants performed a time-estimation task and received symbolic feedback about the correctness of their responses. Performance was in line with previous studies (e.g., Van der Veen *et al.*, 2004b). Participants received about 50% negative feedback and adjusted their estimation in the correct direction in about 80% of the trials. It was found that ATD attenuated the cardiac decelerative response to negative feedback stimuli, whereas it did not affect subjective mood, electro-cortical, and behavioural responses.

To our best knowledge, the present study was the first to study the effects of ATD on cardiac responses to feedback stimuli. We hypothesized that ATD would affect the cardiac response to negative feedback, which was based on the hypothesis that the cardiac response would be more sensitive to the affective properties of the feedback stimulus (Van der Veen *et al.*, 2004a, Van der Veen *et al.*, 2004b). The found attenuated decelerative response, however, was not directly predicted. The effect of ATD on cardiac responses might have been related to the role of serotonin in vagal control of the heart (Jordan, 2005). In his review, Jordan describes the important role of serotonin in the autonomic regulation of heart rate. However, if the reported effects would have been caused by such a peripheral mechanism, an overall effect should have been found leading

[2] To be sure not to miss any interesting effects, we performed some additional analyses. First, we changed the interval in which we determined the maximal difference between negative and positive feedback or the mean amplitude for negative feedback. We used both a longer interval (200-450ms) and a later interval (300-450 ms). Furthermore, we also examined effects for different electrodes (Fz, Pz). Finally, we examined P3 amplitude by computing the maximal amplitude on Pz in an interval between 250 and 500 ms after onset of positive or negative feedback. All these additional analyses did not reveal any significant effects of ATD.

to effects on cardiac responses to both positive and negative feedback. This was clearly not the case. So, it seems more likely that the found effect is related to higher order processes. One possibility is that the found effect is related to a change in punishment prediction. Based on their recent findings in an observational reversal-learning task, Cools *et al.* (2008) have recently proposed a role for serotonin in punishment prediction. They found that ATD improved punishment prediction, and in this way removed the natural bias in favor of reward prediction. In the present study this would mean that ATD could have improved the predictability of the negative feedback stimulus. Previous research has shown that the amplitude of cardiac deceleration following negative feedback is strongly related to violations of performance based expectations (Crone *et al.*, 2003), with higher amplitudes found with stronger violations. Combining these two previous findings this would mean for the present data set that ATD could have reduced the violations of expectations due to better punishment prediction, and therefore cardiac responses to negative feedback were attenuated.

Cardiac responses to positive and negative feedback did not differ significantly, which was somewhat unexpected. In a previous study it was found that cardiac responses to negative feedback showed a stronger deceleration which was independent of the informative value of the feedback stimulus (Van der Veen *et al.*, 2004b). An important difference with the current study was that participants were told that correct responses would be rewarded with a small amount of money and incorrect responses would be punished with small monetary penalty. This could have led to a stronger impact of the feedback stimulus and stronger dissociation of cardiac responses to positive and negative feedback. When, however, the cardiac response to negative feedback followed by correct performance adjustments was compared with the cardiac response to negative feedback followed by incorrect adjustments, the expected stronger cardiac deceleration was found on feedback trials followed by correct adjustments. This means that the current study had enough power to detect these subtle differences. This difference between these two types of negative feedback was not differentially affected by ATD. This can be interpreted in terms of ATD having an effect on the cardiac response to negative feedback in general, and not differentially affecting the response to more or less processed negative feedback stimuli.

ATD did not affect the electro-cortical response to negative feedback. This is in line with earlier studies (De Bruijn *et al.*, 2004, De Bruijn *et al.*, 2006) in which it was found that electro-cortical responses to errors, which are thought to reflect more or less the same underlying process (Ridderinkhof *et al.*, 2004), are not affected by various serotonergic manipulations. However, in line with the idea of improved punishment prediction following ATD possibly causing the attenuated cardiac response, electro-cortical response to negative feedback should decrease as well. This was not the case. This implies that cardiac response appears to be more sensitive to improved punishment

prediction than electro-cortical response. The electro-cortical results are also not in line with a number of fMRI studies (Evers *et al.*, 2005, Evers *et al.*, 2006a, Evers *et al.*, 2006b). A possible explanation for this difference between ERP and fMRI studies could possibly be found in the used measurement technique. In this study we measured electrical activity at the scalp which can be measured with a high temporal resolution, but has a poor spatial resolution. The mentioned fMRI studies measured the blood-oxygen-level-dependent response which has a poor temporal and very good spatial resolution. These differences are important for the sensitivity to pick up the subtle effects of serotonergic manipulations on the measured processes. Nieuwenhuis *et al.* (2005b) have shown that the differences between these techniques are very relevant in this domain. They showed that negative feedback evoked a clear negative deflection in the ERP, whereas no clear additional brain activity accompanying negative feedback could be detected with fMRI in exactly the same task. Finally, the finding that ATD did not affect the FRN was not in line with the findings of Fallgatter *et al.* (2004). They reported an increased ERN in participants with a genetic variation associated with lower serotonin metabolism. This difference is possibly related to the acute nature of the manipulation used in the present study. The participants described in the Fallgatter *et al.* study were all born with variation in the serotonin transporter gene associated with lower serotonin metabolism and therefore their physiological system could have adjusted by changing all kinds of properties of the serotonin system. These long term adjustments are very relevant to the effects of various drugs that act on the serotonin system, which can for instance be seen in the opposing effects of acute and long-term administration of selective serotonin reuptake inhibitors (e.g., Stahl, 1998). The dissociation between the effect of ATD on cardiac and electro-cortical measures suggests that these measures could reflect different aspects of the feedback stimulus. This dissociation fits the hypothesis that the cardiac response might more strongly reflect affective properties (Van der Veen *et al.*, 2004b) and fits the earlier found dissociation in a probabilistic learning task (Van der Veen *et al.*, 2004a).

Performance was unaffected by ATD which is in line with a large number of studies that used other tasks to quantify flexible behaviour (e.g., Evers *et al.*, 2005, Evers *et al.*, 2006b, Rubia *et al.*, 2005) in which no effects on performance were found, whereas brain activation was affected by ATD. In a recent review (Evers *et al.*, 2007) we have discussed a number of possible explanations for the dissociation between effects on performance and physiological measures. The most obvious reason is of course that physiological measures reflect a selection of the processes that are involved in generating a response, whereas behavioural measures reflect the outcome of these processes. In this way both types of measures can be differentially vulnerable to fluctuations in serotonin levels.

In accordance with previous studies subjective mood was not affected by ATD. ATD has been known to only have an effect on subjective mood in vulnerable participants, such

as healthy participants with a family history of affective disorder (Benkelfat *et al.*, 1994, Klaassen *et al.*, 1999, Quintin *et al.*, 2001).

There are some limitations to the present study. First of all the sample size is relatively small. Second, this study only included young healthy males making it difficult to extend these findings to patients with disorders in which a disturbed 5-HT function is thought to be involved, such as depression and anxiety disorder. These findings, however, could implicate a role for 5-HT in affective function. In addition to the reported reappearance of depressive symptoms following ATD in remitted depressed patients (Delgado *et al.*, 1990, Smith *et al.*, 1999) and the mood lowering effects of ATD in healthy control participants with a family history of depression (Klaassen *et al.*, 1999), attenuated cardiac slowing following ATD could be a reflection of affective disturbances in disorders such as depression and anxiety disorder.

This study showed that ATD attenuated cardiac deceleration, possibly reflecting a decreased impact of negative feedback stimuli due to improved punishment prediction. This decreased impact was not visible in electro-cortical responses and did not result in a change in behaviour. Combined with earlier findings this shows that effects of ATD on feedback related behaviour are subtle and that it is important to distinguish different aspects of the feedback stimulus and to use a combination of different measures to monitor the processes of interest. Cognitive and affective aspects of the feedback stimulus appear to be differentially reflected in cardiac and electro-cortical responses and are differentially affected by serotonergic manipulations. For future studies it is therefore of the utmost importance to focus on these separable aspects of feedback and use the appropriate measures to examine them. This study shows that especially phasic heart rate responses are sensitive to lowered 5-HT and that this measure provides important information about the impact of feedback signals.

Chapter 7

Effects of functional polymorphisms in the 5-HTT and TPH2 gene on feedback processing

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Abstract

Rationale and objective Recent studies suggest that serotonin plays an important role in electrophysiological manifestations of error processing. In line with these findings, we investigated the influence of two functional polymorphisms of the serotonin transporter gene (5-HTTLPR) and the tryptophan hydroxylase 2 gene (TPH2-*rs1386493*) on behavioural and electrophysiological correlates of feedback processing.

Methods Behavioural responses to negative feedback and two electrophysiological correlates of feedback processing (i.e., the feedback-related negativity (FRN) and feedback P3), were studied in a group of healthy individuals across a large age span (22-76 years), using a time-estimation task. In addition, we examined gene-age interactions.

Results In the younger group (<51 years), carriers of the less functional 5-HTTLPR variant (S) had a larger FRN and a larger difference between positive and negative feedback in the time window of the feedback P3 than others (LL). TPH2-*rs1386493* did not influence FRN-size, but did influence the feedback P3 in older individuals (>51 years). Carriers of the less functional TPH2-gene variant (T) showed a smaller difference between positive and negative feedback than the CC group. Performance on the task was not influenced by the two polymorphisms.

Conclusions 5-HTTLPR and TPH2-*rs1386493* have differential influences on the electrophysiological responses to feedback, and these effects are different for younger participants than for older participants. The distinct roles of the two genes in the serotonin pathway and alterations in the functionality of the serotonin system with advancing age are likely to underlie these effects.

Introduction

After an error or negative feedback, a negative deflection is observed in the event-related brain potential (ERP) at fronto-central midline recording sites, which is called the error-related negativity (ERN; Falkenstein *et al.*, 1991, Gehring *et al.*, 1993) or feedback-related negativity (FRN; Miltner *et al.*, 1997). The anterior cingulate cortex (ACC) is considered to be the source of these electrophysiological responses to errors and feedback. Although the ERN and FRN are thought to be directly related to changes in dopamine (Holroyd and Coles, 2002, Jocham and Ullsperger, 2009, Nieuwenhuis *et al.*, 2002), several studies have shown that serotonin (5-HT) may also play a role in these manifestations (Althaus *et al.*, 2009, Fallgatter *et al.*, 2004, Jocham and Ullsperger, 2009).

A number of studies have focused on the serotonin-transporter gene (5-HTT). A common polymorphism in the promoter region of this gene, 5-HTTLPR, is known to affect the reuptake of 5-HT by the serotonin transporter in the brain. Individuals with at least one copy of the short (S) allele, which is associated with decreased expression of the 5-HTT protein, had larger ERN responses than individuals who were homozygous for the long (L) allele (Althaus *et al.*, 2009, Fallgatter *et al.*, 2004). A recent study by Olvet *et al.* (2010a), however, did not replicate these findings in a larger sample, casting doubt on the relationship between 5-HTTLPR and error processing.

In contrast to previous studies focusing on the ERN, the present study's primary aim was to investigate the association between 5-HTTLPR and the FRN. A time-estimation paradigm was used to elicit the FRN. Several fMRI studies have shown that the rostral part of the ACC (rACC) is involved in feedback processing in time-estimation tasks (Nieuwenhuis *et al.*, 2005b, Van Veen *et al.*, 2004) whereas error and conflict processing are linked to more dorsal parts of the ACC (e.g., Ridderinkhof *et al.*, 2004). Holmes *et al.* (2010) recently showed that the 5-HTTLPR polymorphism affects the rostral and dorsal part of the ACC differently; individuals with an S allele had increased rACC activity in response to errors compared to LL participants, while they had decreased dACC activity in response to conflict. In line with these findings, we expected that carriers of the S allele would show larger FRN responses than participants with the LL genotype. The same was expected for the feedback P3, a positive-going ERP component following the FRN that is thought to reflect the evaluation of feedback outcome (e.g., Wu and Zhou, 2009).

Our second aim was to investigate a functional polymorphism in the tryptophan hydroxylase 2 gene (TPH2) in relation to feedback processing. Recently, increasing attention is drawn to this gene that encodes for the brain-specific isoform of the enzyme tryptophan hydroxylase, which is involved in the conversion of tryptophan into serotonin. To our knowledge studies investigating the influence of single nucleotide polymorphisms (SNPs) within this gene on error or feedback processing are not available yet. Zhang *et al.* (2010) recently characterized one of these common SNPs (*rs1386493* C/T), showing that

the T variant reduces 5-HT production. We expected TPH2-*rs1386493* to have similar effects on the FRN and feedback P3 as 5-HTTLPR.

Finally, there is evidence suggesting that 5-HT receptor function decreases with advancing age (McEntee and Crook, 1991, Shiroma *et al.*, 2010). An additional goal of this study was, therefore, to investigate whether the effects of 5-HTTLPR and TPH2-*rs1386493* on the FRN and feedback P3 change with advancing age.

Methods

Participants

Data were obtained from 98 healthy volunteers (73 female), aged 22-76 (M=51.7) years, who were recruited as a control group for a study on depression. Individuals with a lifetime history of depression or a first-degree relative with depression, were therefore excluded from participation. Other exclusion criteria were neurological illness, the use of medication affecting the central nervous system, and pregnancy. All participants gave written informed consent and the study was approved by the local medical ethics committee.

Time-estimation task

Participants performed a modified time-estimation task (Mies *et al.*, 2011b) based on the paradigm developed by Miltner *et al.* (1997). Participants had to indicate the end of a one-second interval by pressing the button of a response device. Following the button press, they received performance feedback, i.e., positive feedback if their response occurred within a specified window around the target, and negative feedback if the response occurred outside the window. Unbeknownst to participants, the window was dynamically adjusted to ensure an equal amount of positive and negative feedback stimuli (see Miltner *et al.*, 1997).

The feedback consisted of face stimuli presented against a horizontal or vertical background grid. For reasons beyond the scope of this paper, the background grid communicated to participants whether the feedback was valid or invalid. Valid feedback was based on the participant's performance, while invalid feedback was determined randomly by the computer. Participants received invalid feedback in 50% of the trials. The emotional expression of the face informed participants that their estimate was correct or incorrect (respectively, a happy vs. a fearful face). Finally, in case of incorrect estimates, the gender of the face indicated whether the estimate was too short (e.g., a male face) or too long (e.g., a female face). The faces used in this study were from the Ekman and Friesen pictures set (Ekman and Friesen, 1978). Four versions of the task were counterbalanced across participants to correct for possible effects of the gender of the face stimuli and background grid.

Electrophysiological recordings

The EEG was derived from five electrodes placed at Fz, Cz, Pz, C3 and C4 according to the 10-20 system (Sharbrough *et al.*, 1991). Linked mastoids were used as a reference. The electro-oculogram (EOG) was derived from four electrodes, one placed above and one below the right eye, and one each on the outer canthus of each eye. A ground electrode was placed on the sternum. The EEG was sampled at 256 Hz, low-pass filtered at 30 Hz, and high-pass filtered with a time constant of 0.33 s. Electrode impedance was kept below 8 kΩ. Event-related potentials (ERPs) were locked to the onset of the feedback stimulus, and epochs were extracted between 100 ms preceding and 700 ms following feedback onset. The method of Gratton *et al.* (1983) was used to correct EEG traces for vertical EOG. Epochs were manually checked for artifacts (e.g., noise) and excluded from analysis when necessary. Each ERP was baseline-corrected by averaging the first 100 ms before feedback onset and subtracting this average from the ERP.

Procedure

After the study was fully explained, and participants gave their consent, blood was drawn for the genetic analyses. When blood drawing failed, a saliva sample was taken. Then participants were seated in front of a computer screen, and electrodes were placed for EEG recordings. After a short practice block, participants performed four blocks of the time-estimation task of 10 minutes each.

Genetic analyses

Genomic DNA was isolated from peripheral blood samples (EDTA, n=92), following standard procedures, or from saliva (n=6) using the kit of Oragene (DNA Genotek). In order to determine the 5-HTTLPR genotypes, PCR amplification of the genomic DNA (40 ng) was carried out in a final volume of 20 µl, in the presence of 10 pmol of oligonucleotide primers (forward: 5'-GGCGTTGCCGCTCTGAATGC-3'; reverse: 5'-GAGGGACTGAGCTGGACAACCAC-3'), 2.5 M betaine, 250 µM deoxyribonucleotides, 5x PCR reaction buffer with $MgCl_2$ (Expand high fidelity buffer 2, Roche), and 1 U of Taq DNA polymerase (FastStart Taq, 5 U/µl, Roche). Finally, 5 µl of the PCR product was loaded onto an agarose gel.

In order to determine the *rs1386493* C/T SNP in the TPH2 gene, genomic DNA was amplified through a PCR in a final volume of 25 µl, in the presence of 10x PCR reaction buffer with $MgCl_2$ (Roche), 10 pmol of oligonucleotide primers (forward: 5'-TGGGTGGGTGAATAAATGAATGC-3'; reverse: 5'-GAAATTCCCAGTCGCCAATAGC-3'), 250 µM deoxyribonucleotides, and 1 U of Taq DNA polymerase (5 U/µl). Finally, the SNP was sequenced (BDT v3.1, Applied Biosystems), and the sequences of each participant were analyzed using SeqScape (Applied Biosystems).

Statistical analyses

Participants were assigned to one of two groups on the basis of their 5-HTTLPR genotype: S group (SS + SL) vs. L group (LL). The same was done for *rs1386493*: T group (TT + TC) vs. C group (CC).

Performance on the time-estimation task was analyzed by comparing the two genotype groups on the percentage of correct adjustments after valid negative feedback using independent-samples t-tests. This was done separately for 5-HTTLPR and TPH2-*rs1386493*.

For the electrophysiological analyses we focused on the feedback condition and channels expected to be associated with the most pronounced FRN and feedback P3 responses. Therefore the responses to valid feedback at channels Fz, Cz (FRN), and Pz (feedback P3) were taken into account. The FRN was defined as the peak negativity of the difference wave (valid negative feedback minus valid positive feedback) between 200 and 350 ms after feedback onset measured at Fz and Cz. Two independent-samples t-tests were used to assess the difference between the two genotype groups with regard to feedback-related activity at channel Fz and Cz. This was done separately for 5-HTTLPR and TPH2-*rs1386493*.

The feedback P3 was defined as the mean amplitude of the ERP waveforms for valid positive and valid negative feedback between 350 – 500 ms after feedback onset measured at Pz. The mean amplitude for positive feedback was subsequently subtracted from the mean amplitude for negative feedback, resulting in a difference score. This difference score was used as the dependent variable in an independent-samples t-test with genotype as between-subjects factor.

To investigate whether age influenced the possible effects of genotypes on the FRN and feedback P3, we performed a median age split (median age is 51 years) and then repeated all analyses for the younger and older group, separately.

Results

Twenty-six participants were excluded from analyses. Six participants were not eligible for the ERP analyses due to recording problems, incomplete data, too many EEG artifacts, or an allergic reaction to the electrode paste. Eight participants were outliers on the basis of their mean estimation time, either > 1408 or < 539 ms. Another 8 participants did not show a reliable FRN. Finally the genetic analyses failed for 4 participants.

Of the 72 participants included in the analyses (56 female), 18 were homozygous for the S allele (14 female), 34 were heterozygous (SL; 29 female), and 20 were homozygous for the L allele (13 female). Three participants were homozygous for the T-allele in the TPH2-gene (TT; 2 female), 24 were heterozygous (TC; 20 female), and 45 were

homozygous for the C-allele (CC; 34 female). Table 7.1 shows the demographics and number of participants in each genotype group.

Table 7.1 Demographic data

	5-HTTLPR			**TPH2 (*rs1386493*)**		
	SS/SL	**LL**	p-value[a]	**TT/TC**	**CC**	p-value[a]
Total						
N	52	20		27	45	
Age	50.5 ±13	51.0 ±12	0.88	47.3 ±12	52.6 ±13	0.09
Female	43 (83%)	13 (65%)	0.11	22 (81%)	34 (76%)	0.56
Young (<51 years)						
N	24	12		17	19	
Age	38.7 ±8	43.6 ±9	0.11	40.4 ±9	40.3 ±9	0.98
Female	22 (92%)	7 (58%)	0.02	14 (82%)	15 (79%)	0.80
Old (>51 years)						
N	28	8		10	26	
Age	60.5 ±7	62.0 ±6	0.57	59.1 ±7	61.5 ±6	0.32
Female	21 (75%)	6 (75%)	1.00	8 (80%)	19 (73%)	0.67

[a]independent-samples t-tests were used to assess differences in age between genotype groups, and χ^2-tests to assess differences in gender distribution

5-HTTLPR and feedback processing

The S and L group did not differ in performance ($t(70) = 0.26$, $p = 0.80$). Participants with at least one copy of the S allele correctly adjusted their behaviour on 77.5 ±6% of the valid negative feedback trials, and those without an S allele on 78.0 ±8% of the trials. In addition the groups did not differ in mean estimation time (1056 vs. 1057 ms for the S and L group, respectively, $t(70) = 0.02$, $p = 0.99$), and intra-individual standard deviation of estimation time (270 vs. 273 ms for the S and L group, respectively, $t(70) = 0.12$, $p = 0.90$). Finally, analyses of performance of the younger and older group, separately, failed to reveal significant effects of genotype on performance.

When the whole group was taken into account 5-HTTLPR did not affect the FRN (Fz: $t(70) = 1.01$, $p = 0.32$; Cz: $t(70) = 1.51$, $p = 0.14$). FRN sizes for the S group were -2.4 ±1.4 µV at Fz and -2.4 ±1.3 µV at Cz, and for the L group -2.1 ±0.9 µV at Fz and -1.9 ±0.9 µV at Cz. Separate t-tests for the younger and older group, however, revealed a main effect of 5-HTTLPR on the FRN-response in the younger group at channel Cz ($t(34) = 2.56$, $p = 0.015$), but not in the older group ($t(34) = 0.13$, $p = 0.90$). The S carriers up to 51 years of age had larger FRN-responses, i.e., a larger difference in response to positive and negative

feedback, than their peers with an LL genotype (-2.7 ±1.1 μV vs. -1.7 ±1.0 μV at Cz; see Figure 7.1A and 7.2)[1]. This effect did not reach significance at Fz (-2.6 ±1.5 μV vs. -2.1 ±0.9 μV, $t(34) = 1.07$, $p = 0.29$).

We subsequently investigated the effects of 5-HTTLPR on the feedback P3. In the group as a whole, we found no effect of 5-HTTLPR on the feedback P3 ($t(70) = 1.57$, $p = 0.12$). In the younger group, however, S carriers showed a larger difference in feedback P3 amplitudes between positive and negative feedback than their LL peers ($t(34) = 2.38$, $p = 0.023$, see Figure 7.1C and 7.2). This effect was not present in the older group ($t(34) = 0.32$, $p = 0.75$).

Because the younger group had an unequal gender distribution between the two 5-HTTLPR genotypes (see Table 7.1), we checked whether gender contributed to the effects of 5-HTTLPR on the FRN and feedback P3. For this purpose, we first performed two independent-sample t-tests on the group as a whole including gender as independent variable and the FRN or feedback P3 as dependent variable. The effects failed to reach an acceptable significance level ($t(70) = 1.50$, $p = 0.14$, and $t(70) = 1.75$, $p = 0.085$, for the FRN and feedback P3, respectively). Secondly, we performed the same tests within the older group in which the gender distribution between the two genotype groups was equal ($t(34) = 0.52$, $p = 0.61$, and $t(34) = 0.76$, $p = 0.45$, for the FRN and feedback P3, respectively). These analyses show that it is unlikely that the unequal gender distribution interacts with the effects of 5-HTTLPR on the FRN and feedback P3.

TPH2-rs1386493 and feedback processing

The T group made just as many correct adjustments after valid negative feedback as the C group (77.1 ±7% vs. 78.0 ±6% for the T and C group, respectively, $t(70) = 0.59$, $p = 0.56$). Mean estimation time did not differ between the groups either (1072 vs. 1047 ms, for the T and C group, respectively, $t(70) = 0.95$, $p = 0.35$). By contrast, intra-individual standard deviation of estimation time was larger for the T group than the C group (306 vs. 250 ms, respectively, $t(70) = 2.24$, $p = 0.028$). The analyses on the younger and older group separately showed that the latter effect was mainly due to the older participants ($t(34) = 2.19$, $p = 0.036$).

Rs1386493 neither had a statistically significant influence on the FRN-response in the group as a whole, nor in the younger or older group separately. At channel Fz,

[1] Because Figure 7.2 suggests that there was also a main effect of genotype independent of valence in the time window of the FRN in the younger group, we did an additional analysis on the ERPs for positive and negative feedback separately. We therefore calculated the peak negativity between 200 and 350 ms in both baseline-corrected ERPs. Against what would be expected on the basis of Figure 7.2, a repeated-measures ANOVA with valence as within-subjects factor and genotype as between-subjects factor revealed no significant main effect of genotype in the younger group at Fz or Cz (Fz: $F(1,34) = 2.42$, $p = 0.13$; Cz: $F(1,34) = 2.08$, $p = 0.16$).

however, the younger participants with the CC genotype showed a slightly larger FRN than their T peers ($t(34) = 1.8$, p = 0.080), as can be seen in Figure 7.1B[2].

The group as a whole did not show an effect of *rs1386493* on the feedback P3 ($t(70) = 1.06$, p = 0.29). In the older group, however, participants with the CC genotype showed a larger difference between positive and negative feedback in the time window of the feedback P3 than T carriers ($t(34) = 2.12$, p = 0.042, see Figures 7.1D and 7.3). No such difference was found in the younger group ($t(34) = 0.48$, p = 0.64).

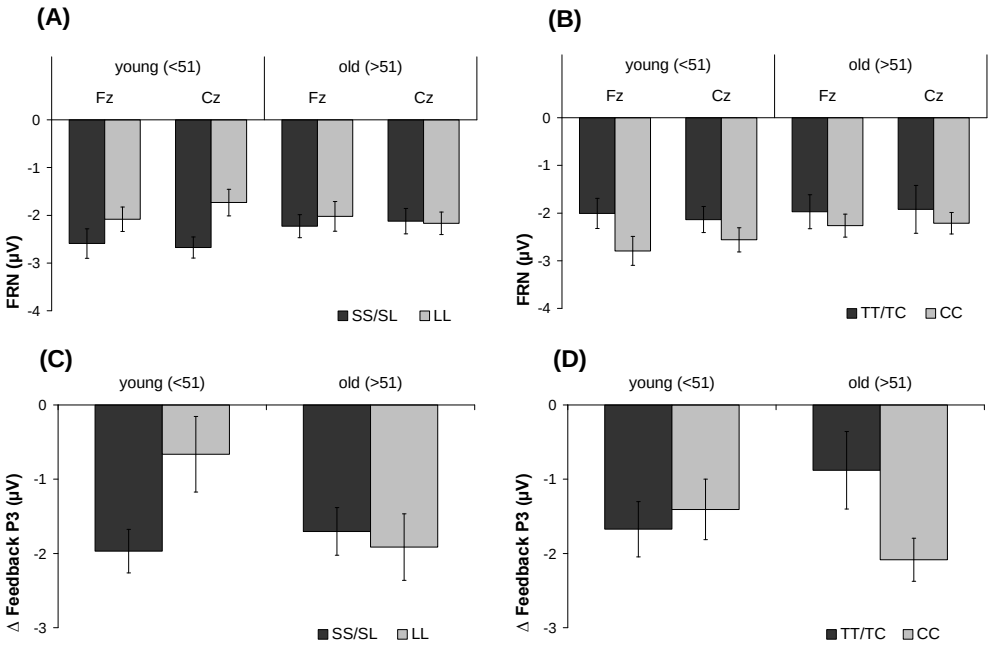

Figure 7.1 Bar graphs (with SEM) showing (A) FRN responses (negative minus positive feedback) at channel Fz and Cz of young (<51 years) and old (≥51 years) individuals for 5-HTTLPR S carriers versus LL individuals; (B) FRN responses of young and old individuals for TPH2-*rs1386493* T carriers versus CC individuals; (C) feedback P3 responses (negative minus positive feedback) at channel Pz of young and old individuals for 5-HTTLPR S carriers versus LL individuals; and (D) feedback P3 responses of young and old individuals for TPH2-*rs1386493* T carriers versus CC individuals.

[2] An additional analysis on the peak negativity of the separate ERPs for positive and negative feedback, as described in Footnote 1, revealed a significant main effect of genotype in the younger group at Fz and Cz (Fz: $F(1,34) = 4.31$, p = 0.046; Cz: $F(1,34) = 5.44$, p = 0.026): the C group had larger (i.e., more negative) amplitudes in the time window of the FRN than the T group. This effect was independent of valence.

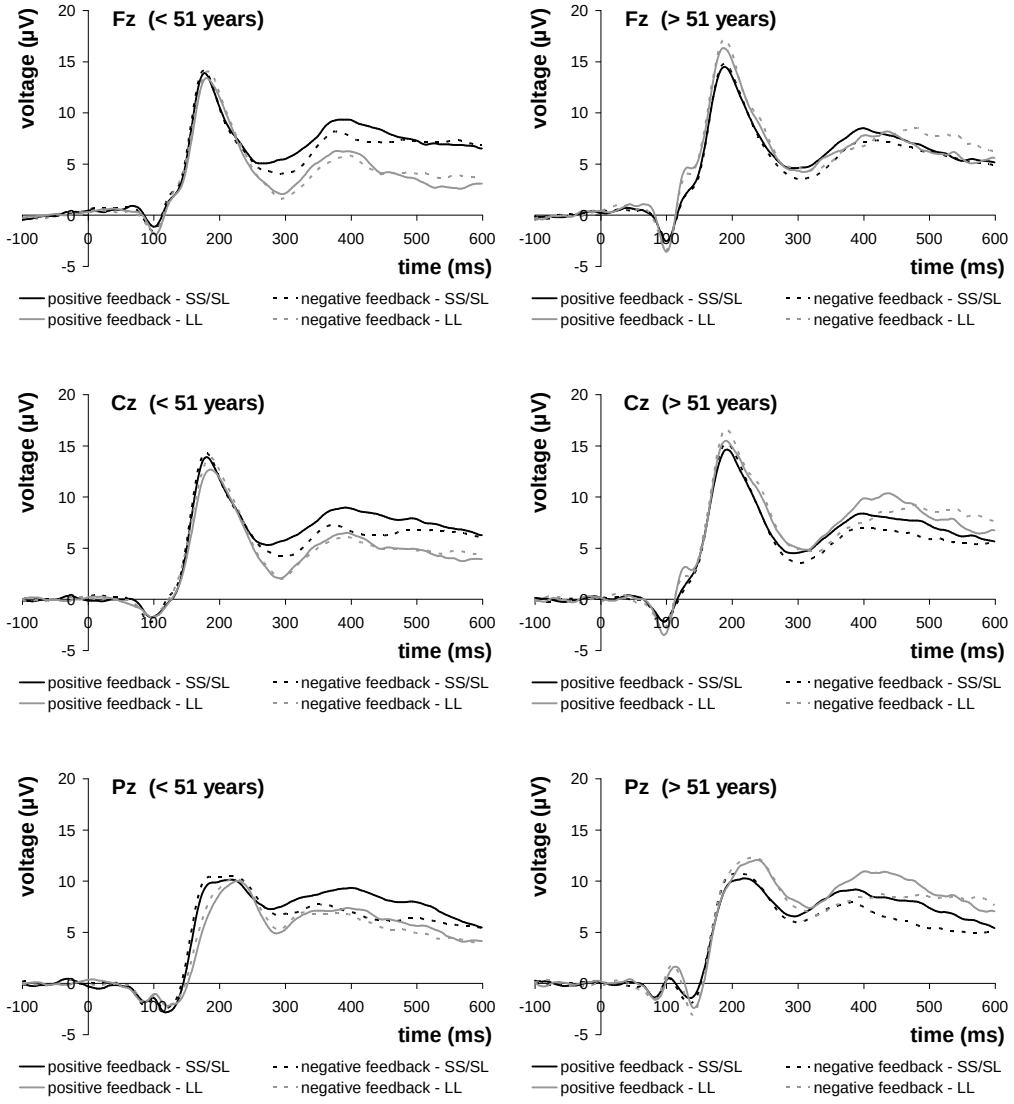

Figure 7.2 Grand-average event-related brain-potentials from channels Fz, Cz and Pz evoked by positive and negative feedback for 5-HTTLPR S carriers and LL individuals for young (<51 years; left panel) and old (≥51 years; right panel) individuals

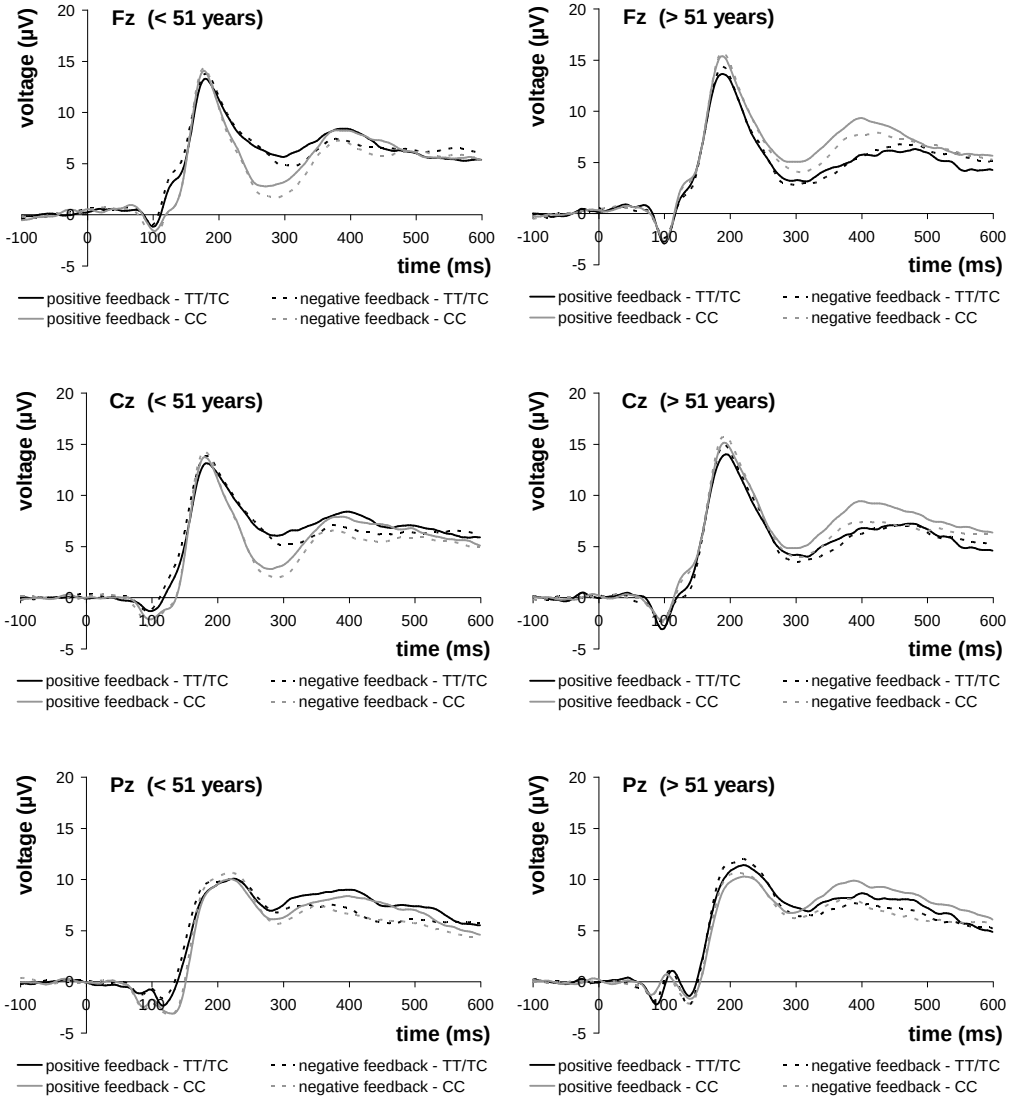

Figure 7.3 Grand-average event-related brain-potentials from channels Fz, Cz and Pz evoked by positive and negative feedback for TPH2-*rs1386493* T carriers and CC individuals for young (<51 years; left panel) and old (≥51 years; right panel) individuals

Discussion

The aim of this study was to investigate the influence of 5-HTTLPR and TPH2-*rs1386493* on feedback processing. Interestingly, the effects of genotype were influenced by the age of the participants. Relatively young participants (up to 51 years of age) who had at least one copy of the short allele of 5-HTTLPR had, in comparison to young participants with the more functional LL genotype, a larger FRN and a larger difference between feedback P3 responses to positive and negative feedback. Older participants who carried the TPH2-*rs1386493* T variant, however, showed a smaller difference between feedback P3 responses to positive and negative feedback than older participants with the more functional CC genotype.

Our 5-HTTLPR findings concerning the FRN are in line with two previous studies that reported larger ERN-amplitudes for S carriers (Althaus *et al.*, 2009, Fallgatter *et al.*, 2004). Our findings also appear to be in line with the fMRI study by Holmes *et al.* (2010) that showed increased rACC activation for S carriers in response to errors. Our results suggest that the rACC response to the valence of feedback is different for individuals with an S allele and those without. Olvet *et al.* (2010a), however, were unable to detect the 5-HTTLPR effect in a young, well-balanced and relatively large sample. This discrepancy might be due to the selection of participants. In contrast to the other studies including our own, Olvet *et al.* (2010a) did not screen participants for psychiatric disorders. Psychiatric disorders such as depression and anxiety, and even mild symptoms of these disorders, such as increased negative affect, have been found to influence the ERN/FRN (Chiu and Deldin, 2007, Hajcak *et al.*, 2003a, 2004, Holmes and Pizzagalli, 2008, Luu *et al.*, 2000, Ruchsow *et al.*, 2006, Ruchsow *et al.*, 2004). If some of the undergraduates in the study by Olvet *et al.* (2010a) suffered from such symptoms, genotype effects might have been overruled.

This is the first time that 5-HTTLPR effects were investigated in middle-aged to older participants, which revealed an age-dependent genotype effect: 5-HTTLPR did not influence the FRN-response in older individuals. This may be due to the combined effects of decreased 5-HT receptor function associated with aging (Sheline *et al.*, 2002, Uchida *et al.*, 2011) and the alleged influence of 5-HTTLPR on the regional distribution and density of 5-HT receptors (Hariri *et al.*, 2006). A recent study showed that 5-HT receptor function exponentially decreases with age, dropping more quickly in early adulthood and more gradually later on (Uchida *et al.*, 2011). Changes in neurotransmission associated with the S allele are still not fully understood, but it is possible that these changes become less prominent when people age, mirroring the exponential decrease in 5-HT receptor function. Combined effects of a reduction in ACC volume associated with S allele carriers (Pezawas *et al.*, 2005) and with aging (e.g., Bergfield *et al.*, 2010) may also contribute to our findings. It could be argued, however, that a general reduction in FRN-size associated with advanced age caused a floor effect, resulting in a lack of the 5-HTTLPR effect in the

older group. In a previous study we confirmed that the FRN-response decreased with age in the current sample (Mies *et al.*, 2011b). This reduction in FRN-size is thought to be due to a decrease in dopamine level (Nieuwenhuis *et al.*, 2002). We would like to argue that a floor effect causing the lack of 5-HTTLPR effect in the older group seems implausible because no floor effect was expected for the feedback P3, while the effects of 5-HTTLPR were the same. The neural system underlying the feedback P3, the locus coeruleus-norepinephrine system, is thought to be less influenced by aging than the neural system underlying the FRN (Mathewson *et al.*, 2008). Similar to the FRN, however, there was an effect of 5-HTTLPR on the feedback P3 in the younger group, but not in the older group. It is therefore most likely that, rather than a floor effect as a result of reduced dopamine function in older age, 5-HTTLPR interacts with the changes in 5-HT function associated with aging, leading to differential effects on feedback processing in younger and older adults.

As already mentioned, the effects of 5-HTTLPR on the FRN were also found on the feedback P3. In the relatively young S carriers the difference in response to positive and negative feedback was larger than in individuals with the LL genotype, but no such effect was found in the older group. The feedback P3 is thought to reflect the significance of feedback outcomes (e.g., Wu and Zhou, 2009), and is known to be influenced by the amount of attention paid to a stimulus (Nieuwenhuis *et al.*, 2005a). The feedback P3 is also known to be larger for unexpected feedback outcomes than for expected outcomes (Hajcak *et al.*, 2007). The results suggest that young S carriers differentiate more in their attention towards feedback outcomes than others, perhaps evaluating positive feedback as more unexpected, and negative feedback as more expected, while this effect diminishes with older age.

TPH2-*rs1386493* also influenced the feedback P3, but only in older participants. Contrary to expectations, older participants with the more functional CC genotype showed a larger difference between positive and negative feedback than individuals with the less functional T variant. Therefore, this SNP appears to affect feedback processing in a different way as compared to 5-HTTLPR. This difference probably reflects the specific roles of the genes in 5-HT function; 5-HTT is involved in the reuptake of 5-HT while TPH2 is involved in the production of 5-HT. Evidence is accumulating that 5-HTTLPR has a substantial influence on brain development and structure rather than on 5-HT metabolism *per se* (Jedema *et al.*, 2010). It is possible that, in contrast to 5-HTTLPR, TPH2-*rs1386493* mainly influences 5-HT metabolism. This may contribute to the differential effects of the two polymorphisms on the electrophysiological correlates of feedback processing.

We should note that there is at least one important limitation to this study. We did not assess the rare A/G SNP (rs25531) within the L allele, which leads to a functionality comparable to the S allele (Hu *et al.*, 2005). However, we do not expect a major impact of this SNP on our findings, because of its low prevalence in Caucasians (see Murphy *et al.*,

2008, Wendland *et al.*, 2006), which implies that only a few participants have possibly been misclassified. Moreover, the triallelic and biallelic approach led to largely similar results in the study by Olvet *et al.* (2010a).

In conclusion, 5-HTTLPR and TPH2-*rs1386493* had differential influences on electrophysiological responses to feedback, and importantly these effects were markedly different for younger participants than for older participants. As expected, relatively young 5-HTTLPR S carriers showed a larger difference between positive and negative feedback in the time windows of the FRN and the feedback P3 than participants with the LL genotype. Relatively old carriers of the TPH2-*rs1386493* T variant, on the other hand, had a smaller difference in feedback P3 responses to positive and negative feedback. On the basis of these findings, future research is warranted on this specific SNP in the TPH2-gene and on gene x age interactions, not only in relation to error and feedback processing, but to cognitive and social function in general.

Chapter 8

General discussion

The aims of this thesis were to deepen our understanding of the disturbances in feedback processing in patients with major depressive disorder (MDD), to gain further insight into the neural basis of feedback processing, especially concerning the role of the anterior cingulate cortex (ACC), and to investigate the role of serotonin (5-HT) in feedback processing. To reach these goals we had both depressed and non-depressed participants perform a time-estimation task while examining their behavioural, electro-cortical, cardiac, and/or blood-oxygen-level-dependent (BOLD) responses to the feedback provided during this task. To examine the role of 5-HT in feedback processing, we employed acute tryptophan depletion (ATD) in healthy volunteers, and we examined two genetic polymorphisms in two serotonin-related genes in another group of healthy volunteers. In this chapter, the main results and conclusions are reviewed, some methodological issues are discussed, and suggestions for further research are given.

Further insights into feedback processing in healthy individuals

One of the main questions we have tried to answer is whether patients with MDD are more sensitive to the emotional value of feedback than to the information conveyed by the feedback. For this purpose patients and healthy volunteers performed a time-estimation task with valid and invalid feedback. We used emotional faces as feedback stimuli to increase the emotional value and ecological validity of the feedback stimuli.

In order to understand the alleged deficits in feedback processing in depression it is necessary to more closely examine the behavioural and neurophysiological responses to feedback in healthy individuals. The sensitivity of different measures (event-related brain potentials (ERPs), heart rate, BOLD) and ERP components (feedback-related negativity (FRN), feedback P3) to the validity of feedback is therefore of interest. In chapter 2 we showed that the behavioural, cardiac and feedback P3 responses were sensitive to the validity of the feedback, but the FRN was not. Furthermore, both the feedback P3 and cardiac slowing were more pronounced when valid negative feedback was followed by a correct adjustment than when it was followed by an incorrect adjustment, suggesting a relationship with remedial action. Therefore, although statistically uncorrelated, the feedback P3 and cardiac response showed many similarities. These similarities suggest a common neural substrate. The underlying substrate of the FRN, on the other hand, appears to be different. Our fMRI results (chapter 3) showed that the rostral part of the ACC (rACC) was mainly sensitive to the valence of the feedback, whereas the rostral cingulate zone (RCZ; dorsal part of the ACC) was mainly sensitive to the validity of the feedback. Together, our results suggest that the RCZ contributes to the feedback P3 and cardiac responses to feedback, being more active in response to valid feedback than to invalid feedback, whereas the rACC contributes to the FRN, being primarily sensitive to the valence of the feedback. The rACC as one of the main generators of the FRN in time-

estimation tasks is in line with results by Nieuwenhuis *et al.* (2005b). We argue that the FRN response elicited in a time-estimation task acts as an 'alert' signal, which is mainly driven by rACC activity, and does not appear to directly signal the need for behavioural adjustments, a function that has been allocated to the RCZ (Holroyd and Coles, 2002, Ridderinkhof *et al.*, 2004, Van der Veen *et al.*, 2011).

The role of the ACC in feedback processing

As argued above, the rACC was mainly sensitive to the valence of feedback, being more active in response to positive feedback than to negative feedback, while the RCZ was mainly sensitive to the validity of feedback, being more active in response to valid than to invalid feedback. In chapter 3 we discussed our findings along the popular dorsal-cognitive/ventral-affective subdivision hypothesis (Bush *et al.*, 2000, Devinsky *et al.*, 1995). We argued that the cognitive evaluative function of the dorsal part of the ACC was reflected in the sensitivity of the RCZ to feedback-validity. The rACC, however, which is more ventrally located, did not clearly show this distinction between valid and invalid feedback, which meshes nicely with the idea that the ventral part of the ACC is more involved in the processing of emotion independent of its validity. However, the dorsal-cognitive/ventral-affective subdivision hypothesis, also referred to as the segregation hypothesis, has recently been challenged on the basis of studies reporting involvement of the dorsal part of the ACC in the processing of negative emotion, fear, pain, and social rejection, and studies reporting ventral ACC areas involved in emotion expression and regulation (Eisenberger *et al.*, 2003, Etkin *et al.*, 2011, Shackman *et al.*, 2011). Shackman *et al.* (2011) postulated the 'adaptive control hypothesis', which suggests that the RCZ uses information about punishment to bias responding when the most adaptive course of action is uncertain. The RCZ, therefore, integrates emotion, pain, and cognitive control. This idea is not new, as Shackman *et al.* noted, but is postulated as a new working hypothesis to explain aversively motivated behaviour and to replace the segregation hypothesis, which in their opinion is not tenable anymore. Although it is very appealing to assume that the dorsal part of the ACC is an important hub integrating all information relevant for performance and social function, such as emotion and cognitive control, the authors, unfortunately, do not take into consideration the possible important role of the rACC in mediating this function. They instead focus more on the interplay between the dACC/RCZ and for instance the amygdala and striatum.

Our findings do not completely match with their working hypothesis. First of all, one would expect on the basis of the adaptive control hypothesis that the RCZ would have been sensitive to the negative valence of feedback given its validity, but we found no interaction between valence and validity in the RCZ, while other areas, such as the amygdala and putamen did show an interaction. This raises the question whether the RCZ is really the important hub integrating all relevant information as suggested by Shackman

et al. (2011). In line with our findings, a study by Somerville *et al.* (2006) on the role of the ACC in social feedback revealed a double dissociation between dorsal and ventral ACC regions. They showed that the *dorsal* ACC was sensitive to expectancy violation (i.e., cognitive conflict), whereas a more *ventral* region of the ACC was sensitive to social acceptance (i.e., emotion).

Second and perhaps more importantly, the brain regions that were sensitive to the valence of feedback in our study were more active in response to *positive* feedback than to *negative* feedback. Several other studies on feedback processing using a time-estimation task (Nieuwenhuis *et al.*, 2005b, Van der Veen *et al.*, 2011), and studies on social reward (Moor *et al.*, 2010, Somerville *et al.*, 2006) reported similar findings. It is, however, unclear to what extent the adaptive control hypothesis can account for reward and appetitively motivated behaviour.

We argued in chapter 5, that our findings do not discard the segregation hypothesis. In most studies the emotional value of feedback is equivalent to its informational content, but with our modified version of the time-estimation task including invalid emotional feedback, we were able to disentangle the emotional content from the informational content. Our findings suggest that the RCZ is primarily involved in the evaluation of the relevance of emotional information, rather than being involved in emotion processing per se. The more ventral part of the ACC, on the other hand, does not appear to exert this cognitive evaluative function, which leads to the rACC being primarily involved in the processing of the emotional value of the feedback per se (see also Somerville *et al.*, 2006). We, therefore, note that feedback processing, which entails both cognitive and affective processing, requires involvement of both subdivisions of the ACC. We do agree with Shackman *et al.* (2011) that a strict segregation of functions of the subdivisions of the ACC is unlikely, partly because the variation between individuals in the mapping of different domains such as cognition, pain and affect to ACC anatomy is probably very large. It has also been suggested that the cognitive and emotional differentiation of the ACC should be viewed along a continuum rather than as a strict segregation (Mies *et al.*, 2011a, Mohanty *et al.*, 2007). It is, on the other hand, known that the cytoarchitecture and receptor architecture of the subdivisions is different, and that these subdivisions can even be further subdivided into smaller areas (Palomero-Gallagher *et al.*, 2009, Vogt and Vogt, 2003). Being so closely related to each other, however, the dorsal and ventral parts of the ACC are probably highly cooperative in order to maintain accurate performance.

To summarize, the adaptive control hypothesis is very appealing because it integrates all aspects in which the RCZ, or more broadly speaking the dorsal ACC, may be involved in. However, our findings, and those of others (e.g., Mohanty *et al.*, 2007, Somerville *et al.*, 2006) point in the direction of a segregation of functions, and are therefore more in line with the segregation hypothesis than with the adaptive control

hypothesis. The segregation hypothesis also fits with studies on depression showing distinct activity patterns of the subdivisions of the ACC, which will be discussed below.

Feedback processing in patients with MDD

One of the main aims was to gain further insight into performance monitoring and feedback processing in depression. To examine whether performance of patients with MDD is more driven by the emotional content of feedback stimuli than by the informational content, we investigated patients' responses to both valid and invalid emotional feedback in one single paradigm (chapter 4). The behavioural responses show that patients performed slightly worse than healthy controls when feedback was valid, but, similar to healthy controls, they did not adjust their behaviour after invalid negative feedback. Because the behavioural results imply that the patients in our study ignored the invalid emotional feedback, it is unlikely that performance disturbances found in depression are due to the affective value of feedback per se.

Importantly, independent of validity, patients showed a larger FRN than controls. In contrast to the behavioural responses, the increased FRN responses suggest that the affective value of feedback underlies this specific electrophysiological correlate of feedback processing. The increased FRN responses are probably due to atypical rACC functioning.

Our results are in line with most previous studies reporting increased error-related negativity (ERN) or FRN responses in depressed patients (Chiu and Deldin, 2007, Holmes and Pizzagalli, 2008, Tucker *et al.*, 2003). Some studies, however, reported opposite findings, i.e., smaller ERNs or FRNs (Ruchsow *et al.*, 2006, Ruchsow *et al.*, 2004, Schrijvers *et al.*, 2008, Schrijvers *et al.*, 2009). As discussed in chapter 4, there are at least two important factors that are likely to explain discrepancies between studies: the use of different types of medication, and the severity of illness. Medication may exert a direct influence on the ACC, but it is also possible that the changes in symptoms and changes in the severity of symptoms associated with the use of medication influence the responses to errors and feedback. Our results suggest, although statistically not significant, that FRN responses are increased in moderate to severe depression, but are less increased in patients with more severe depression. Similarly, the results by Tucker *et al.* (2003) suggested that FRN responses were increased in moderately depressed patients, but this effect disappeared above a certain level of symptom severity. Although the patients included in the study by Tucker *et al.* were not nearly as severely depressed as the patients in our study, it is not unlikely that FRN-size depends on the severity of depression following a bell-shaped pattern, i.e., a normal FRN in healthy to mildly depressed subjects, increased FRNs in patients with moderate to severe depression, and less increased or even smaller FRNs in more severely depressed patients. Specific symptoms of depression, such

as anhedonia, apathy and psychomotor retardation, may underlie these decreases in FRN-size in more severely depressed patients (Schrijvers *et al.*, 2009, Tucker *et al.*, 2003). The patients in our study were rather severely depressed and had typical melancholic features such as anhedonia. On average, they were, however, not as severely depressed as the patients in the studies by Schrijvers *et al.* (2008, 2009), who reported decreased ERN responses in patients with psychomotor retardation (Schrijvers *et al.*, 2008). The discrepancy between the studies might be explained by this difference in severity, and the presumed differences in the occurrence and severity of psychomotor retardation.

ACC function in depression

Atypical rACC function is likely to underlie the increased FRN responses we found in patients with MDD (chapter 4). The rACC is probably hypersensitive to the valence of feedback in these patients. In another study, using fMRI (chapter 5), we examined the responses of the rACC, RCZ and subgenual ACC (sgACC) to feedback in individuals with higher than average depression scores, to further unravel the differences in ACC function associated with depression. Analyses of depressive state in general (high versus low scores on the BDI) did not reveal robust associations with ACC function in relation to feedback processing. The core symptoms of depression, however, *depressed mood* and *anhedonia*, correlated with sgACC and RCZ activity. In depression, it has been demonstrated that the dorsal part of the ACC is *hypo*responsive and the ventral part *hyper*responsive (Davidson *et al.*, 2002, Mayberg, 1997, 2003, Pizzagalli, 2011). Our results concerning anhedonia are in line with these findings.

The sgACC appears to play a crucial role in depression. It is thought to be involved in general emotion processing (e.g., Harvey *et al.*, 2007). In chapter 5 we showed that activity in the sgACC was influenced by several symptoms and personality characteristics related to depression (i.e., depressed mood, anxiety, neuroticism) including anhedonia. Other studies also found effects of anhedonia on this brain region. Keedwell *et al.* (2005) and Harvey *et al.* (2007) reported a positive correlation between anhedonia and activity in the ventromedial prefrontal cortex, a region corresponding to the rACC/sgACC, in response to positive emotional stimuli compared to neutral stimuli. In addition, Wacker *et al.* (2009) and Pizzagalli *et al.* (2004) found a positive correlation between anhedonia and resting EEG delta activity in the sgACC, which implies reduced resting brain activity in this area. Reduced activity in this region during rest seems at odds with hyperactivity, but during task engagement this region should be deactivated, which is thought to occur less in more anhedonic individuals (Wacker *et al.*, 2009). Therefore, the hyperactivity we found in the sgACC in response to feedback might be due to less deactivation in individuals with higher levels of anhedonia.

Depressed mood was also associated with hyperactivity in the sgACC. Studies using negative mood induction can shed more light on the effects of depressed mood on brain

activation. After negative mood induction, increased activity has been reported in response to negative stimuli in the sgACC, rACC, and ventromedial and orbitofrontal prefrontal cortex in healthy individuals (Berna *et al.*, 2010, Habel *et al.*, 2005, Wang *et al.*, 2006). The association we found between depressed mood and sgACC activity is therefore in line with these studies which clearly indicate that a change in mood state influences activity in the ventral-rostral area of the ACC and prefrontal cortex.

The main conclusion on the basis of our findings reported in chapter 5 is that the neural circuitry involved in feedback processing is influenced by specific symptoms of depression rather than by the depressive state in general. This emphasizes the need for further investigations into the variability of these symptoms in patients with MDD. These findings may have implications for the treatment of individual patients. Especially anhedonia has shown to be an important modulator of brain function in relation to feedback processing, and needs further investigation.

To summarize, atypical functioning of all subdivisions of the ACC appears to play a role in depression, and the RCZ and rACC seem to underlie behavioural and electrophysiological manifestations of (disturbances in) feedback processing. Depending on the task at hand, which may recruit certain brain regions more than others, the use of psychotropic substances, severity of illness, and presence of specific symptoms, performance and neurophysiological responses to feedback may appear 'normal', increased, or decreased.

The role of serotonin in feedback processing

Finally, we examined the role of serotonin in feedback processing. In chapter 6, we found that the electro-cortical responses to feedback were not influenced by ATD, and therefore not susceptible to a transient decrease in central serotonin availability. The cardiac response to feedback, however, was attenuated after ATD. We explained this in terms of increased punishment prediction associated with lower 5-HT levels (Cools *et al.*, 2008), and therefore argued that ATD influences cognitive processing. It is of interest to note that we also found an attenuated cardiac response to incongruent stimuli in an Eriksen flanker task after ATD, but not in response to congruent stimuli and errors (Van der Veen *et al.*, 2010). Together these findings suggest that ATD has a larger influence on cognitive processing than on affective processing, and that this cognitive processing is reflected in heart rate. ATD appears to specifically attenuate the cardiac response to stimuli that need cognitive control, without changing the electro-cortical and behavioural responses.

A recent review by Feenstra and Van der Plasse (2010), however, raised doubts about the presumed mechanisms by which ATD exerts its effects, and posed the possibility of a more direct effect of ATD on the peripheral cardiovascular system, that is, on heart rate. The authors further proposed that effects of ATD on cognition and behaviour may

occur in line with the somatic-marker hypothesis (Damasio, 1996), which entails that affective signals from the body, including changes in heart rate, influence cognition and behaviour. Although we acknowledge the importance of the somatic-marker hypothesis, the model postulated by Feenstra and Van der Plasse cannot easily explain why we found a smaller difference in cardiac response to positive and negative feedback, rather than a general increase or decrease in heart rate. Since ATD did not affect heart rate itself, but selectively attenuated the cardiac response to negative feedback stimuli, it is unlikely that our effects were caused by a peripheral effect of ATD (Van der Veen *et al.*, 2010, Van der Veen *et al.*, 2008).

Van Donkelaar *et al.* (2011) emphasized some other mechanisms by which ATD might exert its cognitive and behavioural effects. One of these mechanisms is a decrease in local cerebral blood flow (CBF) associated with ATD. They argue that the rather consistent evidence of memory impairments after ATD, found mostly in animal studies, but also in human studies, are most likely due to reduced local CBF in hippocampal areas, which might in its turn be influenced by a decrease in nitric oxide, also triggered by ATD (Van Donkelaar *et al.*, 2011). In line with this, Talbot & Cooper (2006) showed that whereas CBF *increased* in the sgACC and striatum with increasing sadness after ATD, CBF *decreased* in the dACC after ATD, independent of mood change. If heart rate is under influence of the dACC, as hypothesized on the basis of the findings described in this thesis (see also Critchley *et al.*, 2003), decreases in CBF in this brain region might influence cognitive processing, which might in its turn explain our cardiac effects. In other words: decreased CBF in the dACC or RCZ might lead to decreased cognitive control and therefore to an attenuation of cardiac responses to negative feedback.

It is possible that the effects of ATD on behavioural and neurophysiological correlates of mood and cognition are based on other mechanisms than a purely serotonergic one. Results from genetic studies, however, may shed more light on involvement of 5-HT in cognitive and affective processing. In chapter 7 we investigated the effects of two genetic polymorphisms on feedback processing. We found an effect of 5-HTTLPR on the FRN and feedback P3. Individuals with a short allele (SL or SS) had larger FRNs than homozygotes for the L allele. Previous studies showed the same effect of this polymorphism in relation to error processing (Althaus *et al.*, 2009, Fallgatter *et al.*, 2004; but see Olvet *et al.*, 2010a). Our finding suggests larger alert signals in response to feedback in S carriers. The presumed developmentally-induced morphological differences in the rACC between S carriers and L homozygotes (Jedema *et al.*, 2010) possibly underlie this effect.

We also found an effect of a functional polymorphism in the TPH2-gene (*rs1386493*). The T variant has been found to be associated with a decrease in 5-HT production (Zhang *et al.*, 2010). Against expectations, the difference between positive and negative feedback in the time window of the feedback P3 was *smaller* in individuals with

the less functional variant, but, interestingly, this effect was only present in older participants. Because this is the first time this polymorphism was investigated in relation to performance monitoring, future studies should further examine this polymorphism to assess the strength and importance of our finding.

Similar to our ATD effects, the effects of these genetic polymorphisms on the FRN and feedback P3 may have been caused by differences in local CBF associated with these polymorphisms. Indeed, associations between the 5-HTTLPR polymorphism and local CBF/perfusion have been reported in the amygdala, hippocampus, and ventromedial PFC (Canli *et al.*, 2006, Rao *et al.*, 2007). Viviani *et al.* (2010), however, did not find a correlation between the 5-HTTLPR polymorphism and whole-brain baseline brain perfusion in a much larger sample of healthy volunteers, casting doubt on this association. It should be noted, however, that they only zoomed in on the amygdala and orbitofrontal cortex as regions of interests, and not on the dACC and rACC, which are of main interest here.

To summarize, the studies described in chapter 6 and 7 provide further evidence for an involvement of 5-HT in the cardiac and electro-cortical responses to feedback. The role of 5-HT in feedback processing, however, is perhaps only indirect, and appears to be small. The genetic polymorphisms had, for instance, no clear effects on the group as a whole, but depended on the age of the participants, and ATD had no effects on the electro-cortical responses to feedback. It is also important to note that the behavioural responses to feedback were largely unaffected by both ATD and the genetic polymorphisms under investigation. It is therefore difficult to assess to what degree this knowledge contributes to impairments in feedback processing as seen in depression.

What remains unclear is whether the role of 5-HT in neurophysiological and autonomic correlates of feedback processing is direct or indirect. In addition to the effects of 5-HT on local CBF, it is possible that the effects found are more closely related to dopamine (DA) than to 5-HT, and that the interactions between 5-HT and DA are reflected in these responses. In the dACC, for example, the density of DA fibres is very high, and 5-HT is known to modulate DA function (Smith *et al.*, 1997, Talbot and Cooper, 2006). As we have shown, two genetic polymorphisms can have different effects due to their specific functions in the 5-HT pathway. The same will apply for dopamine-related genetic polymorphisms. Complex gene-gene interactions and gene-environment interactions (e.g., stress, mood state) may underlie the responses to feedback. Future studies might want to focus on these interactions in relation to error and feedback processing. In addition to genetic analyses, the influences of pharmacological 5-HT and DA challenges on local CBF, especially in the ACC, might prove beneficial for a deeper understanding of the role of these neurotransmitters in error and feedback processing.

Dysfunctions of the 5-HT system have been associated with depression for a long time, although the evidence is rather indirect (e.g., response to SSRIs). Depression is a heterogeneous disease which makes it difficult to assess if, or to what extent 5-HT dysfunctions contribute to the illness from person to person. Furthermore, the 5-HT system does not operate independently from other neurotransmitter systems, making it virtually impossible to precisely pinpoint the effects caused by differences in 5-HT function to a direct effect of 5-HT. Despite these limitations, our findings point in the direction of a role of 5-HT in feedback processing. It should be noted that the brain regions that are mostly affected by ATD (i.e., dorsal and subgenual ACC, posterior cingulate cortex, striatum, and hippocampus; e.g., Smith *et al.*, 1999, Talbot and Cooper, 2006, Van Donkelaar *et al.*, 2011), have been found dysfunctional in depression, further emphasizing the link between 5-HT and depression. The role of 5-HT may also be an important link between specific symptoms of depression and disturbances in performance monitoring.

Clinical implications

The practical implications of the studies described in this thesis are rather limited. For example, individual variability in cardiac and ERP responses was very high, rendering these measures not useful for assessments of individual patients. At the group level, however, the findings increase our knowledge of the underlying mechanisms of feedback processing in depression, and therefore contribute to a better understanding of disturbances in performance monitoring in depression. What can be learned from these findings is the special role of the ACC and its subdivisions in the link between depression and atypical feedback processing.

Our findings point in the direction of shifting the focus of treatment development from the depressive state in general to a focus on alleviating the severity of specific symptoms of depression (see also Holtzheimer and Mayberg, 2011), not only at the subjective level but also at the neural level. Anhedonia, for instance, might require a different treatment program than depressed mood, or sleep disturbances.

Limitations

Several limitations of the studies described in this thesis should be mentioned. First of all, although our results imply that 5-HT plays a role in feedback processing, effects were small and not very consistent. It is therefore difficult to form a unified account of this role. The two approaches we used to further unravel the role of 5-HT were difficult to compare, because the two methods (ATD vs. genetic approach) are completely different, the groups under investigation were different (young healthy males vs. mainly middle-aged healthy

females), and the versions of the time-estimation task were different (valid feedback only vs. valid and invalid feedback; symbols vs. emotional faces). However, despite these differences, both studies are largely in line with other studies and are suggestive of a role of 5-HT in feedback processing, although this role may eventually be proven indirect.

Unfortunately, we were unable to investigate the effects of the genetic polymorphisms on the cardiac responses to feedback, due to the large variability of this specific measure, and therefore the lack of power to perform these analyses. Due to the small sample size, and therefore a lack of power, we were also unable to investigate the relationship between the genetic polymorphisms and the electro-cortical and cardiac responses to feedback in the group of MDD patients. Large sample sizes are needed to solve these problems.

Although our modified version of the time-estimation task revealed interesting results concerning the sensitivity of the different measures to the validity of feedback, and the specificity of the disturbances in feedback processing in MDD, the complexity of the task, together with the large age span, caused some noise, which may have resulted in weak effects.

Finally, we only used one paradigm to investigate feedback processing, i.e., a time-estimation paradigm. Although this limits us in a way that it is difficult to extrapolate our findings to feedback processing and performance monitoring in general, we were able to more closely examine several aspects and correlates of feedback processing within this specific paradigm.

Recommendations for future research

To more clearly understand the involvement of the different subdivisions of the ACC in different aspects (validity, valence) of feedback processing, future studies might want to include several (intermediate) versions of the time-estimation task. In most studies described in this thesis the time-estimation task contained several new aspects (emotional faces, feedback validity). By decreasing the amount of new aspects one by one, and including a more homogeneous group of participants, i.e., students, it would be easier to better comprehend and interpret our present findings. One of the unresolved issues in this thesis is our finding that FRN responses were decreased when feedback was followed by correct adjustments. This needs further examination in future studies.

Most studies focus on young students in examining responses to errors and feedback. Although this has the advantage of homogeneity and therefore less noise, it does not represent the general population. Whereas young individuals are important in further unravelling basic mechanisms of feedback processing, it is also necessary to examine individuals from a large age span to gain a better representation of feedback processing in the adult brain, and the changes that occur during adulthood. Our finding

that genetic polymorphisms exert different effects in young to middle-aged individuals than in older individuals emphasizes the need to incorporate large age spans in future studies, not only in studies on error and feedback processing, but in studies on social function in general. Robust tasks and large sample sizes are required to decrease the noise that is inherent to the inclusion of such a large age group.

There is also a need for longitudinal studies on error and feedback processing in (young) individuals at risk for depression. This way changes in the neural circuitry underlying feedback processing in individuals who develop MDD can be examined and compared with the neural circuitry in individuals who do not develop MDD. The ACC and its subdivisions, and especially the connectivity between these subdivisions and other brain regions involved in feedback, reward and punishment processing, such as the amygdala, and striatum, are important research areas to focus on.

In line with this, manipulation of the ACC, by means of medication, psychotherapy or other treatments (e.g., ECT, transcranial magnetic stimulation, deep brain stimulation), may lead to both functional and structural changes that may prevent depressed individuals from relapsing, and perhaps even prevent at-risk individuals from developing MDD.

Finally, a focus on specific symptoms of depression such as anhedonia and depressed mood is likely to be more beneficial in unravelling the etiology and pathophysiology of depression than a focus on the depressive state in general (see also Holtzheimer and Mayberg, 2011).

Conclusions

Several conclusions can be formulated on the basis of the studies described in this thesis.

1) We showed that performance disturbances after negative feedback in moderately to severely depressed drug-free inpatients depend on the validity of the feedback, and not necessarily on the affective value of negative feedback per se. Disturbances in performance are therefore context-dependent. At a neural level, however, depressed patients do respond differently to the affective value or valence of feedback than healthy individuals, because, independent of validity, FRN-responses to feedback were increased. This increased FRN-response probably reflects an atypical, or hypersensitive, rACC response to the valence of feedback.
2) We showed that whereas the rACC was primarily sensitive to the valence of the feedback in a time-estimation task, the rostral cingulate zone was more sensitive to the validity of the feedback. The RCZ, therefore, appears to be involved in the evaluation of the importance of the feedback stimulus for future behaviour, while the rACC appears to act as an 'alert' system.

3) We found further evidence for a role of serotonin in feedback processing, although it remains unclear whether this role is direct or indirect. Non-depressed individuals with one or two short alleles of the serotonin-transporter gene (5-HTTLPR) showed an increased FRN response compared to non-depressed individuals with two long alleles, which suggests that developmental differences in brain structure and function associated with genetically-lowered serotonin-transporter function influences electrophysiological responses to performance feedback. A role of serotonin in feedback processing was also reflected by an attenuation of the cardiac response to negative feedback after acute tryptophan depletion.
4) Finally, specific symptoms of depression appear to be more informative in unravelling disturbances in the neural circuitry underlying feedback processing than a depressive state in general. We found that responses of the rostral cingulate zone and subgenual ACC to feedback were associated with self-reported depressed mood and anhedonia, which suggests that 'atypical' neurophysiological responses to feedback are also present in subclinical individuals.

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Summary

Major depressive disorder (MDD) is a common and complex mental illness, which involves disturbances in both affective and cognitive processing. These disturbances come together in feedback processing. Many studies have shown that depressed individuals perform worse than others after making an error or after receiving negative feedback on their performance. Studies using event-related brain potentials (ERPs) have shown aberrant electrophysiological responses to errors and negative feedback. These studies have, however, not clarified whether these disturbances are due to the affective value of the feedback or to the cognitive evaluation of the relevance of the feedback for future performance. The affective value of feedback (valence; positive versus negative) and the relevance or validity of feedback are often intertwined. It is also not clear yet how the brain region thought to underlie these responses to feedback, the anterior cingulate cortex (ACC), responds to these two modalities of feedback. Finally, the neurotransmitter dopamine is thought to be involved in the electrophysiological responses to errors and feedback. There is, however, increasing evidence that serotonin (5-hydroxytryptamine, 5-HT) plays an important role in these manifestations of feedback processing as well. In *chapter 1* these research issues were briefly introduced and the specific aims of this thesis were stated. The aims of this thesis were to deepen our understanding of the disturbances in feedback processing in patients with MDD, to gain further insight into the neural basis of feedback processing, especially concerning the role of the ACC, and to investigate the role of 5-HT in feedback processing.

First, feedback processing in healthy individuals was examined. In *chapter 2*, using a time-estimation task, we investigated the behavioural, cardiac and electro-cortical responses to the valence and validity of feedback in 84 non-depressed volunteers across a large age span. Participants had to produce 1s intervals followed by positive and negative feedback that was valid or invalid (i.e., related or unrelated to the preceding time estimate). The background grid indicated whether feedback was valid or invalid, and emotional faces (happy vs. fearful) were used to communicate the valence (positive vs. negative) of the feedback. We found that participants used the valid negative feedback to adjust their performance on the next trial, and performed around chance level after receiving invalid negative feedback. Negative feedback was associated with a transient cardiac slowing only when feedback was valid, and correct adjustments after valid negative feedback were associated with a more pronounced cardiac slowing. Validity, however, did not affect the feedback-related negativity (FRN), except when remedial action was taken into account. Furthermore, we found that the FRN and cardiac response to feedback decreased with advancing age, but performance did not. We interpreted these findings to suggest that the FRN and cardiac response signal ‘alert’ and that the cardiac response, but not the FRN, is implicated in the mechanisms invoked in remedial action.

In *chapter 3* we used fMRI to examine the roles of the two subdivisions of the ACC, the dorsal and the rostral part, in the processing of feedback valence and validity in 29 non-depressed participants, using the same time-estimation task as described in chapter 2. Again, we found that participants used the validity information to adjust their time estimates to negative feedback. The rostral cingulate zone (RCZ), which mainly overlaps with the dorsal ACC, was more active after valid feedback than after invalid feedback, but was insensitive to the valence of the feedback. The rostral ACC (rACC), posterior cingulate and right superior frontal gyrus, however, appeared to be primarily sensitive to the valence of the feedback. These regions were more active after positive feedback than after negative feedback. Our results imply that the RCZ is primarily involved in the evaluation of the relevance of the feedback, while the rACC is primarily involved in the processing of the emotional value of the feedback. These results are in line with the dorsal-cognitive ventral-affective subdivision hypothesis of the ACC, also known as the segregation hypothesis.

In *chapter 4* we compared behavioural and electro-cortical responses to feedback valence and validity in patients with MDD with those of non-depressed participants. Fifteen moderately to severely depressed drug-free inpatients were matched on the basis of their gender, age and level of education to 30 non-depressed individuals (subsample of the participants reported on in chapter 2). Similar to the controls, patients did not use the invalid negative feedback to adjust their time estimates. Patients, however, made fewer correct adjustments after valid negative feedback than controls, and they had larger FRN-responses. This study therefore showed that the behavioural responses of depressed individuals to negative feedback are context-dependent. The FRN-results, on the other hand, suggest that depressed drug-free patients have an atypical, or hypersensitive, rACC response to feedback, which is independent of feedback-validity.

The same time-estimation task was again used in *chapter 5*, in which performance and ACC responses were compared between students who had mild depressive symptoms, as measured with the Beck Depression Inventory (BDI, ≥10), and students who had virtually no symptoms (BDI<3). In this way we were able to examine whether disturbances in feedback processing and dysfunctions in the cortico-limbic circuit (including the ACC and amygdala) were present in subclinical individuals, who might be at risk of developing MDD. Forty-two young undergraduates participated in this fMRI study. In addition to the BDI, they were asked to fill out two questionnaires assessing the core symptoms of depression: the Profile of Mood States (POMS) assessing depressed mood, and the Snaith-Hamilton Pleasure Scale (SHAPS) assessing anhedonia or the loss of pleasure. No difference in performance was found between the two BDI groups, nor was performance related to depressed mood or anhedonia. The subgenual ACC, however, was more active

in response to feedback in students with higher levels of depressed mood and anhedonia. The RCZ, on the other hand, was less active in response to feedback in the more anhedonic individuals. Interestingly, the differential response of the RCZ to the validity of the feedback was decreased in individuals with higher levels of depressed mood. This study therefore showed differential neurophysiological correlates of depressed mood and anhedonia on feedback processing. We suggested that these different ACC responses might be related to a vulnerability to depression.

In the last two research chapters we examined the role of serotonin in feedback processing. In *chapter 6* we examined the effect of acute tryptophan depletion (ATD), a method to transiently lower serotonin in the brain, on behavioural, cardiac and electro-cortical responses to positive and negative feedback in healthy volunteers. Twenty healthy young males were included in this study and they were subjected to a different version of the time-estimation task than reported on in the other chapters. In this version of the task only valid feedback was given, and the feedback stimuli consisted of symbols instead of emotional faces. We found no effect of ATD on subjective mood, performance and FRN responses. We did find an effect of ATD on the cardiac response to negative feedback: cardiac slowing to negative feedback was attenuated after ATD compared to the placebo condition (sham depletion). These results imply that the electro-cortical and cardiac manifestations of feedback processing reflect different aspects of feedback processing. We argued that the attenuation of cardiac slowing in response to negative feedback might reflect decreased impact of the negative feedback stimuli due to increased punishment prediction associated with lower levels of serotonin in the brain.

In *chapter 7* we investigated the effects of two genetic polymorphisms influencing serotonin function (5-HTTLPR and TPH2-*rs1386493*) on behavioural and electro-cortical correlates of feedback processing. Seventy-two participants across a large age span were selected from the non-depressed participants reported on in chapter 2. We found no difference in performance, FRN responses and feedback P3 responses between individuals with less functional genotypes (5-HTTLPR-SS/SL and TPH2-*rs1386493*-TT/TC) and those with the more functional genotypes (LL and CC). When we split the group in half, however, on the basis of the median age (51 years), we found that relatively young carriers of the less functional 5-HTTLPR variant had a larger FRN and a larger difference between positive and negative feedback in the time window of the feedback P3 than others. TPH2-*rs1386493* did not influence FRN-size, but did influence the feedback P3 in older individuals (>51 years). Carriers of the less functional TPH2-gene variant showed a smaller difference between positive and negative feedback than the others. These results show that these two polymorphisms have differential influences on the electrophysiological responses to feedback, and that these effects depend on the age of the participants. The

distinct roles of the two genes in the serotonin pathway and alterations in the functionality of the serotonin system with advancing age are likely to underlie these differences.

In *chapter 8* a general discussion of the main findings is given. We concluded that 1) depressed patients' disturbances in performance after negative feedback are context-dependent, and that the increased FRN-responses in depression probably reflect an atypical, or hypersensitive, rACC response to the valence of feedback; 2) the RCZ appears to be involved in the evaluation of the relevance of feedback, while the rACC appears to be mainly involved in the processing of the emotional value of feedback, and therefore acts as an 'alert' system; 3) serotonin plays a role in feedback processing, although the mechanisms are still unclear; and 4) specific symptoms of depression appear to be more informative in unravelling disturbances in the neural circuitry underlying feedback processing than a depressive state in general. Furthermore, in this chapter, a recent theory about the function of the dorsal part of the ACC was discussed in more detail, and new theories elaborating on the mechanisms by which ATD exerts its effects were discussed. On the basis of our findings some suggestions for future research were given, including longitudinal studies on error and feedback processing in individuals at risk for depression. Furthermore, studies with a focus on specific symptoms of depression, such as anhedonia, might be more beneficial in unravelling the etiology and pathophysiology of depression than studies investigating the depressive state in general.

Summary in Dutch (Samenvatting)

Depressie is een veel voorkomende en complexe psychiatrische aandoening met verstoringen in zowel het cognitieve als het affectieve domein. Deze verstoringen komen beiden tot uiting in de verwerking van feedback. Eerdere studies hebben aangetoond dat depressieve mensen na het maken van een fout of na het krijgen van negatieve feedback slechter presteren. Met behulp van hersenpotentialen (ERPs) is aangetoond dat depressieve patiënten afwijkende elektrofysiologische reacties op fouten en negatieve feedback laten zien. Deze studies hebben echter niet duidelijk gemaakt of deze stoornissen te wijten zijn aan de affectieve waarde van de feedback of aan de cognitieve evaluatie van de relevantie van de feedback voor aanpassingsgedrag. De affectieve waarde van de feedback (valentie; positief versus negatief) en de informatiewaarde of validiteit van feedback zijn vaak met elkaar verweven. Het is ook nog niet duidelijk hoe het hersengebied dat vermoedelijk ten grondslag ligt aan deze reacties op feedback, de anterior cingulate cortex (ACC), reageert op deze twee aspecten van feedback. Ten slotte wordt verondersteld dat de neurotransmitter dopamine betrokken is bij de elektrofysiologische reacties op fouten en feedback. Er is echter steeds meer bewijs dat ook serotonine (5-hydroxytryptamine, 5-HT) een belangrijke rol speelt bij deze manifestaties van feedbackverwerking. In *hoofdstuk 1* werden deze onderzoeksvragen geïntroduceerd en staan de specifieke doelstellingen van dit proefschrift vermeld. Het doel van dit proefschrift was om ons begrip van de verstoringen in de verwerking van feedback bij depressieve patiënten te vergroten, om meer inzicht te krijgen in de neurale basis van feedbackverwerking, in het bijzonder met betrekking tot de rol van de ACC, en te onderzoeken of 5-HT een rol speelt bij de verwerking van feedback.

Eerst hebben we feedbackverwerking bij gezonde personen onderzocht. Met behulp van een tijdschattingstaak onderzochten we in *hoofdstuk 2* de gedrags-, hartslag- en elektrocorticale responsen op de valentie en de validiteit van feedback van 84 vrijwilligers zonder depressie tussen de 22 en 76 jaar. Deelnemers moesten intervallen van 1 seconde produceren/schatten en kregen vervolgens positieve of negatieve feedback, die informatief of niet-informatief was (dat wil zeggen, al dan niet gerelateerd aan de vorige schatting). De achtergrond van de feedbackstimuli gaf aan of de feedback informatief was of niet, en emotionele gezichten (blij vs. angstig) werden gebruikt om de valentie van de feedback (positief vs. negatief) te communiceren. De deelnemers gebruikten de informatieve negatieve feedback om hun gedrag aan te passen tijdens de volgende schatting, en presteerden rond kansniveau na het ontvangen van niet-informatieve negatieve feedback. Alleen informatieve negatieve feedback was geassocieerd met een tijdelijke hartslagvertraging, en deze hartslagvertraging was het sterkst als vervolgens een correcte aanpassing werd gemaakt. Informatiewaarde/validiteit was echter niet van invloed op de feedback-related negativity (FRN), behalve als aanpassingsgedrag mee werd genomen. Verder zagen we dat de FRN en hartslagrespons afnamen met leeftijd, maar

aanpassingsgedrag niet. Onze bevindingen suggereren dat de FRN en hartslagrespons op feedback representaties van een waarschuwings- of alertheidssignaal zijn en dat de hartslagrespons, maar niet de FRN, betrokken is bij de mechanismen die ten grondslag liggen aan gedragsaanpassing.

In *hoofdstuk 3* werd met dezelfde tijdschattingstaak als beschreven in hoofdstuk 2 de functies van de twee subdivisies van de ACC, te weten het dorsale en rostrale gedeelte, in de verwerking van de valentie en validiteit van feedback bij 30 niet-depressieve deelnemers onderzocht, door middel van fMRI. Weer vonden we dat de deelnemers de validiteitinformatie gebruikten om hun schattingen aan te passen na negatieve feedback. De rostral cingulate zone (RCZ), die voor een groot deel overlapt met de dorsale ACC, was actiever na informatieve feedback dan na niet-informatieve feedback, maar was ongevoelig voor de valentie van de feedback. De rostrale ACC (rACC), posterior cingulate en rechter superior frontal gyrus, daarentegen, bleken vooral gevoelig voor de valentie van de feedback. Deze gebieden waren actiever na positieve feedback dan na negatieve feedback. Onze resultaten impliceren dat de RCZ in de eerste plaats betrokken is bij de evaluatie van de relevantie van de feedback, terwijl de rACC vooral betrokken is bij de verwerking van de emotionele waarde van de feedback. Deze resultaten komen overeen met de hypothese dat de ACC is onder te verdelen in een dorsaal-cognitief en ventraal-affectief gedeelte, ook wel bekend als de segregatiehypothese.

In *hoofdstuk 4* hebben we gedrags- en elektrocorticale responsen op de valentie en validiteit van feedback vergeleken tussen depressieve en gezonde deelnemers. Vijftien medicatievrije opgenomen patiënten met een matig tot ernstige depressie werden op basis van hun geslacht, leeftijd en opleidingsniveau gekoppeld aan een subgroep van 30 niet-depressieve personen waarover we eerder gerapporteerd hebben in hoofdstuk 2. Net als de controlepersonen, gebruikten de patiënten de niet-informatieve negatieve feedback niet om hun tijdschattingen aan te passen, maar de informatieve negatieve feedback wel. Patiënten maakten echter minder correcte aanpassingen na informatieve negatieve feedback dan de controlegroep, en ze hadden grotere FRN-responsen. Deze studie laat daarom zien dat de gedragsresponsen van depressieve patiënten op negatieve feedback contextafhankelijk zijn. De FRN-resultaten, daarentegen, suggereren dat depressieve medicatievrije patiënten een atypische of overgevoelige rACC respons hebben op feedback, die onafhankelijk is van de validiteit van de feedback.

Dezelfde tijdschattingstaak werd opnieuw gebruikt in *hoofdstuk 5*, waarin gedrags- en ACC-responsen werden vergeleken tussen studenten met milde depressieve symptomen, gemeten met de Beck Depression Inventory (BDI, ≥ 10), en studenten die vrijwel geen symptomen hadden (BDI <3). Op deze manier konden we nagaan of verstoringen in de

verwerking van feedback en disfuncties in het cortico-limbische circuit (inclusief de ACC en de amygdala) aanwezig waren in subklinische personen die mogelijk risico lopen op het ontwikkelen van een depressie. Tweeënveertig studenten namen deel aan deze fMRI studie. In aanvulling op de BDI, vroegen we ze twee vragenlijsten in te vullen waarmee de kernsymptomen van depressie gemeten kunnen worden: depressieve stemming gemeten met de Profile of Mood States (POMS), en anhedonie of het verlies van plezier gemeten met de Snaith-Hamilton Pleasure Scale (SHAPS). We vonden geen verschil tussen de twee BDI-groepen in het percentage gedragsaanpassingen na negatieve feedback, noch vonden we verschillen in relatie tot depressieve stemming of anhedonie. De subgenuale ACC van studenten die hoger scoorden op depressieve stemming en anhedonie was echter actiever in reactie op feedback. De RCZ, daarentegen, was minder actief in reactie op feedback in de studenten met hogere anhedonie-scores. De gevoeligheid van de RCZ voor de validiteit van feedback nam af bij mensen met een hogere mate van depressieve stemming. Deze studie laat zien dat de neurofysiologische correlaten van depressieve stemming en anhedonie op feedbackverwerking verschillen. Deze verschillen in ACC reacties zouden mogelijk te relateren zijn aan een kwetsbaarheid voor depressie.

In de laatste twee onderzoekshoofdstukken onderzochten we de rol van serotonine bij de verwerking van feedback. In *hoofdstuk 6* onderzochten we het effect van tryptofaandepletie (ATD), een methode waardoor de hoeveelheid serotonine in de hersenen tijdelijk verlaagd wordt, op gedrags-, hartslag- en elektrocorticale responsen op positieve en negatieve feedback bij gezonde vrijwilligers. Twintig gezonde jonge mannen deden mee aan dit onderzoek en er werd een andere versie van de tijdschattingstaak gebruikt dan in de studies beschreven in de andere hoofdstukken. In deze versie van de taak werd enkel informatieve feedback gegeven, en de feedbackstimuli bestonden uit symbolen in plaats van emotionele gezichten. We vonden geen effect van ATD op subjectief ervaren stemming, gedragsaanpassingen en FRN-responsen. Wel vonden we een effect van ATD op de hartslagrespons op negatieve feedback: de hartslagvertraging in reactie op negatieve feedback was verminderd na ATD vergeleken met de placeboconditie. Deze resultaten impliceren dat de elektrocorticale respons en de hartslagrespons verschillende aspecten van feedbackverwerking representeren. De vermindering van de hartslagvertraging in respons op negatieve feedback zou verklaard kunnen worden door een verminderde impact van de negatieve feedbackstimuli als gevolg van een verhoogde anticipatie op negatieve feedback die geassocieerd wordt met lagere serotonineniveaus in de hersenen.

In *hoofdstuk 7* onderzochten we de effecten van twee genetische polymorfismes (varianten) die de serotoninefunctie beïnvloeden (5-HTTLPR en TPH2-*rs1386493*) op de gedrags- en elektrocorticale correlaten van feedbackverwerking. Tweeënzeventig

deelnemers werden geselecteerd uit de groep niet-depressieve deelnemers waarover we eerder gerapporteerd hebben in hoofdstuk 2. We vonden geen verschil in percentage gedragsaanpassingen, FRN-responsen en feedback P3-responsen tussen de individuen met minder functionele genotypen (5-HTTLPR-SS/SL en TPH2-*rs1386493*-TT/TC) en individuen met de meer functionele genotypen (LL en CC). Wanneer we de groep echter in tweeën splitsten op basis van de leeftijdsmediaan (51 jaar), vonden we dat de relatief jonge dragers van de minder functionele 5-HTTLPR variant een grotere FRN hadden en een groter verschil tussen positieve en negatieve feedback lieten zien op de feedback P3 dan de anderen. TPH2-*rs1386493* had geen invloed op de grootte van de FRN, maar wel op de feedback P3 in oudere deelnemers (> 51 jaar). Dragers van de minder functionele TPH2-genvariant lieten een kleiner verschil zien tussen positieve en negatieve feedback dan de anderen. Deze resultaten tonen aan dat deze twee polymorfismes een verschillende invloed hebben op de elektrofysiologische reacties op feedback, en dat deze effecten afhankelijk zijn van de leeftijd van de deelnemers. De verschillende rollen van de twee genen in het serotoninemetabolisme en veranderingen in de functionaliteit van het serotoninesysteem met het ouder worden liggen waarschijnlijk ten grondslag aan deze verschillen.

In *hoofdstuk 8* werden de belangrijkste bevindingen bediscussieerd. We concludeerden dat 1) de verstoringen in het gedrag van depressieve patiënten na negatieve feedback contextafhankelijk zijn, en dat de verhoogde FRN-respons bij depressie waarschijnlijk veroorzaakt wordt door een atypische of overgevoelige rACC respons op de valentie van feedback, 2) de RCZ met name betrokken is bij de evaluatie van de relevantie van de feedback, terwijl de rACC zicht vooral bezighoudt met de verwerking van de emotionele waarde van de feedback, en dus fungeert als een waarschuwingssysteem, 3) serotonine een rol speelt bij feedbackverwerking, hoewel de mechanismen nog onduidelijk zijn, en 4) specifieke symptomen van depressie informatiever lijken te zijn voor het onderzoeken van verstoringen in de neurale circuits die ten grondslag liggen aan feedbackverwerking dan een depressieve staat in het algemeen. In dit hoofdstuk werd verder een recente theorie over de functie van het dorsale deel van de ACC in meer detail besproken, en nieuwe theorieën over de mechanismen van ATD werden bediscussieerd. Op basis van onze bevindingen werd een aantal suggesties gedaan voor vervolgonderzoek, waaronder longitudinale studies naar fouten- en feedbackverwerking bij personen met een verhoogd risico op depressie. Daarnaast kunnen studies met een focus op specifieke symptomen van depressie zoals anhedonie, wellicht meer inzicht geven in het ontstaan, de ontwikkeling en ziektemanifestatie van depressie dan een depressieve staat in het algemeen.

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About the author

Curriculum Vitae

Gabry Wilhelmina Mies was born in Weert, the Netherlands, on April 20, 1981. After completing secondary education (VWO) at the Philips van Horne Scholengemeenschap in Weert, she started studying biology at Wageningen University in 1999. In the specialization phase of her study, she focused on animal behaviour. She carried out her major thesis on anticipatory behaviour in dairy cows together with a fellow student at the Department of Ethology and Welfare, Animal and Society, Faculty of Veterinary, University of Utrecht. Then she went to Maastricht to study the effects of acute tryptophan depletion on facial emotion perception in healthy volunteers and first-degree relatives of depressed patients by means of fMRI for her minor thesis at the Department of Psychiatry and Neuropsychology, University of Maastricht. Before graduating in 2005, she studied the effects of delay and health consciousness on healthy snack choice for her second minor thesis at the Department of Human Nutrition, Wageningen University. In 2006 she worked as a research assistant at the same department on a project on the effects of omega-3-fatty acids on cognitive performance and mental wellbeing in older people. In February 2007 she started her PhD research at the Department of Psychiatry, Erasmus MC, University Medical Centre Rotterdam of which the results are described in this thesis. This PhD research was carried out under the supervision of Dr. Frederik M. van der Veen, Prof.dr. Michiel W. Hengeveld and Prof.dr. Maurits W. van der Molen (Department of Psychology, University of Amsterdam). From March until September 2011 she worked as a scientific researcher at the O3 Research Centre Mental Healthcare Rijnmond, at the Department of Psychiatry.

Publications

Van der Veen, F.M., Mies, G.W., Van der Molen, M.W., & Evers, E.A. (2008). Acute tryptophan depletion in healthy males attenuates phasic cardiac slowing but does not affect electro-cortical response to negative feedback. *Psychopharmacology (Berl)*, 99(2): 255-63.

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Mies, G.W., Van der Veen, F.M., Tulen, J.H.M., Hengeveld, M.W., & Van der Molen, M.W. (2011). Cardiac and electrophysiological responses to valid and invalid feedback in a time-estimation task. *Journal of Psychophysiology*, 25(3): 131-142.

Mies, G.W., Van den Berg, I., Franken, I.H.A., Smits, M., Van der Molen, M.W., & Van der Veen, F.M. Neurophysiological correlates of depressed mood and anhedonia on feedback processing. *Manuscript submitted.*

Mies, G.W., Van der Veen, F.M., Oostra, B.A., De Graaf, B.M., Hengeveld, M.W., & Van der Molen, M.W. Effects of functional polymorphisms in the 5-HTT and TPH2 gene on feedback processing. *Manuscript submitted.*

PhD portfolio

Name PhD student: G.W. Mies	Promotor 1: Prof. dr. M.W. Hengeveld
Erasmus MC Department: Psychiatry	Promotor 2: Prof. dr. M.W. van der Molen
PhD period: February 2007 – June 2011	Co-promotor: Dr. F.M. van der Veen

1. PhD training	**Year**	**Workload**
General courses		
- Classical Methods for Data-analysis (NIHES)	2007	160
- Research Integrity (Erasmus MC)	2008	60
- Biomedical English Writing and Communication (Erasmus MC)	2008	110
Specific courses		
- Tool-kit of Cognitive Neuroscience (Donders Institute)	2007	40
- SNPs and Human Diseases (MolMed)	2007	40
- Neuropsychopharmacology (ONWAR)	2008	56
- Tool-kit of Cognitive Neuroscience: Advanced fMRI data analysis (Donders Institute)	2009	24
- Cognitive Neuroscience (ONWAR)	2009	40
- Tool-kit of Cognitive Neuroscience: Advanced topics in MR imaging of the brain (Donders Institute)	2011	24
National and international conferences, seminars, and workshops		
- Conference Integrating Imaging and Genetics, Amsterdam	2007	20
- Onderzoeksdagen Psychiatrie, Zandvoort (oral presentation)	2008	20
- Endo-Neuro-Psycho Meeting, Doorwerth	2008	8
- 14th World Congress of Psychophysiology, St. Petersburg, Russia (poster presentation)	2008	30
- International Symposium on the Neural Basis of Decision Making, Groesbeek	2009	8
- Workshop 'Solving the serotonin paradox', Nijmegen	2009	8
- Endo-Neuro-Psycho Meeting, Doorwerth (oral presentation)	2009	20
- 9th World Congress of Biological Psychiatry, Paris, France	2009	20
- 49th annual meeting Society for Psychophysiological Research (SPR), Berlin, Germany (poster presentation)	2009	30
- 7th FENS Forum of European Neuroscience, Amsterdam (poster presentation)	2010	20
- 50th annual meeting Society for Psychophysiological Research (SPR), Portland, USA (poster presentation)	2010	20
- Endo-Neuro-Psycho Meeting, Lunteren (oral presentation)	2011	20
Didactic skills		
- Basistraining didactiek Teach-the-Teacher (Desiderius School)	2008	16
2. Teaching activities		
Supervising Master's theses (4 MSc theses, 1 BSc thesis)	2007-2009	250
- 2e jaars keuzeonderwijs	2007-2010	160
- Tutoraat	2008-2009	80
Total (hours)		1304