Pharmaco-fMRI Challenges before and after short-term Treatment of Major Depression with Escitalopram, Mirtazapine, Agomelatine or Placebo and the Relation to the Hypothalamus-Pituitary-Adrenal-Axis Activity

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Contents

1. Introduction

1.1. Major Depressive Disorder (MDD)

According to the World Health Organization (WHO) depressive disorders are of outstanding health-economic importance as they are the psychiatric disorders that most frequently cause psychosocial disability (WHO 2017). Intensive biologically oriented psychiatric research over the last decades contributed to a deeper insight in miscellaneous pathophysiologic mechanisms playing a role in the etiology of Major Depressive Disorder (MDD) (Schüle, Baghai et al. 2007). Nevertheless, the etiology of depressive disorders still is not fully understood. A multifactorial genesis is supposed and has been elucidated in increasing detail. Besides psychological and social factors biological variables apparently play a major role, which lead in their whole to a disturbed central nervous homeostasis. MDD is a chronic stress related disorder and a complex clinical syndrome characterized not only by depressed mood but also by vegetative and cognitive symptoms. Moreover, genetic, neuroendocrine and neurochemical biomarkers may predict impaired processing and regulation of emotions related to major depression as well as antidepressant treatment response in general. In patients suffering from acute depression a wide range of negative influence on the processing of emotional information is manifested. This is believed to contribute to the etiology and maintenance of the depressed state (Beck 2008).

1.2. HPA axis activity

A dysregulation of the activity of the hypothalamic-pituitary-adrenocortical (HPA) system is one of the major neuroendocrine abnormalities in major depressive disorder (Holsboer and Barden 1996). These include elevated circulating plasma levels of both corticotropin (adrenocorticotropic hormone, ACTH) and cortisol (Holsboer and Barden 1996) in addition with elevated levels of corticotropin releasing hormone (CRH) in the cerebrospinal fluid (Nemeroff, Widerlov et al. 1984). One of the first neuroendocrine function tests investigating HPA axis dysregulation in depression was the dexamethasone suppression test (DST). In contrast to the suppressibility of ACTH and cortisol secretion after administration of the synthetic glucocorticoid dexamethasone (Dex) in healthy volunteers there was an enhanced proportion of patients suffering from affective disorders with escape from adequate cortisol suppression (Carroll 1982). In addition, a change of the DST results in dependency from the clinical outcome was described (Greden, Gardner et al. 1983). In contrast to the DST the combined dexamethasone suppression / corticotropin releasing hormone stimulation (Dex/CRH) test seems to be an even better and more sensitive diagnostic tools for the

assessment of the HPA system dysregulation in depression available so far. A test sensitivity of 80 up to 90% has been shown in depressed patients (Heuser, Yassouridis et al. 1994). It has been suggested that the hyperactivity of the HPA system during depression may be considered as a neuroendocrine sign of a disturbed HPA system homeostasis which is rather used as a state than as a trait marker of diagnostic entities (Heuser, Yassouridis et al. 1994). Despite partly conflicting results, the amygdala and the hippocampus seem to have faciliatory or regulatory roles in the generation of HPA axis responses, at least this was shown in animal experiments (Goursaud, Mendoza et al. 2006). Also, a relationship between hypercortisolemia and cognitive dysfunction in acutely depressed patients has been published some years ago (Behnken, Bellingrath et al. 2013). An involvement of the amygdala in the processing of facial emotional expressions such as anger, sadness and disgust was hypothesized. Porter and Gallagher described a dysfunction of monoaminergic transmitter systems in depression including cognitive impairment which is facilitated by the HPA-system (Porter and Gallagher 2006). Adolphs described an activation of the HPA system during the perception of fear. This results in an activation of the hypothalamus after perception of the threatening emotional information by projection of the stress-signaling impulses via the amygdala (Adolphs 2008). Thus, it is still discussed, whether the specific role of the amygdala is only the processing of fear or whether it is responsible for the accurate identification of facial emotional expressions in general (Loughead, Gur et al. 2008). There exists strong evidence for the very important role of the amygdala in regulating the response of the HPA axis to stress, but also to positive emotional faces (van Marle, Hermans et al. 2009), although the exact kind of emotion processing by the amygdala is less clear up to now (Ellenbogen, Carson et al. 2010). In MDD both types of processing are abnormal, HPA axis function (investigated using the DST) and facial emotion processing (Maes, Calabrese et al. 1994). Patients suffering from MDD demonstrate a biased and increased attention during the processing of negative facial expressions (Bourke, Douglas et al. 2010). This seems to be true even for non-affected high risk probands for depression, in first-degree relatives of patients with MDD who – in addition – show an elevated HPA axis activity (Ising, Lauer et al. 2005, Le Masurier, Cowen et al. 2007). Therefore, in depression an interdependency between the perception of emotional facial expressions and the HPA-axis regulation, which in turn appears to be influenced by major depression, has been confirmed by these earlier studies.

1.3. Antidepressant treatment

1.3.1. Antidepressants

A multifactorial genesis of MDD is supposed and has been elucidated in increasing detail during the last years. Accordingly, treatment of depression uses also multilayer approaches. In case of moderate depression national and international guidelines recommend either antidepressants or psychotherapy respectively, in case of severe or treatment resistant depression both approaches are recommended (DGPPN 2015). In case of medium to severe depression the combination of antidepressants with psychotherapy, psychoeducation and social support is associated with the highest probability of a fast response to the treatment.

Over the last decades, our understanding of the neurochemical mechanisms of antidepressant drug action has advanced considerably, but, although the knowledge about the pharmacodynamic mechanisms of action of antidepressants increased steadily, there is still a lack of information about the exact mechanisms of action in the human brain and which of these are mandatory for the antidepressant efficacy. Moreover, it remains to be elucidated, how far changes in emotional processing may account for the resolution of this multiple symptom domains during successful treatment with antidepressants. Recent fMRI neuroimaging studies suggest that a change in emotional processing could also explain partly the global improvement because attention and appraisal are of fundamental importance in brain function. The influence of antidepressant treatments on HPA axis activity and the potential to predict treatment response with repeated Dex/CRH tests are well known (Schule, Baghai et al. 2009), nevertheless, it remains unclear up to now whether the activity in central areas such as amygdala or hippocampus or other brain regions have the potential to contribute to an earlier and more reliable prediction of treatment effects. For some classes of antidepressants, e.g. for selective serotonin reuptake inhibitors (SSRIs) changes in the emotion processing circuitry and area specific changes in the brain activation were associated with treatment response (Anderson, McKie et al. 2008) or efficacy (Cipriani, Furukawa et al. 2009). However, most pharmaco-fMRI studies deduced effects in brain activation by antidepressants from the investigation in healthy subjects, not in depressed patients who showed dysfunctions in emotional and cognitive processing in the brain associated with depressive disorders (Akimova, Lanzenberger et al. 2009, Lanzenberger, Wadsak et al. 2010). A huge number of studies have considered the effect of a single dose of an SSRI on emotional processing. These data are useful in addressing the following issue: Overall, it appears that a single dose of the SSRI (e.g. citalopram) can result in increased fear recognition and increased emotion potentiated startle response (Harmer,

Bhagwagar et al. 2003, Browning, Reid et al. 2007, Grillon, Levenson et al. 2007). Importantly, in addition to this anxiogenic-like effect, positive changes in emotional bias are also observed in single dose studies, in terms of attentional bias to positive words (Browning, Reid et al. 2007) and increased recognition of happy faces (Harmer, Bhagwagar et al. 2003). This suggests that a single administration of an SSRI produces a mixture of negative and positive emotional effects in cognitive models. However, after seven days of SSRI treatment there is evidence for a decreased fear response in term of diminished recognition of fearful faces and reduced emotional startle reaction (Harmer, Shelley et al. 2004). This fits well with the clinical observation that acutely administered SSRIs may first increase anxiety, while repeated treatment has sustained anxiolytic effects.

There is a growing body of evidence that antidepressants can affect emotional processing very early after starting the treatment and independently from changes in subjective mood. For example, after a one-week treatment with the SSRI citalopram or the selective noradrenaline reuptake inhibitor (NARI) reboxetine in healthy volunteers a change in emotional processing could be seen which indicated responses in the opposite direction of the changes seen generally in depressive disorders (Harmer, Shelley et al. 2004). Moreover, according to studies in healthy volunteers, antidepressant drugs have some effects on emotional processing very quickly after administration in the absence of discernible changes in mood. One study shows that short term SSRI treatment was associated with reduced blood oxygenation level-dependent (BOLD) response in the amygdala to negative facial expressions presented outside of conscious awareness (Harmer, Mackay et al. 2006). Such a finding suggests thinking about the role of the amygdala in antidepressant treatment, because the amygdala plays a key role in the processing of threat or fear-relevant cues as described before.

Taken together, findings from the single dose and seven-day pharmaco-fMRI studies with antidepressants in healthy volunteers indicate that cognitive models of antidepressant drug action reveal positive changes in emotional processing. A reversible increase in negative emotional processing can be found very early after initiation of the treatment, which is in line with clinical observations of early, but reversible increase of anxiety after starting an SSRI treatment, whereas positive effects can be found to be sustained.

As described before, it seems that CRH levels are in relationship with amygdala hyperactivity in MDD patients, because MDD is accompanied by both cognitive impairment and a hyperactivity of the HPA system, resulting in an enhanced glucocorticoid secretion. Cortisol

acts via mineralocorticoid and glucocorticoid receptors densely located in the hippocampus, a brain area that is important regarding cognitive functions and especially memory functions.

Antidepressants such as SSRIs can influence emotional processing very early on during the treatment and independently from changes in subjective mood. On the other hand, hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis in MDD is one of the most reliably reported neurobiological characteristics of affective disorders. Whether these alterations in HPA axis regulation are limited to the acute stage of MDD or whether they persist after recovery, at least in some patients, remains ambiguous. A relationship between hypercortisolemia and cognitive dysfunction in acutely depressed patients has been repeatedly observed and it was also demonstrated in several studies that a discrete cognitive impairment often persists in the remitted state of depression (Behnken, Bellingrath et al. 2013). On the other hand, MDD is accompanied by morphological changes of brain structures like the hippocampus and the amygdala which are of great importance in the neural circuitry mediating depression. Hyperactivity of the HPA system resulting in enhanced glucocorticoid secretion can often be observed during depression and has been thought to play an important role in inducing these morphological changes (Schuhmacher, Mossner et al. 2012). The SSRI (es)citalopram, used frequently as a first line treatment of MDD, influenced emotional processing shortly after the start of treatment (Harmer, Bhagwagar et al. 2003, Browning, Reid et al. 2007) as described before. Also studies with mirtazapine, an α_2-adrenoreceptor blocker with additional influence on histaminic H_1-, $5HT_{2a}$-, $5HT_{2c}$-, and $5HT_3$-receptors, resulted in a reduction of emotional fear processing: a significantly impaired recognition of fearful facial expressions and reduced eye-blink responses in the emotion potentiated startle task, an effect similar to that of SSRIs, was reported (Arnone, Horder et al. 2009, Komulainen, Heikkila et al. 2016). Agomelatine attenuated fMRI activations in several regions involved in face processing (fusiform gyrus, bilateral inferior frontal gyrus, anterior thalamic nucleus) and showed an antidepressant-like ability to modulate early visual processing of faces in healthy controls without affecting amygdala responses (Lees J 2010). In depressed patients, agomelatine had short- and long-term effects on brain structures involved in emotional regulation such as the dorsomedial prefrontal cortex and precuneus. The development of corresponding biomarkers for a biomarker-based treatment of MDD was suggested (Delaveau, Jabourian et al. 2016). Agomelatine is the first antidepressant with a thus far unique mode of action. It acts as an agonist at melatonin MT_1 and MT_2 receptors in combination with $5HT_{2c}$-antagonistic properties. Randomized double-blind placebo-controlled clinical studies showed an antidepressant and anxiolytic efficacy of agomelatine in the treatment of patients suffering from MDD (Kennedy and Emsley 2006, Olie

and Kasper 2007) and anxiety disorders (for review see: (Eser, Baghai et al. 2007)). MDD patients displayed greater amygdala activation when anticipating negative pictures and greater prefrontal activation when confronted with them without the anticipatory cues. After antidepressant treatment, both amygdala and prefrontal activation decreased significantly in the treated MDD patients relative to controls. These findings show that the neural mechanisms of emotional anticipation and processing are altered in patients with MDD and that the functional neuroanatomy of emotional processing is normalized after successful treatment with an antidepressant (Rosenblau, Sterzer et al. 2012).

1.3.2. Psychotherapy

There are a number of studies investigating neural activation after treatment with antidepressants, but only a very limited number of fMRI studies investigating the effects of psychotherapy on the processing of facial emotions can be found. Fu et al. investigated the neural correlates of implicit processing of sad facial expressions in depression before and after 16 weeks of weekly *Cognitive Behavioral Therapy* (CBT) treatment. Elevated amygdala-hippocampal activity in patients compared to healthy individuals could be demonstrated following therapy. In addition, the dorsal anterior cingulate activity showed a significant relationship with the post-treatment clinical response in depressed patients (Fu, Williams et al. 2008). Also, Siegle et al. could show a changed amygdala activity after psychotherapy. Their patients were treated successfully with CBT and displayed low sustained reactivity to emotional stimuli in the subgenual cingulate cortex (Brodmann's area 25) and high activity in the amygdala with the strongest improvement. Furthermore, they suggested the presence of emotion regulation disruptions, which are targeted in CBT, may be the key to recovery with this intervention (Siegle, Carter et al. 2006). Dichter et al. investigated the effects of *Behavioral Activation Therapy for Depression* which was designed and administered to increase engagement with positive stimuli and a reduction in avoidance behaviors. A high responder rate of 75% was reported together with a decreased activation due to cognitive control in prefrontal structures including paracingulate gyrus, the right orbital frontal cortex, and the right frontal pole. Moreover, the magnitude of the activation in the paracingulate gyrus before treatment was related to the magnitude of the change of depressive symptoms after psychotherapy (Dichter, Felder et al. 2010).

Therefore, it can be stated, that there are findings suggesting that also psychotherapeutic treatment affects processing of emotional stimuli with changes in the activity of the amygdala and other brain regions, which even may be interrelated to the effectiveness of the treatment.

1.4. The processing of facial expressions

Facial expressions are an important component of communication and the correct interpretation of emotional expression is an essential key of successful social relationship. There are some studies suggesting that difficulties in interpersonal communication in depressed patients may be related to abnormalities in affective facial processing (Fu, Williams et al. 2004). There have been published several studies investigating facial emotional processing in depressed patients. Some of them are focused on facial expressions because this may facilitate a better understanding of facial emotion processing due to impaired cognition regarding to different neural regions within the central nervous system (CNS). In addition, impaired facial expression processing may link to other affective and social symptoms in depressed patients. Finally, a change in facial emotional processing during response to antidepressant treatments may help to predict this treatment response in depressed patients (Venn, Watson et al. 2006). Therefore, presenting facial emotional expression stimuli may be a valid and reliable approach for emotion processing investigations in order to activate brain areas responsible for emotion processing in healthy volunteers and in MDD patients with impaired cognition (Fusar-Poli, Placentino et al. 2009).

Ekman and Friesen (1971) suggested that six basic emotional expressions could be recognized. The investigated basic emotional expressions were shown in happy, sad, fearful, angry, disgusted, and surprised faces. In addition, they developed a standardized set of stimuli on the basis of these six emotions which were widely used in behavioral, cognitive and neuroimaging investigations (Ekman and Friesen 1971). Since then imaging studies in affective cognition often used Ekman stimuli for the measurement of responses to emotional faces.

1.5. Influence of Major Depression on processing of facial expressions

According to the cognitive theories of depression, symptoms coming from mood-congruent emotions cause a processing bias. Consequently, patients suffering from MDD tend to attend more to negative emotional stimuli (Scher, Ingram et al. 2005, Beck 2008). Several studies are reporting a negative bias on the perception of social key signals such as emotional facial expressions. This was demonstrated also in cognitive and behavioral investigations (Gur, Erwin et al. 1992, Bouhuys, Geerts et al. 1999). In addition, neuropsychologic studies have reported that MDD patients have a significant bias toward sad emotions (Gotlib, Krasnoperova et al. 2004). Another study has shown that MDD patients interpret happy facial expressions as neutral and neutral facial expressions as sad emotions (Persad and Polivy 1993). Such biases have been associated with aberrant responses across a network of neural areas involved in emotional

processing, including an increase in the response of the amygdala to negative facial expressions in depressed patients compared to matched controls (Sheline, Barch et al. 2001, Surguladze, Young et al. 2004, Suslow, Konrad et al. 2010, Victor, Furey et al. 2010).

1.6. Magnetic resonance imaging and functional MRI

Magnetic resonance imaging (MRI) is a medical imaging technique using strong magnetic fields to create images of biological tissue. Functional magnetic resonance imaging (fMRI) uses standard MRI scans to investigate changes in brain function over time by determining changes in blood flow (Huettel 2014). Blood-oxygen-level dependent (BOLD) contrast is used to detect changes in deoxyhemoglobin concentration consequent to task-induced and spontaneous modulations of oxygen metabolism in response to neural activity. This method has been widely employed in cognition studies for different clinical applications such as surgical planning, for monitoring of treatment outcomes, and as a biomarker in pharmacologic and training programs (Glover 2011).

Over the past three decades neuroscientists have tried to clarify the neural mechanisms that support face perception (Posamentier and Abdi 2003, Haxby and Ida Gobbini 2007, Fusar-Poli, Placentino et al. 2009). Indeed, fMRI studies play a major role in investigations facilitating the understanding of human brain function and of neurophysiological substrates of emotional processing. Despite the growing number of fMRI studies, some individual imaging studies indicate inconclusive findings (Neumann, von Cramon et al. 2008). Predominantly, the difficulty to definitively characterize which specific brain region is associated with each specific emotional expression has been discussed. Most of the studies employ different imaging techniques, analysis methods, and task designs. Nevertheless, fMRI studies showed that emotional faces elicit enhanced response in the limbic, frontal, and visual cortical regions contrasted to responses evoked by neutral faces (Vuilleumier, Armony et al. 2001, Haxby, Hoffman et al. 2002, Winston, O'Doherty et al. 2003, Ishai, Pessoa et al. 2004).

1.6.1. fMRI and pharmacological MRI

Pharmacological MRI (phMRI) is a fMRI investigation method to study the effects of medication on brain function in response to a chemical substance administration. Bryant and Jackson have introduced the application of pharmaco-fMRI for the first time (Bryant and Jackson 1998). There are two main approaches in the pharmaco-fMRI field, the first measuring acute fMRI signal change following drug administration, while the second estimates modulatory effects of a drug on brain networks. (Wandschneider and Koepp 2016).

While fMRI is widely used in research and clinical investigation, where it is commonly combined with a sensorimotor task, phMRI is an adaptation of fMRI enabling the investigation of a specific neurotransmitter system, such as the serotoninergic neurotransmission or the melatonin system. These systems are investigated under physiological or pathological conditions following an activation via the administration of a specific medication such as selective serotonin (5HT) reuptake inhibitors (SSRIs) or melatonin agonists (Klomp, Tremoleda et al. 2012).

1.6.2. Brain regions involved in the processing of facial expression

Investigations trying to identify brain regions responsible for emotional face processing can be subdivided in two major research directions which integrate neuro-anatomical models involved in the representation and generation of emotions. These are also thought to influence the etiology of MDD (Mayberg 1997, Phillips, Drevets et al. 2003, Phillips, Ladouceur et al. 2008). First, encoding of emotional expressions depends on multiple interactions between complimentary systems: a neural core system for the visual analysis of faces refers to bilateral inferior occipital gyri, the lateral fusiform gyrus and the superior temporal sulcus. The second aspect focused on the processing of facial information, such as meaning and significance referring to different brain regions such as amygdala, insula, orbitofrontal areas, and somatosensory cortex (Haxby, Hoffman et al. 2002). Notably, emotional facial expression investigations have been frequently used in neuroimaging studies in depressed patients with regards to neurobiological models of depression (Phillips, Drevets et al. 2003, Mayberg 2007, Phillips, Ladouceur et al. 2008).

1.6.2.1. Amygdala

Functional neuroimaging studies have shown a relevant specific amygdala activation during the presentation of fearful facial expressions of emotion (Morris, Frith et al. 1996, Morris, Buchel et al. 2001) and during verbally guided anticipation of shock (Phelps, O'Connor et al. 2001). This amygdala activation was also reported after brief presentations of fearful facial expressions of emotions which were masked to prevent conscious perception (Whalen, Rauch et al. 1998) or when presented in the cortically blind field (Morris, DeGelder et al. 2001, Pegna, Khateb et al. 2005). There is a large number of functional MRI studies which have shown an increased amygdala BOLD response to masked happy or fearful faces (Sheline, Barch et al. 2001). An fMRI study detected decreased activity in MDD patients in comparison to healthy controls in the left amygdala, during the presentation of sad facial expressions (Fu, Williams et al. 2004).

Furthermore, fMRI investigations of affective facial processing of sad and angry faces in medicated depressed patients in comparison to healthy controls reported an increased BOLD response in the left middle cingulum and the right prefrontal cortex (Frodl, Scheuerecker et al. 2009). The same study found no differences in amygdala activation between MDD patients and healthy controls at baseline before antidepressant treatment (Frodl, Scheuerecker et al. 2011). In contrast to these reports, there are a huge number of imaging investigations of facial expressions in MDD patients in comparison to healthy controls showing greater amygdala BOLD response to emotional facial expressions in MDD (Sheline, Barch et al. 2001, Fu, Williams et al. 2004, Lawrence, Williams et al. 2004, Surguladze, Brammer et al. 2005, Fu, Williams et al. 2008, Matthews, Strigo et al. 2008, Peluso, Glahn et al. 2009, Suslow, Konrad et al. 2010, Victor, Furey et al. 2010, Zhong, Wang et al. 2011).

1.6.2.2. Hippocampus

Neuropathology of MDD suggests that an impaired hippocampus may be a key hub within the limbic system (Campbell and Macqueen 2004). According to a recent review, hippocampal volume reduction is one of the most replicated findings in neuroimaging studies in MDD (Roddy, Farrell et al. 2018). For example, reductions in the gray matter volume and functional impairment in MDD patients have been reported (Bertolino, Frye et al. 2003, Sheline, Gado et al. 2003). Furthermore, some meta-analyses examining MRI studies of the hippocampus, solely (Videbech and Ravnkilde 2004, McKinnon, Yucel et al. 2009, Cole, Costafreda et al. 2011) or as part of a greater limbic system analysis (Koolschijn, van Haren et al. 2009, Kempton, Salvador et al. 2011, Arnone, McIntosh et al. 2012), have found volume reductions of 4% to 10% in depressed patients. Although some imaging studies in depression observed activations in parts of the amygdala extended to (para)hippocampal regions in response to emotional facial expression, patients suffering from MDD showed predominantly an elevated amygdala-hippocampal BOLD response to sad conditions when compared to healthy individuals (Fu, Williams et al. 2008). There are not many studies in depressed patients demonstrating a decreased BOLD response to sad facial expression directly in the hippocampus (Lee, Seok et al. 2008). In contrast, a study by Videbech et al. reported that MDD patients showed increased activity of the hippocampus and the cerebellum relative to the healthy controls (Videbech and Ravnkilde 2004).

1.6.2.3. Insula

Fusar-Poli et al. performed a metanalysis of 105 fMRI studies on healthy subjects and reported the processing of facial expressions (emotional and neutral) is associated with an elevated activation in temporoparietal areas, such as the parietal lobe, the middle temporal gyrus and the insula. That was specifically true for the contrast of neutral facial expressions versus baseline in the left insula, the processing of happy facial expressions versus baseline in the left insula, of sad facial expressions versus baseline in the left insula, and of disgusted facial expressions compared with baseline in the right insula (Fusar-Poli, Placentino et al. 2009).

According to a review by Stuhrmann (Stuhrmann, Suslow et al. 2011), an imaging study by Surguladze et al. investigated the BOLD response to faces displaying different degrees of disgust in MDD patients in contrast to a healthy control group. They identified greater left insula activation in the depressed patients in comparison to the group of healthy controls (Surguladze, El-Hage et al. 2010). Irrespective of an altered processing of disgust in major depressed patients, also an altered activation in the insula to other emotional facial expressions has been published (Suslow, Konrad et al. 2010). Zhong et al. demonstrated a greater activation in the insula and the parahippocampal gyrus (PHG) to sad facial expression and a decreased activation to happy facial expression (Zhong, Wang et al. 2011). Fu et al. have shown an increased insula activation to fearful/angry (combined contrast) facial expressions in a sample of young MDD patients (Fu, Williams et al. 2004). Notably, they could demonstrate a thalamic hyper-responsiveness to sad facial expressions.

Currently in most of the reviewed imaging studies on processing of emotional facial expression there is a clear trend for similar activation patterns between the insula, the parahippocampal gyrus area and the amygdala, demonstrating an emotional bias of the activity in limbic structures in MDD patients in contrast to healthy individuals. This includes a greater BOLD response to negative facial expressions and an increased BOLD response to happy facial expressions. One study reported also a different pattern of decreased activity in the insula in a combined contrast of sad and fear facial expressions in MDD patients (Townsend, Eberhart et al. 2010).

Some studies identified aberrant activity in striatal structures which also had similar activation patterns like that reported in the amygdala and insula. It was demonstrated that predominantly the putamen and caudate nucleus show a greater BOLD response to sad/angry facial expressions

and increased BOLD response to happy facial expressions (Fu, Williams et al. 2004, Lawrence, Williams et al. 2004, Fu, Williams et al. 2008, Scheuerecker, Meisenzahl et al. 2010).

1.6.2.4. Motor cortex and prefrontal cortex

The role of the prefrontal cortex (PFC) in facial emotional processing in depression so far is less clear (Stuhrmann, Suslow et al. 2011). Using different imaging methods such as positron emission tomography (PET), neuroscientists have focused on the PFC and specially on the dorsolateral prefrontal cortex (DLPFC) function in MMD patients. There are studies reporting a reduced cerebral blood flow and metabolism in the left DLPFC and hypermetabolism in the right DLPFC in acute MDD (Mayberg 2003, Phillips, Drevets et al. 2003, Grimm, Beck et al. 2008).

Mayberg's limbic-cortical dysregulation model based on evidence from a series of PET studies is consistent with the findings of decreased activation in dorsal neocortical regions such as the DLPFC and dorsal anterior cingulate cortex (ACC), and of increased activation in paralimbic regions such as the insula, amygdala and hippocampus (Mayberg 1997, Mayberg 2003). In the lateral PFC no consistent activity pattern in different imaging studies on facial processing in MDD in contrast to healthy subjects can be found. Both findings, increased activation and decreased activation in the dorsolateral DLPFC in MDD patients to sad and angry facial stimuli, can be found nearly equally often (Lawrence, Williams et al. 2004, Keedwell, Andrew et al. 2005, Frodl, Scheuerecker et al. 2009, Suslow, Konrad et al. 2010, Zhong, Wang et al. 2011). Although Davidson et al. reported a hypoactivation of the lateral prefrontal cortex (LPFC) and the ACC (Davidson, Irwin et al. 2003), Anand et al. identified the paradoxical imaging study results which shows greater activation of prefrontal regions, such as the DLPFC (Anand, Li et al. 2005), the medial prefrontal cortex (MPFC) (Anand, Li et al. 2005, Johnstone, van Reekum et al. 2007) and the dorsal ACC (Beauregard, Paquette et al. 2006) during the presentation of affective stimuli in depressed patients. These reports and their changes after antidepressant treatment are summarized and reviewed by Rosenblau et al. (Rosenblau, Sterzer et al. 2012). Alterations of neural responses in the MPFC have been reported after successful antidepressant combination treatment with the selective noradrenalin and serotonin reuptake inhibitor (SNRI) venlafaxine in combination with light therapy (Benedetti, Radaelli et al. 2009).

Some inconsistencies were reported regarding neural responsiveness to happy facial stimuli in the DLPFC and in more ventral, lateral PFC areas. So far, is not possible to draw valid conclusions about a general hyper- or hypoactivation of the DLPFC during facial emotion

processing in unipolar depression although altered neuronal responses in the DLPFC are a prevalent finding in MDD patients due to the high variability in all published neuroimaging studies. In the orbitofrontal cortex (OFC) several independent studies detected a decreased activation in inferior and medial OFC areas in response to either sad, fearful or angry facial stimuli (Lawrence, Williams et al. 2004, Keedwell, Andrew et al. 2005, Lee, Seok et al. 2008). Furthermore, Surguladze et al. reported hyperactivation to disgust in the OFC in patients suffering from MDD (Surguladze, El-Hage et al. 2010).

In addition, some facial-processing imaging studies report similar results about activation in the prefrontal cortex and the motor cortex. A greater BOLD response to angry facial expressions in the motor cortex (Brodmann's area (BA) 6, BA 4) of MDD patients in contrast to healthy controls was shown (Fu, Williams et al. 2004, Keedwell, Andrew et al. 2005, Fu, Williams et al. 2008, Scheuerecker, Meisenzahl et al. 2010).

1.6.2.5. Fusiform gyrus

In the above mentioned meta-analysis of studies in healthy subjects, the processing of facial expressions (emotional and neutral) was associated with an elevated activation in visual areas such as the fusiform gyrus. That was reported specifically for the contrast of neutral facial expression, fearful facial expressions, and for the processing of disgusted facial expressions compared to baseline (Fusar-Poli, Placentino et al. 2009).

A study by Ho et al. on adolescent MDD patients in contrast to healthy controls identified that the fMRI BOLD signal in the left fusiform gyrus during emotional facial processing was significantly associated with greater individual-level estimates of perceptual processing efficiency. Furthermore, they suggested a facial processing bias in younger MDD patients characterized by greater perceptual processing efficiency of emotional visual information in sensory brain regions responsible for the early processing of visual information (Ho, Zhang et al. 2016). In addition, a study on MDD patient shows increase BOLD response in the fusiform gyrus after the presentation of sad facial stimuli (Fu, Williams et al. 2008).

1.6.2.6. Summary of brain regions involved in the processing of facial expression in MDD

According to the cognitive models of depression and behavioral studies, MMD patients suffer from abnormal emotional processing. There are many neuroimaging investigations pointing to an emotional processing bias in depressed patients. In addition, reviews of neuroimaging studies

have shown that depressed patients have abnormalities within the common face processing network, including a mood-congruent processing bias influencing the responsiveness especially in the regions amygdala, insula, parahippocampal gyrus (PHG), fusiform face area, and putamen. Very often, neuroimaging studies in MDD patients have shown that amygdala hyperactivity is associated with negatively biased facial emotion processing. Notably, the amygdala, the ACC, OFC, and the DLPFC are core components of a network for emotion regulation which is pathologically altered in depressive disorders (Stuhrmann, Suslow et al. 2011). Furthermore, neuroimaging studies in depression need to extend these findings, especially by replicating data with the same activation paradigms and larger sample sizes in order to enable researchers to make more valid assumptions on neural emotional processing mechanisms before and after administration of antidepressant medications. This may contribute to a better understanding of the etiology and mechanisms during effective treatments of depressive disorders.

1.7. Research questions

In the following study the evidence for a cognitive neuropsychological model of antidepressant drug action has been examined. Using pharmaco-fMRI in a double-blind randomized placebo-controlled design we investigated the effects of short-term antidepressant treatment in patient groups receiving differential treatments. We then compared the BOLD % signal change in the defined brain regions of drug treated patients to patients who received placebo treatment. The effect of antidepressants (escitalopram, mirtazapine, agomelatine) or placebo on amygdala, dorsolateral prefrontal cortex, fusiform gyrus, hippocampus and insula (all bilateral) was investigated. BOLD responses to facial expressions in patients with MDD were investigated. In addition, the HPA axis activity of a subgroup of patients was studied before treatment and after one week of acute treatment to elucidate the relationship between amygdala response during facial processing and HPA-axis hyperactivity in relation to the clinical outcome before and after short-term treatment.

The following research questions should be answered with our study:

1) Is the fMRI BOLD % signal change during facial processing of MDD patients in several regions of interest (ROIs: amygdala, DLPFC, hippocampus, insula, and fusiform gyrus) influenced by a short-term antidepressant treatment?
2) Are there detectable differences of the BOLD response to facial processing between the drug-treated (escitalopram, mirtazapine, or agomelatine) patients and and the placebo-treated patients after one week of short-term treatment?
3) Are there detectable differences of the BOLD response to facial processing between the four different treatment groups (antidepressants escitalopram, mirtazapine, agomelatine or placebo) after one week of active or placebo treatment?
4) Is there a detectable correlative relationship between clinical outcome (measured using the Hamilton rating scale for depression) and the BOLD % signal change in different ROIs during facial processing before and after one week of treatment with the antidepressants escitalopram, mirtazapine or agomelatine, versus placebo?
5) Is there a correlative relationship between the HPA-axis (hyper)activity in MDD and the BOLD % signal change during facial processing? Is this putative correlation altered after one week of antidepressant-drug treatment?

2. Methods

2.1. Study design

This study is part of the project "Relevance of the gut-microbiome composition für subtypes of depression, response and side effects during antidepressant treatment" which is a part of the German reseach network "Novel strategies for the optimized treatment of depression (OptiMD)" funded by the Federal Ministry of Education and Research (Bundesministerium für Bildung und Forschung, BMBF, www.bmbf.de, support code 01EE1401B).

The single center study was carried out as a randomized, double-blind and placebo-controlled trial in a parallel group design. Ethical approval was granted by the local Ethics committee of the University Regensburg. The clinical trial was registered and approved by the Federal Institute for Drugs and Medical Devices (Bundesinstitut für Arzneimittel und Medizinprodukte, BfArM, www.bfarm.de).

2.1.1. Study sample and patient selection

All patients were included in the study after adequate explanation of the study procedures and after they provided written informed consent. They were investigated at the Department of Psychiatry and Psychotherapy of the University Regensburg, located in the Bezirksklinikum Regensburg, Universitätsstraße 84 in 93053 Regensburg, Germany. We included 33 major depressed in-patients admitted for the treatment of a major depressive disorder independently of study participation. Patients were not treated with antidepressants or other psychotropic substances except lorazepam for agitation or zopiclone for insomnia during the first pre-treatment fMRI and during the first combined Dex/CRH-test. Table 2 shows clinical and demographic data for the patients. In all participants we assessed the presence of current and past psychiatric disorders using the Mini-International Neuropsychiatric Interview (M.I.N.I.) (Sheehan, Lecrubier et al. 1998) for the 10th version of the International Classification of Diseases (WHO 2016). The depressed patients met criteria for a primary diagnosis of major depressive disorder (MDD) and were not suffering from other psychiatric diagnoses. In addition they were physically healthy and not suffering from serious somatic diseases. Medical history, psychiatric history, vital signs, a laboratory screening and an electrocardiogram (ECG) were assessed. The principles of informed consent were implemented according to the current revision of the Declaration of Helsinki, the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Guideline for Good Clinical Practice and regulatory requirements. All patients are included in the study after

adequate explanation of the study procedure and after written informed consent. Withdrawal of consent was possible at any time during the study.

Our detailed study inclusion criteria were:

- Male and female in-patients in the age between 18 and 65 years;
- Major depressive disorder;
- Admission on a voluntary basis independent of our study;
- Written informed consent for trial participation after the scope and nature of the investigation have been explained to the patients before starting trial-related activities;
- Indication for antidepressant therapy independent of the clinical trial;
- Primary unipolar depression (ICD-10: F32, F33) or bipolar depression (ICD-10: F31.3-5), current episode of depressed state for at least 2 weeks prior to baseline;
- Physically healthy;
- Right handedness (assessed by the Edinburgh Handedness Inventory (Oldfield, 1971)).

The detailed exclusion criteria were:

- Schizophrenia, substance dependence as define by ICD-10 or any other psychiatric primary diagnosis (according to the ICD-10 criteria);
- major somatic or neurological disorder;
- abnormalities in the laboratory screening at baseline (e.g. hypo- or hyperthyroid state, hyperhidrosis, elevated liver enzymes, blood cell dyscrasias);
- lacking ability to give informed consent;
- have been admitted to the clinic involuntarily during their present episode;
- pregnancy or breast-feeding;
- in case of the inclusion of premenopausal female patients insufficient contraception leads to exclusion from the study;
- contraindications to magnetic resonance imaging patient with heart pacemaker or implanted metal in the scull;
- or concurrent medication, which could alter emotional processing;
- known history of alcohol or drug abuse during 6 month prior to the screening;
- being at clinically risk of suicidal behavior (HAM-D Item 3 > 2 or clinical impression);
- involuntary admission to the hospital;

- known allergies or hypersensitivity reactions or other contraindications for escitalopram, agomelatine or mirtazapine;
- being treated with psychotropic medication < 3 weeks before study (5 weeks in case of fluoxetine pretreatment);
- Unusual diets leading to malnutrition;
- Pretreatment with antibiotics or corticosteroids.

Discontinuation criteria were:

- Withdrawal of consent at any time during the study period;
- If necessary concerning clinical reasons, investigators and sub-investigators could stop the study participation of an individual patient;
- Noncompliance to the study protocol.

Our goal was to provide a better understanding of the interactions between neural systems during antidepressant treatment, the effect of antidepressants on emotional processing, the relationship between amygdala hyperactivity as well as other brain regions BOLD response to emotional facial expression, and the HPA axis activity in relation to clinical effectiveness of the treatment measured using the 21-item version of the Hamilton Rating Scale for depression (HAM-D21) (Hamilton 1967).

In this study firstly, we considered the evidence for a cognitive neuropsychological model of antidepressant drug action. We used pharmaco-fMRI (3T) to investigate the effect antidepressant treatment on the amygdala (right (R)+left (L)), caudate (R+L), dorsal lateral pre-frontal cortex (DLPFC) (R+L), fusiform gyrus (R+L), hippocampus (R+L) and insula (R+L) BOLD response to facial expression at baseline and after one week of antidepressant treatment (antidepressants or placebo in addition to psychotherapy) in major depressed patients.

Secondly, we investigated the activity of the hypothalamus-pituitary-adrenal (HPA)-axis using the combined dexamethasone suppression / corticotropin releasing hormone (Dex/CRH) stimulation test before and after one week of treatment to elucidate the relationship between BOLD response signal change and HPA-axis activity.

Thirdly, we clinically assessed the patients before, during and after treatment periods using the HAM-D21 (Hamilton 1967) rating scale as the primary outcome criterion.

2.1.2. Study design and drug treatment

Double-blind, placebo-controlled, parallel group study in 33 depressed patients who were randomly assigned to 4 groups. The patients in each group received either 10 mg Escitalopram, 30 mg Mirtazapine, 25 mg Agomelatine or placebo for at least 7 days. The patients receive the medication at 8:00 am in case of escitalopram or at 10:00 pm in case of agomelatine and mirtazapine treatment according to usual clinical procedures. For blinding a double-dummy technique was used. On study day 4 and 11, i.e. the days of pharmaco-fMRI scans, the patients received the medication in the morning at 8:00 am (about 4 hours before the fMRI scan). All four groups then received the fMRI scan before treatment and after the seventh day of the treatment. In addition, on the 2nd and 9th day of the study we investigated the levels of cortisol secretion by using combined Dexamethasone suppression / corticotropine-stimulation (combined Dex/CRH-) tests. Mood and anxiety were assessed primarily using the HAMD-21 scale by trained psychiatrists immediately before starting treatment, on the 7th day of escitalopram, mirtazapine, agomelatine or placebo treatment and then in weekly intervals until discharge of the hospital. After the second fMRI scan in case of partial response (defined as at least 10% reduction in HAMD-21 scale) treatment was continued without change, in case of nonresponse a dose increase or change of the treatment was offered to the patients. The same was true if no reduction of at least 20% was seen after 2 weeks of treatment according usual clinical procedures. On the day of inclusion a laboratory screening and on the days of the fMRI scan blood withdrawals for the estimation of drug plasma levels of the used antidepressants were performed. The measurement of plasma levels was performed after unblinding the medication. For the study flow chart see Table 1.

In case of clinical necessity a concomitand treatment with hypnotics, including lorazepam (up to 3 mg/d), zopiclone (up to 15 mg/d) or zolpidem (up to 20 mg/d) was allowed. On the days before and the days of the pharmaco fMRI scan and the Dex/CRH-test concomitant medication was avoided. After study participation all patients received further treatment according to clinical indications. Each patient advised independently of the study only according to clinical reasons. In case patients responded well to the treatment, it was considered to continue pharmacotherapy. In case of nonresponse other pharmacological and non-pharmacological interventions including augmentation strategies were offered.

Table 1: Study flow chart and investigation plan for the blinded short-term treatment period (MED = medication)

Day 1	Day 2	Day 4	Day 5-8	Day 9	Day 10	Day 11	Day 11+
Study inclusion HAM-D21	combined Dex/CRH -test 1	fMRI 1	blinded medication or placebo (MED)	MED & combined Dex/CRH -test 2	MED	MED & fMRI 2 HAM-D21	treatment according to clinical requirements

2.1.3. Functional magnetic resonance imaging data acquisition

fMRI data collection was done using three different 3 Tesla (3T) scanners due to a replacement of the research scanner of the University Regensburg. From the total of 33 patients, 2 were investigated using a Siemens MAGNETOM Allegra head scanner for 3T brain imaging (Siemens AG, Erlangen, Germany), fitted with a birdcage headcoil, which was located at the department of neuroradiology of the University Regensburg, Center for Clinical Magnetic Resonance Research. Eight of the patients were investigated using a Siemens MAGNETOM Skyra full-body scanner for 3T imaging, located at the Institute of Radiodiagnostics at the University Hospital Regensburg, and 23 of the patients were scanned using a Siemens MAGNETOM Prisma head scanner for 3T brain imaging with the 20-channel headcoil, located at the Bezirksklinikum Regensburg (clinical center of the district Upper Palatinate), Center for Clinical Magnetic Resonance Research. The study participants completed a neuroimaging battery including high-resolution structural, resting state, and functional task scans. Only the data from the facial emotion processing task described in the following was used for the current analysis.

2.1.4. Functional MRI experimental task

Before each fMRI task investigation all patients were screened and asked to disclose any ferromagnetic implants and devices such as cardiac pace makers, before they entered the MRT scanner completely metal free. During the fMRI scanning, participants were asked to process visual stimuli and complete a simple gender discrimination task, involving the rapid presentation of emotional and neutral facial expressions or scrambled pictures. In this task, they viewed male and female emotional faces. As a starting basis the Averaged Karolinska Directed Emotional Faces (AKDEF) were used. This is a set of totally 70 pictures of averaged human facial expressions. The material was developed in 1998 by Daniel Lundqvist and Jan-Eric Litton

at Karolinska Institutet, Department of Clinical Neuroscience, Section of Psychology, Stockholm, Sweden (Lundqvist D 1998). All faces were modified from the original pictures of the KDEF set to provide a similar proportion and distribution of light and dark areas to prevent confounding effects independent from facial expressions. Therefore, we masked all pictures to cover parts of the hair and to present identical black and white pictures to keep the attention only on facial expression. The patients were asked to report the gender of the face via an MRI compatible keypad. Stimuli were presented on a computer using *A simple framework* (ASF) for behavioral and neuroimaging experiments (Schwarzbach 2011) based on the psychophysics toolbox (Brainard 1997) for the MATLAB software package (MATLAB_R2016b, Adalperostraße 45, 85737 Ismaning, Germany, https://de.mathworks.com/products/matlab.html) and on a cloned projection display to patients on an opaque screen located at the head of the scanner bore, which subjects view using angled mirrors. Subject responses were registered via an MRI-compatible keypad. Immediately before scanning, all subjects received training with another set of stimuli to ensure that they fully understood the requirements of the task.

There were 18 blocks of the emotional task that contained 10 images (for a given condition), and each image was presented for 1.6 seconds (s). The task had seventeen 16s blocks of a baseline fixation point, an interleave with eighteen 16s blocks of the facial or scrambled task blocks of afraid, angry, happy, neutral, and sad faces, and of scrambled pictures (figure 1). There was no masking between the images. They were merely presented back-to-back within a block. Each condition was repeated 3 times per run (i.e., 6 conditions [afraid, angry, happy, neutral, sad, scrambled]*3 repetitions = 18). Each run was repeated 3 times in a given session (i.e., pre or post).

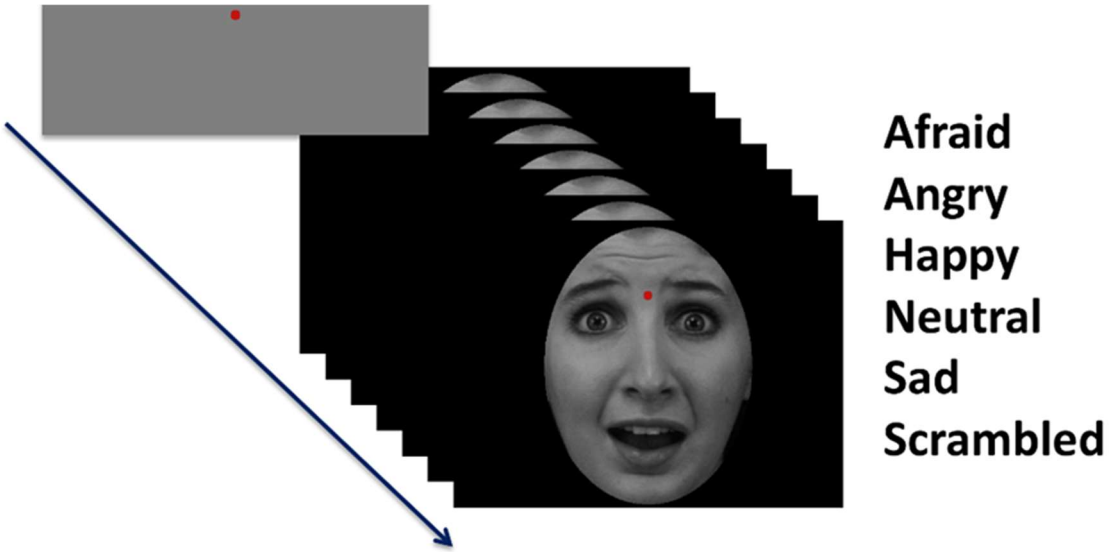

Figure 1: Presented emotional faces during the scan time. The task was to press a button corresponding to the recognized gender in facial pictures or the localization of dark areas in scrambled pictures.

During each emotional block, participants viewed 10 emotional faces (male and female). Each face was presented for 1.6 s and subjects were asked to report the gender of the face via a MRI compatible keypad to ensure patients remaining on focus during the task. They were instructed to press one key with the index finger when they recognized a female face and another key with the middle finger when they recognized a male face. In case the scrambled pictures were presented, they should press one key with their index finger when a picture with more darkness at the bottom was shown and another key with their middle finger in case darker areas are located in the upper parts of the pictures. The patients were asked to lie calmly without moving their head. The total duration of the fMRI procedure was about 60 minutes per session. Each session consisted of 30 min facial task, the results of which are presented here. This was combined with 20 min resting state and 12 min diffusion tensor imaging (DTI) sequences which have been analyzed in another subproject.

2.1.5. Dex/CRH Test

To investigate the activity of the HPA axis, two Dex/CRH-tests in each patient were performed. The first test was performed before treatment on days 1 and 2, the second after the short-term treatment of one week on day 8 and 9.

The patients received 1.5mg Dexamethasone (Dex) (Fortecortin®, Merck KG, Darmstadt, Germany) orally at 11:00 pm on the day 1 (first day of the test) before the CRH challenge (second day of the test). On the day of the CRH stimulation test at 2:30 pm an intravenous forearm catheter was inserted and the first blood samples were collected. The patients had to

stay supine on a bed in a single room under resting conditions. After a 30min adaptation period baseline probes were acquired. At 3:00 pm a bolus of 100µg human CRH lyophilisate (CRF human, Clinalfa® AG, Läufelfingen, Switzerland) was injected within 30s. Blood samples were collected at 3:00, 3:30, 4:00 and 4:15 pm. Blood sampling and CRH injection were performed using the "through-the wall"-technique to avoid disturbance of the patients.

Serum for cortisol determination was stored frozen at –80 °C until en bloc assessment. Cortisol was quantified in 20µl plasma using a commercial enzyme linked immunosorbent assay (ELISA) (Cortisol-ELISA RE52061, IBL international, Flughafenstraße 52a, D-22335 Hamburg, Germany). The detection range is 20-800 ng/mL.

2.1.6. Clinical assessment and psychiatric ratings

The HAM-D sum score was assessed after an interview of trained psychiatrists. The HAM-D21 scale consists of 21 single items related to depression. Their severity was determined in a semi-structured interview. The items were depressed mood, feelings of guilt, suicide, insomnia - early, insomnia - middle, insomnia - late, work and activities, retardation, anxiety - psychic, anxiety - somatic, somatic symptoms – gastrointestinal, somatic symptoms – general, genital symptoms, hypochondriasis, loss of weight, insight, diurnal variation, depersonalization and derealization, paranoid symptoms, and obsessional and compulsive symptoms.

2.1.7. Statistical analyses

2.1.7.1. Demographic and clinical variables

Statistical analyses included descriptive variables (mean, standard deviation, and standard error), the Kolmogorov-Smirnov testing for normal distribution, non- parametric and parametric (if applicable) comparisons of the mean, comparisons of frequencies in the treatment groups (crosstabs, χ^2-tests, Fisher´s exact-tests). Comparisons of the mean of more than two groups are performed using an univariate analysis of variance (ANOVA) with post-hoc comparisons of means (corrected for multiple testing). Cortisol secretion profiles are compared between different groups using the repeated-measurement ANOVA. Statistics were calculated using the software IBM SPSS Statistics Version 24 (https://www.ibm.com/products/spss-statistics).

2.1.7.2. fMRI data analysis

2.1.7.2.1. Preprocessing

For the preprocessing of all images the software package *Statistical Parametric Mapping* SPM12 (Wellcome Centre for Human Neuroimaging, http://www.fil.ion.ucl.ac.uk/spm/) and the MATLAB software package were used. For each individual patient the functional images were slice-time corrected to time repetition (TR) divided by 2 (TR/2). This funcion corrects differences in slice acquisition times and is an important step in fMRI preprocessing (Parker, Liu et al. 2017). The next step proceeded with the Realignment Estimate and Reslicing. This means, each individual imaging dataset was realigned to the first volume by rigid body transformation to correct for head motions of the participants. Ashburner and Friston noted that the aim of this realignment is to remove movement artefact in fMRI or PET times- series (Ashburner J 1997). In a third step, before performing the coregistration and normalization steps in SPM, all individual structural images were reset to their origin in the anterior commissure (AC). The next step was to proceed with coregistration and estimation of each patient´s structural image. In general, proceeding with the registration based on the work of Collignon et al. who recommended the coregistration of structural images to the mean of realigned functional images using a 12-parameter affine transformation (Collignon A 1995). The "reference image" is the image supposed to remain static while the structural source image is moved to match it. Furthermore, the structural images were segmented according to the standard procedure in SPM12 (Ashburner and Friston 2005). Then the spatial normalization to the standard Montreal Neurological Institute (MNI) space) was applied to the functional images to allow for intrasubject analysis. Then spatial smoothing using an 8 mm Gaussian kernel full width at half maximum was applied to increase the signal-to-noise ratio. The reason using smoothing in preprosseing steps was is suppress noise and effects due to residual differences in functional and gyral anatomy during inter-subject averaging. All procedures were performed with consideration of the SPM12 manual (Ashburner 2018).

2.1.7.2.2. Statistical analysis of the images - First level (individual) analysis

All statistical first and second-level analyses were conducted with SPM12 for each individual patient based on the general linear model (GLM). Six types of events were distinguished: afraid, angry, happy, sad, and neutral facial expressions, and scrambled pictures, which were repeated in 3 runs of two scanning sessions. In the event design, a general linear model was then applied

to the time course of activation in which stimulus onsets were modelled as single impulse response functions and then convolved with the canonical hemodynamic response function (HRF) (Friston, Worsley et al. 1994). The data were high-pass filtered with a frequency cutoff at 128 seconds. Statistical parametric maps (SPMs) were generated for each subject by t-statistics derived from contrasts utilizing the HRF (Friston, Penny et al. 2002). After that model parameters estimated using classical (ReML - Restricted Maximum Likelihood) or Bayesian algorithms.

2.1.7.2.3. Contrasts computed on the individual analysis level

The following contrasts of interest were computed on the individual analysis level, while the the comparison of each emotional face condition with neutral facial expressions or scrambled picture conditions or with baseline were the main focus. Therefore, 19 t-test contrasts were calculated comparing neutral faces or scrambled pictures with emotional facial conditions. In addition, the different emotions were compared with each other. Therefore, a set of nineteen subsequent contrasts was acquired for each individual subject:

1)	afraid	>	neutral
2)	afraid	>	scrambled
3)	angry	>	neutral
4)	angry	>	scrambled
5)	happy	>	neutral
6)	happy	>	scrambled
7)	neutral	>	scrambled
8)	sad	>	neutral
9)	sad	>	scrambled
10)	afraid	>	happy
11)	angry	>	happy
12)	sad	>	happy
13)	all facial expressions	>	scrambled
14)	afraid	>	baseline
15)	angry	>	baseline
16)	happy	>	baseline
17)	neutral	>	baseline
18)	sad	>	baseline
19)	scrambled	>	baseline.

2.1.7.2.4. Group analysis

To assess differences in BOLD response to facial expressions or scrambled pictures in different stages of treatment (before and after one week of active treatment) or in different medication groups (one of the three antidepressants or placebo), all groups results were subdivided with regard to our three main questions:

20) All patients pre-post comparison: To access % signal change differences before and after short-term treatment, we assessed all patients together, divided to two sessions (the pre-treatment session compared to the post-treatment session);
21) Post-treatment sessions: The group of all medicated patients together (agomelatine, mirtazapine, escitalopram groups pooled) compared to the placebo group;
22) Post-treatment sessions: The pairwise comparisons of four treatment groups (agomelatine, mirtazapine, escitalopram and placebo).

In the group analysis regarding the three different questions all the groups engaged the same step of group analysis procedure after defining the contrast and model estimation.

2.1.7.2.5. Second level analysis

These single-subject first-level contrast images from the weighted beta-images were engaged into a second-level random-effects analysis to start the group analysis. For each contrast a one-sample t-test was conducted. All fMRI results reported here are subdivided to two different group analysis views. The first reported results are based on voxel statistics computed with SPM for the whole-brain exploratory analysis. The resulting set of significant voxel values for each contrast constituted an SPM map. The maps were thresholded at $T = T = 5.45$ [$p < 0.05$ (Family-Wise Error, FWE)] and overlaid on the MNI template, and labelled according to MNI coordinates. For graphical purposes in those brain regions showing significant effects mean cluster values (parameter estimates) were extracted by using the SPM12 software. The other group analysis results reported here were computed with ROI analysis, based on 10 regions of interest.

2.1.7.2.6. Region of Interest (ROIs) analysis

Defining of region of interest: According to our hypotheses we choose 10 regions of interest as follows (ROI, coordinates; radius of ROI = 5 mm):

23) Left amygdala	(-23.5, -1.95, -18.5)
24) Right amygdala	(27.1, -0.573, -18)
25) Left hippocampus	(-25.3, -22, -11.4)
26) Right hippocampus	(28.9, -21, -11.6)
27) Left insula	(-35.4, 5.44, 2.17)
28) Right insula	(38.7, 5.02, 0.814)
29) Left fusiform gyrus	(-31.4, -41.4, -21.6)
30) Right fusiform gyrus	(33.7, -40.2, -21.5)
31) Left dorsolateral prefrontal cortex (DLPFC)	(−46, 38, 12)
32) Right DLPFC	(46, 38, 12)

The ROIs no. 1 to 8 were selected using the standard automated anatomical labelling (AAL) procedure for structural ROIs described more in detail in Tzourio-Mazoyer et al. (Tzourio-Mazoyer, Landeau et al. 2002). The ROI contains ROIs in the format of the MarsBaR region of interest toolbox for SPM (Brett M 2002). ROIs 9 and 10 were selected using the Montreal Neurological Institute (MNI) coordinates of the regions of interests in the default mode network (DMN) and in the executive control network (De Pisapia, Bacci et al. 2016).

2.1.7.2.7. The MarsBaR / SPM interface

The ROI analysis was done with the SPM toolbox MarsBaR (Brett M 2002) using the following procedure: First, the ROI was characterized by defining the center of the mass (ROI coordinates) and defining a 5 mm radius sphere around the center. Then, the % signal change was produced from computed results of the 1st level of the analysis for each session of each individual patient and 19 differential contrasts in all ROIs separately.

Finally, differential mean values were calculated and compared. For the comparison of the % signal change for each group and ROI before and after antidepressant treatment (pre / post comparison) using SPSS the repeated measures analysis of variance (rmANOVA) was utilized. For the comparison of the treatment groups described on page 31 an univariate ANOVA was performed.

2.1.7.2.8. Final statistical evaluation

To evaluate significant time effects on the BOLD % signal change before and after one week of antidepressant short-term treatment an ANOVA for repeated measurements (rmANOVA) with time as within-subjects factor was performed. After using contrasts between different conditions (all facial and scrambled pictures) in all regions of interest (ROIs) predefined according to our hypotheses, differential contrasts between different emotional and neutral faces and scrambled pictures were computed. All analyses were first done for baseline values of the BOLD % signal change in the group of all patients together. Then different treatment regimes were compared by rmANOVA with group as a between subjects factor. Different therapeutic conditions were summarized first: the group of patients receiving antidepressant medications agomelatine, escitalopram or mirtazapine were compared to the placebo treated patients. In a final step each treatment group (agomelatine, escitalopram, mirtazapine and placebo) were analyzed separately. Furthermore, correlative analyses with the BOLD % signal change of all contrasts to baseline and all contrasts between emotional conditions and scrambled pictures were performed. For that purpose, areas under the curve (AUC) for cortisol concentrations vs. time were calculated by numerical integration using the trapezoidal rule. Then, correlations were calculated for fMRI data and clinical variables (HAM-D21 sum scores) and for fMRI data and the HPA-axis activity (cortisol areas under the curve (AUC), baseline corrected AUCs and peak values). Since some of the variables were not normally distributed, the nonparametric Spearman correlation coefficient was calculated. In addition, the Pearson correlation coefficient was used after clarifying the normal distribution of each used variable with the Kolmororov-Smirnov test.

3. Results

To reject or confirm our hypotheses and to answer the main questions of the three experiments, first the demographic variables are displayed to characterize the investigated sample of 33 major depressed patients investigated before and after one week of antidepressant treatment.

The first experiment is subdivided in two main parts: In the first part, in a pharmaco-fMRI study the BOLD % signal changes before and after treatment during the presentation of emotional facial expressions (afraid, angry, happy, sad, neutral) or scrambled pictures in predefined regions of interest (ROI analysis) were compared. These results represent the main findings of the present study. In the second part, the peak voxel activation was assessed in an exploratory study part using a whole brain analysis.

The second experiment was designed to clarify the effects of antidepressants and placebo on different brain regions of interest. Therefore, the results are subdivided into two sections. In the first part, the BOLD % signal changes between medicated patients and placebo treated patients were compared. In the second part, effects of three different antidepressants or placebo on the BOLD % signal changes in the same predefined ROIs were investigated.

Finally. in the third experiment, interdependencies between the BOLD % signal change in the ROIs and the clinical outcome measured with the HAM-D21 scale and the HPA-axis activity considering cortisol secretion during both combined Dex/CRH-tests were investigated in correlative analyses.

3.1. Demographic and clinical variables

We investigated a total of 33 patients suffering from MDD who were randomized to four treatment goups. Their mean age was 32.6 ± 12 years (mean ± standard deviation, STD). Differences in their mean age in the treatment groups were not statistically significant (ANOVA $F [3, 32] = 1.96$, $p > 0.05$). Nor was the sex distribution significantly different in the treatment groups ($\chi^2 [3, 32] = 2.63$, $p > 0.05$) (table 2).

Table 2: Demographic data and clinical variables.

	n	age ± SEM	sex (m/f)	HAM-D21 day 1 ± SEM	HAM-D21 day 11 ± SEM	HAM-D21 discharge ± SEM
placebo	9	25.9 ± 1.6	5 / 4	24.8 ± 1.8	18.3 ± 1.7	(13.4 ± 2.3)
agomelatine	7	38.4 ± 6.7	5 / 2	27.7 ± 1.8	21.3 ± 1.6	17.6 ± 2.5
escitalopram	9	*31.3* ± 3.2	8 / 1	25.9 ± 1.6	19.7 ± 1.9	12.7 ± 1.5
mirtazapine	8	36.4 ± 4.1	5 / 3	27.3 ± 1.7	21.1 ± 1.3	15.1 ± 1.9
total	33	32.6 ± 2.1	23 / 10	26.3 ± 0.86	20.0 ± 0.83	14.5 ± 1.0
P (ANOVA, χ^2)	33	0.14, n.s.	0.45, n.s.	0.64, n.s.	0.57, n.s.	0.38, n.s.

Our patients showed a significant improvement between the study inclusion before the 1st fMRI scan on day 1 and the day of the 2nd fMRI scan (day 11) in the HAM-D21 score (table 2). The repeated measurement ANOVA revealed a significant time effect ($F [2, 32] = 25.5$, $P < 0.0001$) (table 2 and figure 2).

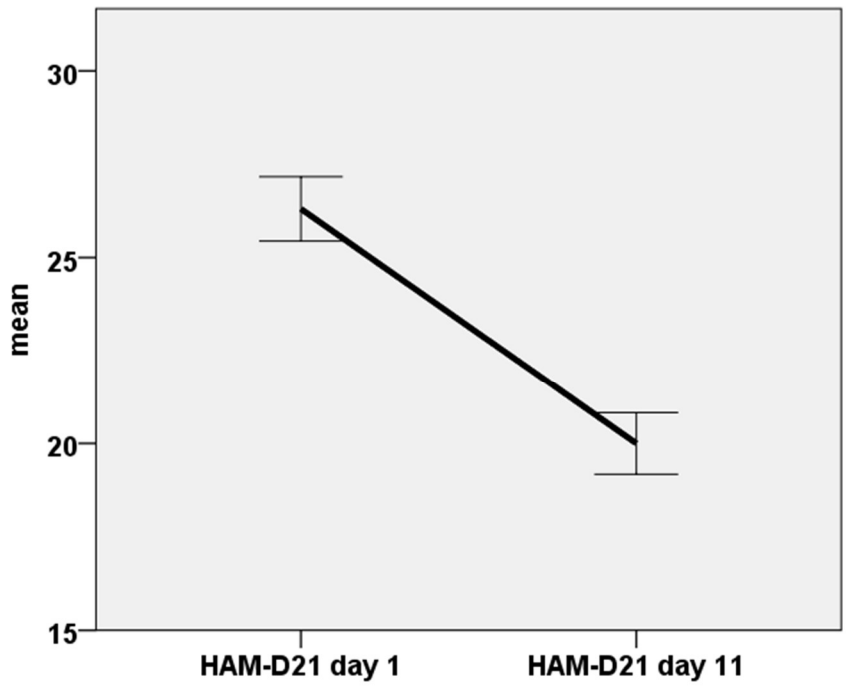

Figure 2: Clinical ratings (HAM-D21 scores), all patients summarized before and after the fMRI scans (error bars ± standard error of the mean, SEM).

3.2. Pharmaco-fMRI challenge before and after antidepressant treatment

3.2.1. BOLD % signal change in different ROIs in all MDD patients before and after treatment

3.2.1.1. BOLD % signal change in the left and right amygdala

The BOLD % signal change in the left and right amygdala in response to facial expressions (afraid, angry, happy, neutral) and scrambled pictures compared to baseline was reduced in the fMRI sessions after short-term antidepressant treatment (post session) in comparison to the fMRI sesssions before treatment (pre sessions) in MDD patients. There are statistically significant differences in the BOLD % signal change between pre and post in the left amygdala during the presentation of angry faces versus baseline (rmANOVA: F [1, 32] = 7.01, p = 0.012) (figure 3), happy faces versus baseline (rmANOVA: F [1, 32] = 4.23, p = 0.048), and sad faces versus baseline (rmANOVA: F [1, 32] = 4.23, p = 0.048). In addition, there are statically significant differences between pre and post BOLD % signal change in the right amygdala during the presentation of happy faces (rmANOVA: F [1, 32] = 8.08, p = 0.008) and neutral faces versus baseline (rmANOVA: F [1, 32] = 4.54, p = 0.042).

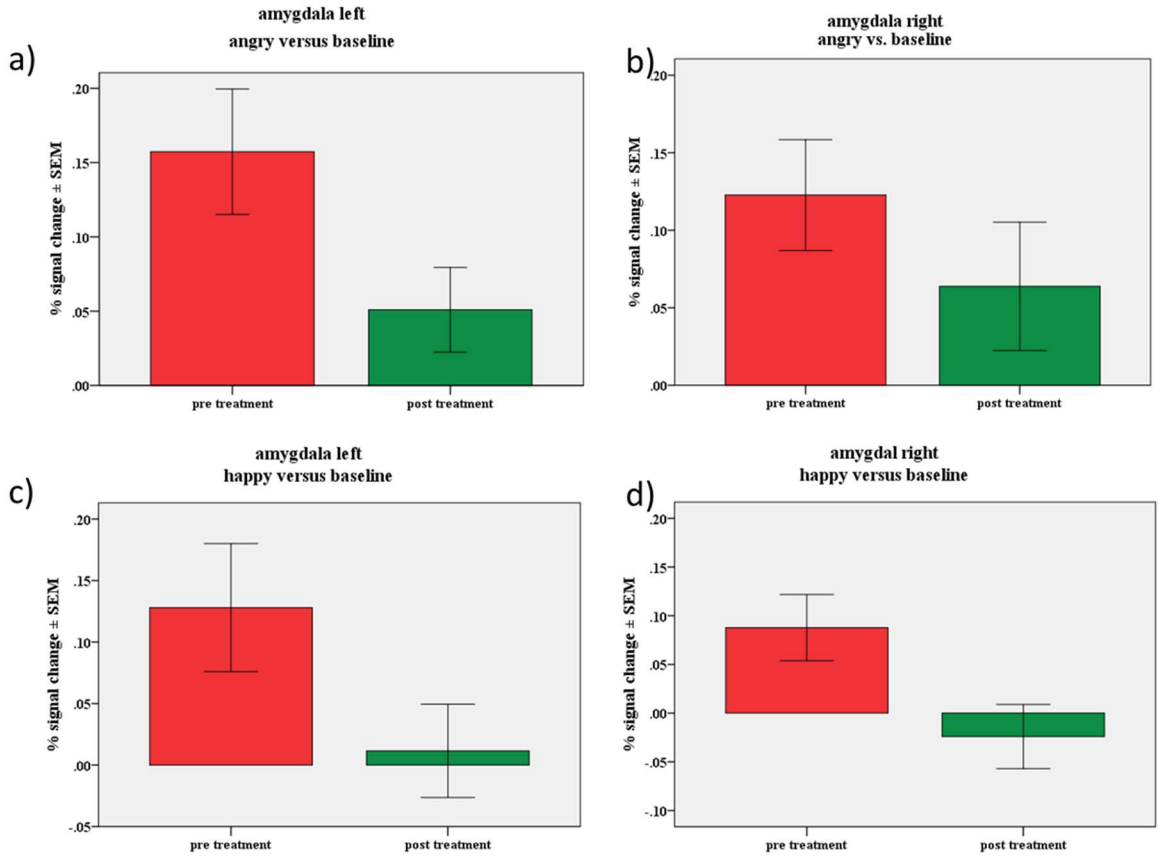

Figure 3: Statistically significant BOLD % signal change ± SEM after treatment in the left amygdala during the presentation of angry (a) and happy (c) faces vs. baseline and in the right amygdala during the presentation of happy faces vs. baseline (d). Angry faces vs. baseline in the right amygdala showed only a nonsignificant trend (b).

Furthermore, there are following statistically significant treatment effects in the left amygdala of MDD patients during the presentation of sad faces in contrast to neutral faces (rmANOVA: F [1, 32] = 7.03, p = 0.012), sad faces in contrast to scrambled pictures (rmANOVA: F [1, 32] = 5.10, p = 0.031), angry in contrast to happy faces (rmANOVA: F [1, 32] = 5.96, p = 0.020) and all faces versus scrambled pictures (rmANOVA: F [1, 32] = 6.03, p = 0.020). In addition. there are significant time effects in the amygdala after one week of antidepressant treatment during the presentation of afraid versus neutral faces (rmANOVA: F [1, 32] = 4.19, p = 0.049), angry versus neutral faces (rmANOVA: F [1, 32] = 6.41, p=0.016), angry faces versus scrambled pictures (rmANOVA: F [1, 32] = 5.18, p = 0.030), happy versus neutral faces (rmANOVA: F [1, 32] = 8.12, p = 0.008), happy faces versus scrambled pictures (rmANOVA: F [1, 32] = 8.05, p = 0.008), neutral faces versus scrambled pictures (rmANOVA: F [1, 32] = 4.75, p = 0.037), angry versus happy faces (rmANOVA: F [1, 32] = 5.13, p = 0.030), afraid versus neutral faces (rmANOVA: F [1, 32] = 9.63, p = 0.004), sad versus happy faces (rmANOVA: F [1, 32] = 7.52, p = 0.010), and all faces versus scrambled pictures (rmANOVA: F [1, 32] = 8.27, p = 0.007).

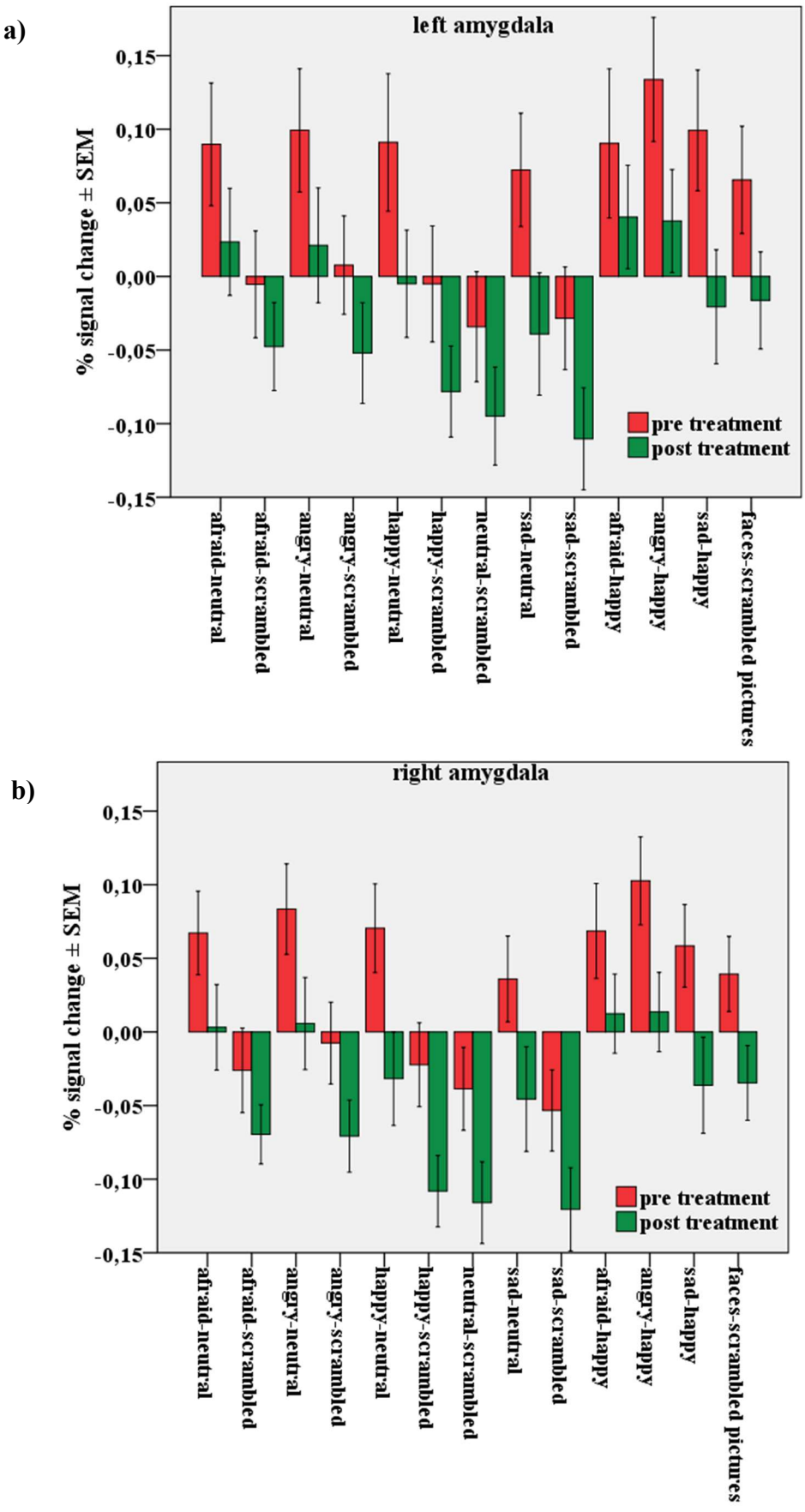

Figure 4: Mean BOLD % signal change ± SEM in the amygdala during the presentation of emotional faces. Contrasts between different emotions or contrasts between emotions and neutral faces or scrambled pictures before and after treatment.

3.2.1.2. *BOLD % signal change in the left and right DLPFC*

The BOLD % signal change in the left dorsolateral prefrontal cortex of MDD patients during the presentation of afraid, angry, and neutral facial expressions after one week of antidepressant treatment was increased. In the left DLPFC during the presentation of happy or sad faces and scrambled pictures in contrast to baseline the BOLD % signal change was reduced. Notably, the BOLD % signal change was reduced in the right DLPFC during the presentation to all emotional facial expressions and scrambled pictures compared to baseline in the post-treatment fMRI sessions. From the described changes, only the BOLD % signal change in the right DLPFC was statistically significant reduced during the presentation of angry faces in contrast to baseline (rmANOVA: F [1, 32] = 8.65, p = 0.006).

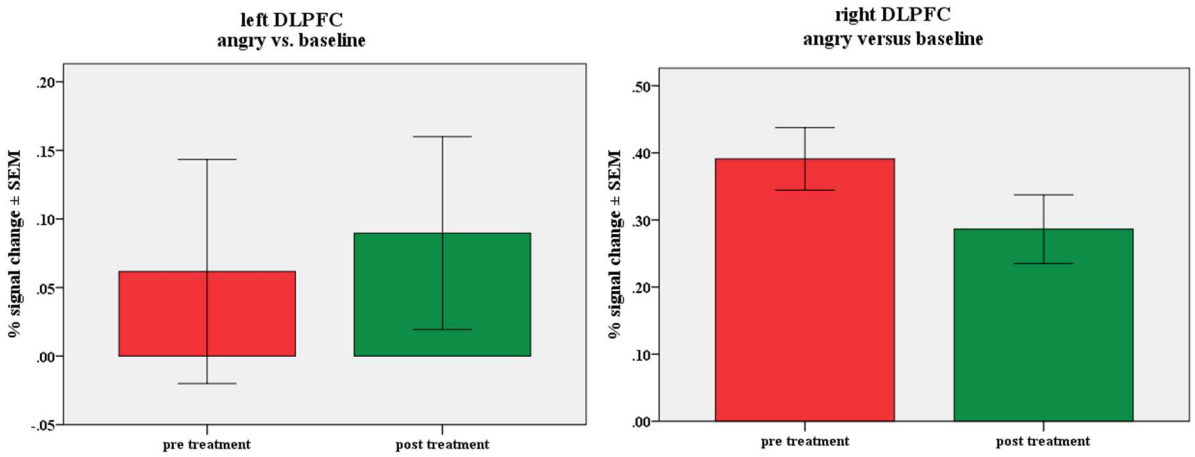

Figure 5: Statistically significant BOLD % signal change ± SEM reduction after treatment in the right DLPFC during the presentation of angry faces versus baseline. In the left DLPFC only nonsignificant changes could be detected.

In addition, the following statistically significant treatment effects after short-term antidepressant treatment were detected in the left DLPFC during the presentation of afraid vs. neutral faces (rmANOVA: F [1, 32] = 17.49, p < 0.001), and in the contrast between all faces versus scrambled pictures (rmANOVA: F [1, 32] = 25.39, p < 0.001). Furthermore, there are a number of significant time effects in the right DLPFC during the presentation of angry faces in contrast to neutral faces (rmANOVA: F [1, 32] = 8.22, p = 0.007), angry faces in contrast to scrambled pictures, happy in contrast to neutral faces (rmANOVA: F [1, 32] = 5.59, p = 0.04), angry versus happy faces (rmANOVA: F [1, 32] = 4.55, p = 0.041), and sad versus happy (faces (rmANOVA: F [1, 32] = 7.12, p = 0.012).

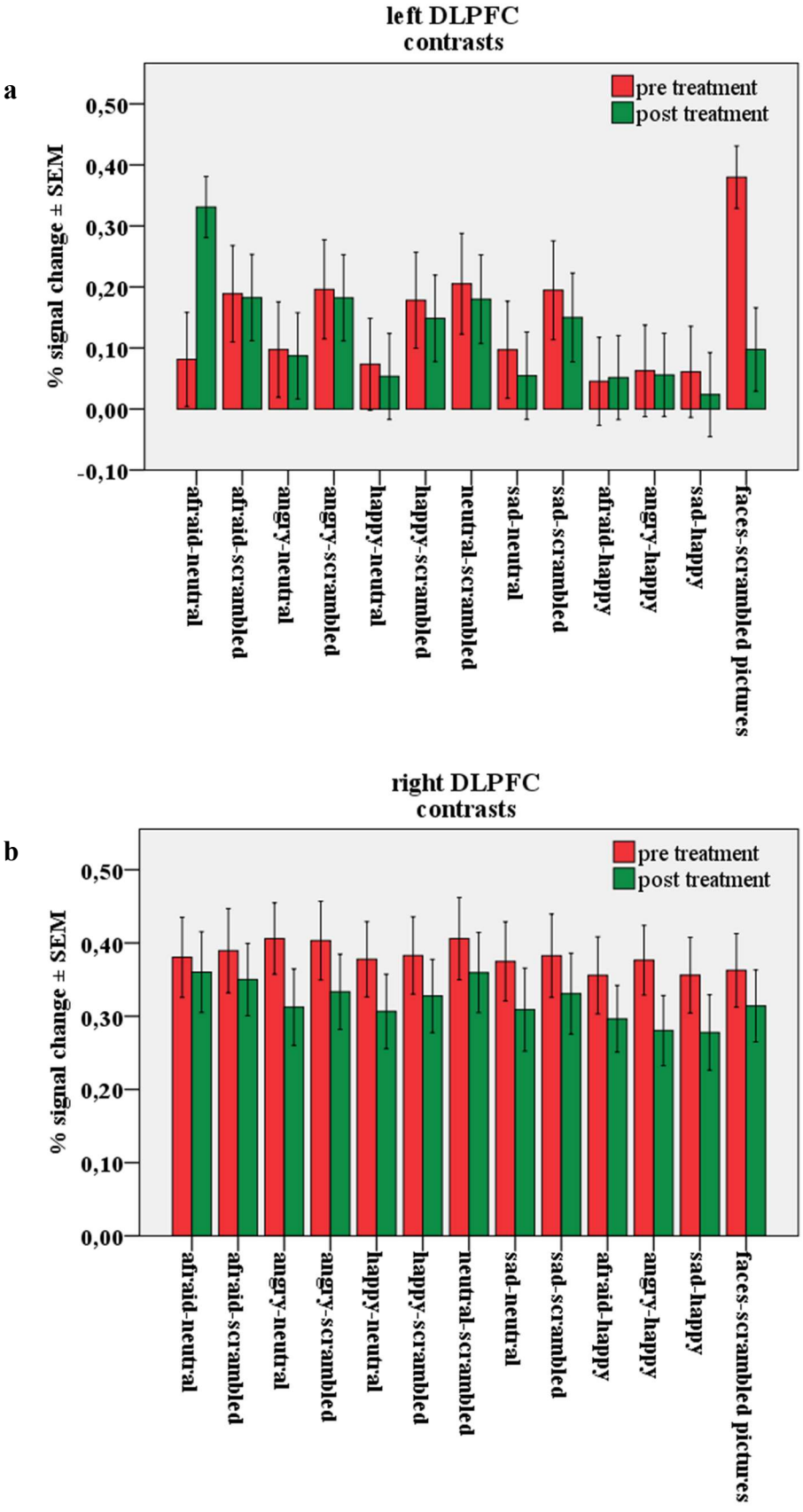

Figure 6: Mean BOLD % signal change ± SEM in the left (a) and right (b) DLPFC during the presentation of emotional faces. Contrasts between different emotions or contrasts between emotions and neutral faces or scrambled pictures before and after treatment.

3.2.1.3. *BOLD % signal change in the left and right fusiform gyrus*

The BOLD % signal change in the left and right fusiform gyrus of MDD patients during the presentation of all facial (afraid, angry, happy, sad and neutral) expressions after short term treatment in contrast to baseline was reduced. The reduction was statistically significant in the right fusiform gyrus after neutral faces in contrast to baseline (rmANOVA: $F [1, 32] = 4.41$, $p < 0.044$).

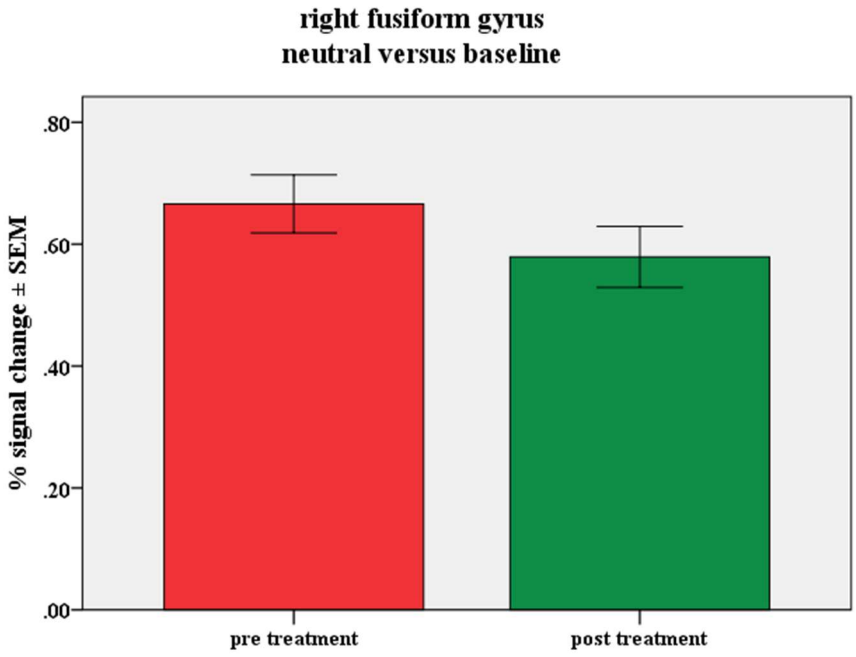

Figure 7: Statistically significant BOLD % signal change reduction after treatment in the right fusiform gyrus during the presentation of neutral faces versus baseline.

Furthermore, there were the following statistically significant time effects after short term treatment in the left fusiform gyrus during the presentation of afraid in contrast to neutral faces (rmANOVA: $F [1, 32] = 35.53$, $p < 0.001$), and all emotional faces in contrast to scrambled pictures (rmANOVA: $F [1, 32] = 31.97$, $p < 0.001$).

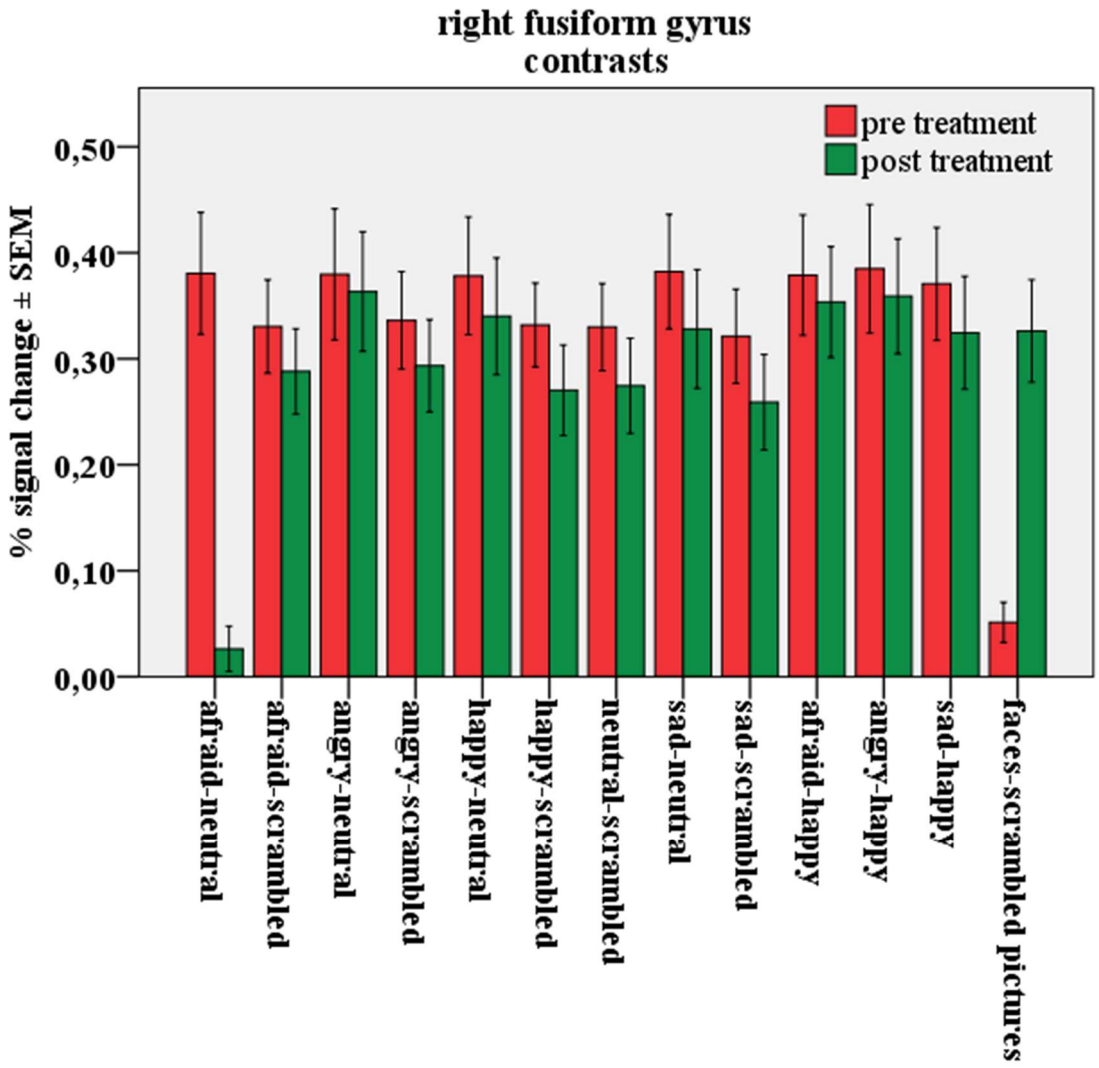

Figure 8: Mean BOLD % signal change ± SEM in the right fusiform gyrus during the presentation of emotional faces. Contrasts between different emotions or contrasts between emotions and neutral faces or scrambled pictures before and after treatment.

3.2.1.4. BOLD %signal change in the left and right hippocampus

The BOLD % signal change in post fMRI sessions was increased in the left hippocampus during presentation of afraid, angry, and neutral faces and scrambled pictures. The same was true in the right hippocampus during the presentation afraid, happy, and sad faces as well as scrambled pictures. Furthermore, the % signal change was reduced in the left hippocampus during presentation of happy and sad faces. In the right hippocampus % signal change were decreased during the presentation of angry and neutral faces (all conditions in contrast to baseline).

3.2.1.5. BOLD %signal change in the left and right insula

The % signal change were reduced in the left and right insula during the presentation of all facial (angry, happy and neutral) expression and scrambled picture except afraid compare to baseline after short term treatment.

3.2.2. Peak activation and exploratory whole brain analysis in all MDD patients before and after treatment

Peak activation clusters were revealed by calculating contrasting responses to all faces or scrambled pictures versus baseline, all faces versus scrambled pictures, all emotional faces versus neutral faces and all negative emotional faces (afraid, angry, and sad) versus happy faces. The calculations were done with the fMRI data of the pre-treatment, and the post-treatment sessions.

Table 3: The peak voxel activation according to the whole brain analysis were exploratorily assed and the results were shown in the table below ($p < 0.05$ with FEW adjustment, the cluster with the global maxima were chosen and overlaid on the MNI template, and labelled by using the MNI coordinates in SPM12.

Session (group)	Condition	Volume	t-test	MNI peaks X -Y-Z	Anatomic region
pre	afraid	1565	13.84	-3, -37, 38	left posterior cingulate gyrus (PCgG)
post	afraid	576	13.66	6, -40, 44	right precuneus (PCu)
pre	angry	895	14.21	-9, 38, -4	left anterior cingulate gyrus
post	angry	2077	13.28	-9, -37, 38	left posterior cingulate gyrus
pre	happy	2376	14.40	0, -40, 44	left posterior cingulate gyrus (PCgG)
post	happy	3280	12.83	0, -40, 44	left posterior cingulate gyrus (PCgG)
pre	neutral	1917	12.54	0, -40, 44	left posterior cingulate gyrus (PCgG)
post	neutral	2091	12.09	-6, -40, 41	left posterior cingulate gyrus (PCgG)
pre	sad	1868	11.97	0, -40, 44	left posterior cingulate gyrus (PCgG)
post	sad	2571	13.20	0, -43, 44	left precuneus (PCu)
pre	scrambled	1232	14.03	0, -61, 26	left precuneus (PCu)
post	scrambled	219	13.23	-51, -70, 26	left angular gyrus (AnG)
pre	afraid > scrambled	2332	12.90	-42, -40, 44	left supramarginal gyrus
post	afraid > scrambled	936	12.59	-33, -46, 44	left superior parietal lobe
pre	angry > scrambled	2113	13.30	45, -34, 44	right supramarginal gyrus
post	angry > scrambled	1101	13.18	-42, -43, 44	left supramarginal gyrus
pre	happy > scrambled	2400	13.63	48, -34, 41	right supramarginal gyrus
post	happy > scrambled	3124	15.00	-39, -43, 41	left supramarginal gyrus
pre	neutral > scrambled	426	14.69	27, 2, 53	right middle frontal gyrus
post	neutral > scrambled	1112	13.74	-33, -46, 44	left superior parietal lobe
pre	sad > scrambled	2062	12.67	48, -34, 44	right supramarginal gyrus
post	sad > scrambled	2875	13.83	-24, -70, 35	left superior parietal lobe
pre	faces > scrambled	2490	13.48	48, -34, 41	right supramarginal gyrus
post	faces > scrambled	2476	14.66	-33, -46, 44	left superior parietal lobe

For each cluster, voxel volume, maximum t-test value, voxel coordinates, and the main structures included in the cluster and only the peak activation were mentioned. For all results the height threshold was T = 5.451732 [p < 0.05t (FEW)], the extent threshold was k = 0 voxels.

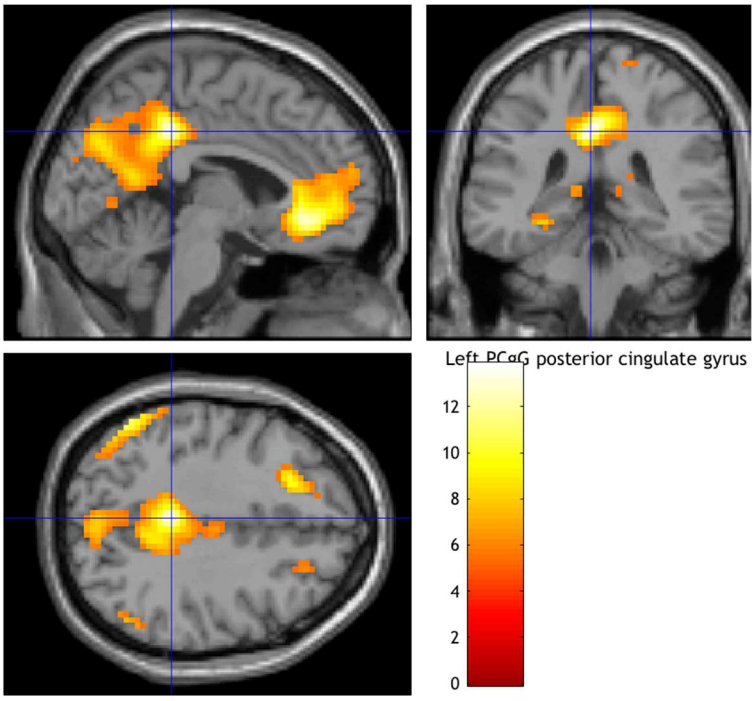

Figure 9: Left posterior cingulate gyrus (-3 -37 38), T: 13.48, KE: 1565; Peak activation (global maxima) in MDD patients before treatment during the presentation of afraid facial pictures compared to baseline.

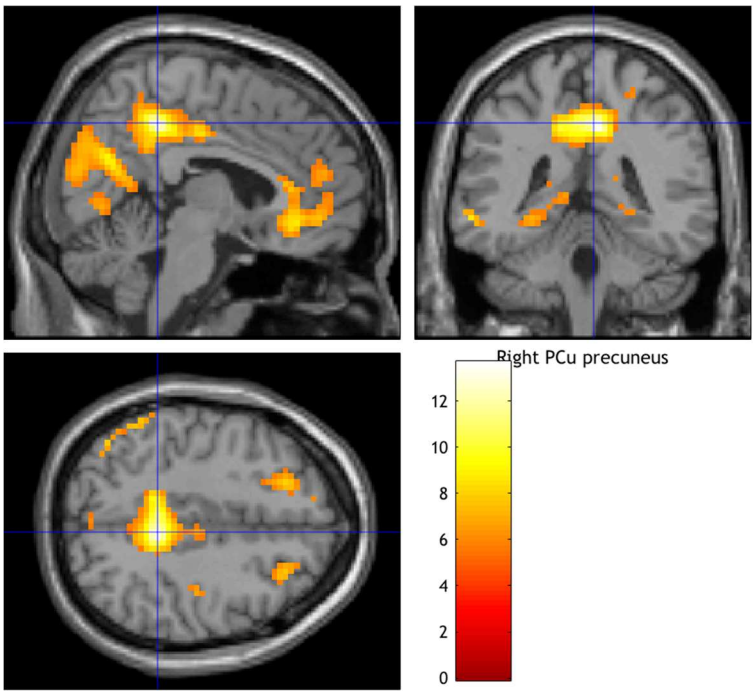

Figure 10: Right precuneus (PCu) (6, -40, 44), T:13.66, KE: 576; Peak activation (global maxima) in MDD patients after treatment during the presentation of afraid facial pictures compared to baseline.

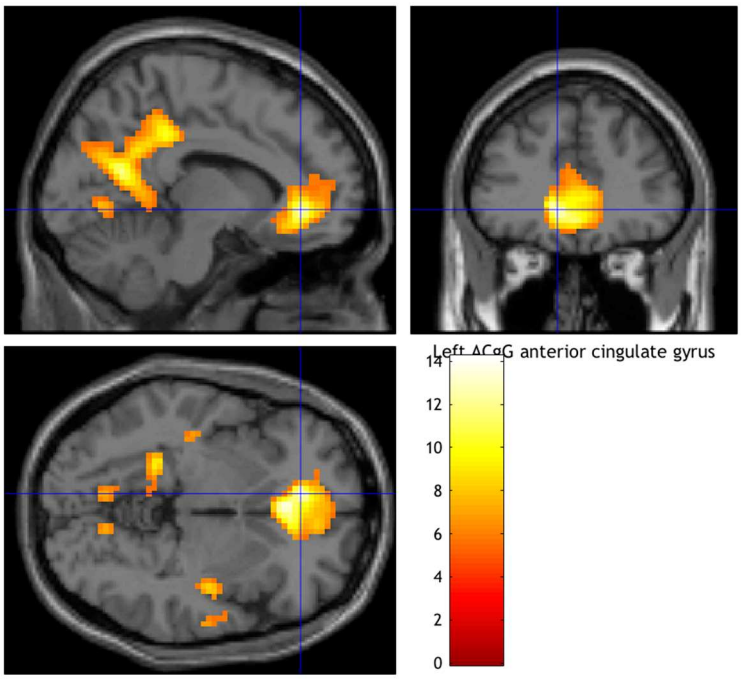

Figure 11: Left anterior cingulate gyrus (-9 38 -4), T: 13.31, KE: 865; Peak activation (global maxima) in MDD patients before treatment during the presentation of angry facial pictures compared to baseline.

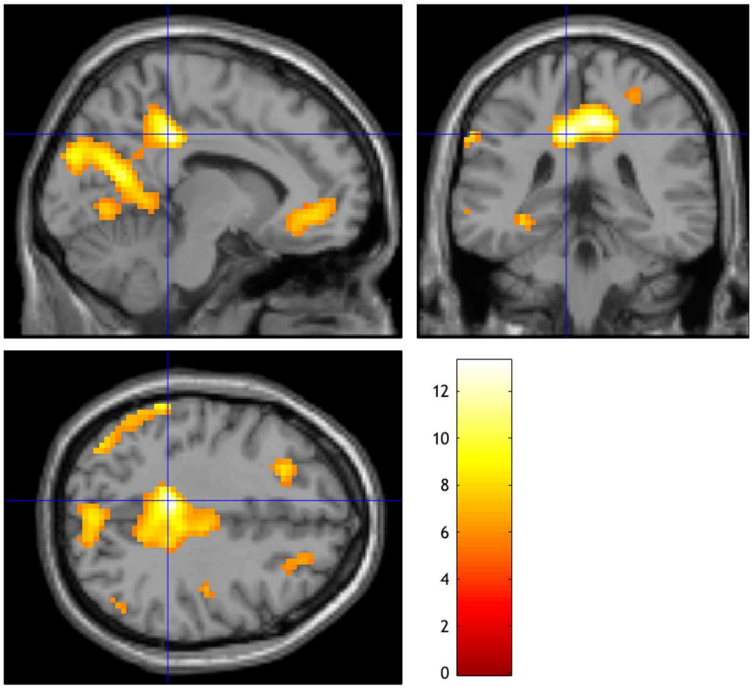

Figure 12: Left posterior cingulate gyrus (-9, -37, 38), T: 13.28, KE: 2077; peak activation (global maxima) in MDD patients after treatment during the presentation of angry facial pictures compared to baseline.

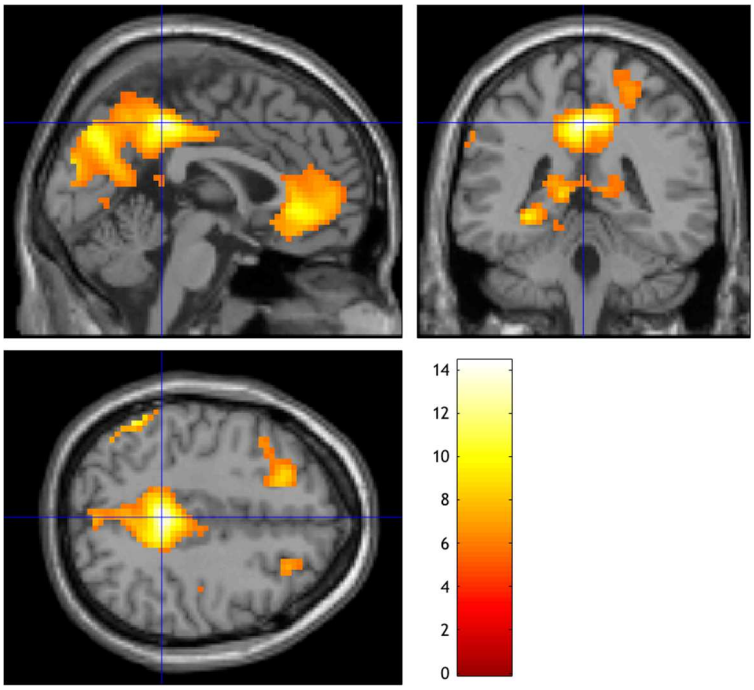

Figure 13: Left posterior cingulate gyrus (0 -40 44), T: 14.40, KE: 2376; Peak activation (global maxima) in MDD patients before treatment during the presentation of happy facial pictures compared to baseline.

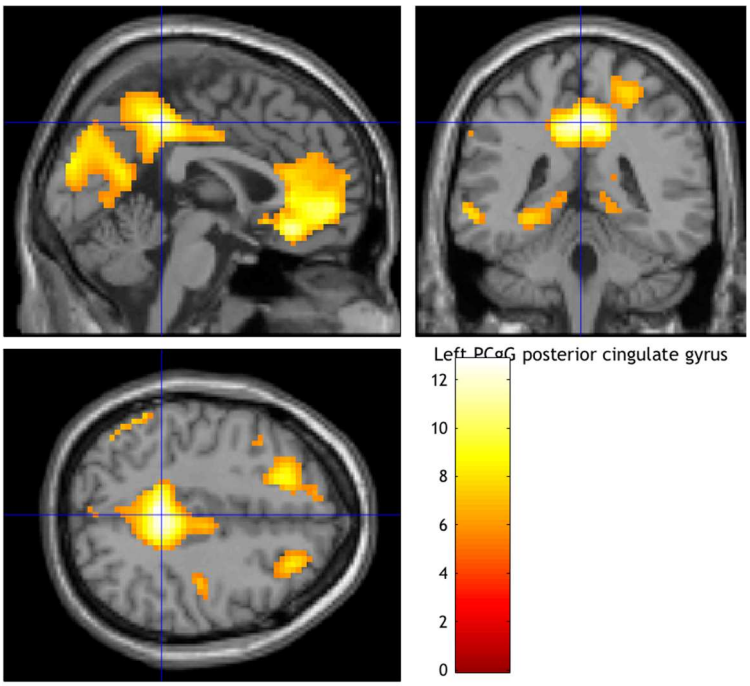

Figure 14: Left posterior cingulate gyrus (0 -40 44), T: 12.83, KE: 3280; Peak activation (global maxima) in MDD patients after treatment during the presentation of happy facial pictures compared to baseline.

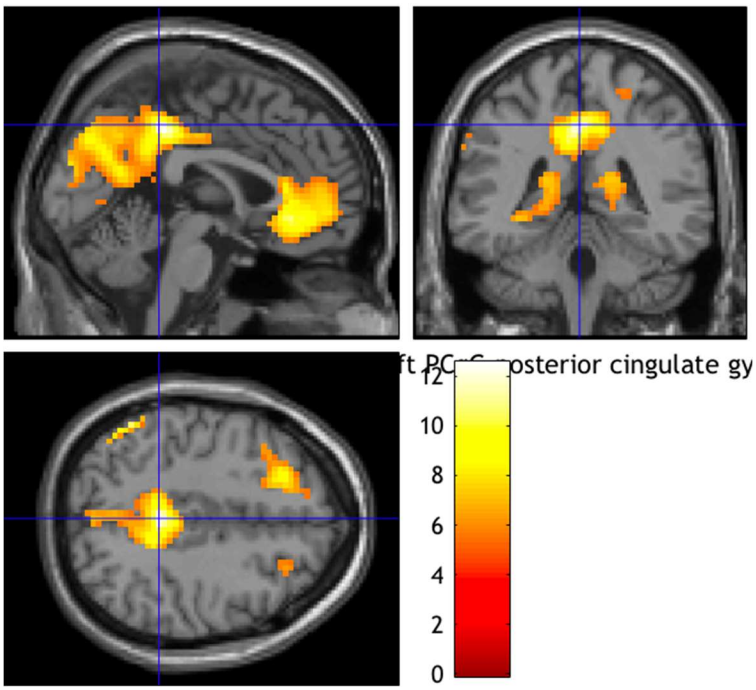

Figure 15: Left posterior cingulate gyrus (0 -40 44), T: 12.54, KE: 1917; Peak activation (global maxima) in MDD patients before treatment during the presentation of neutral facial pictures compared to baseline.

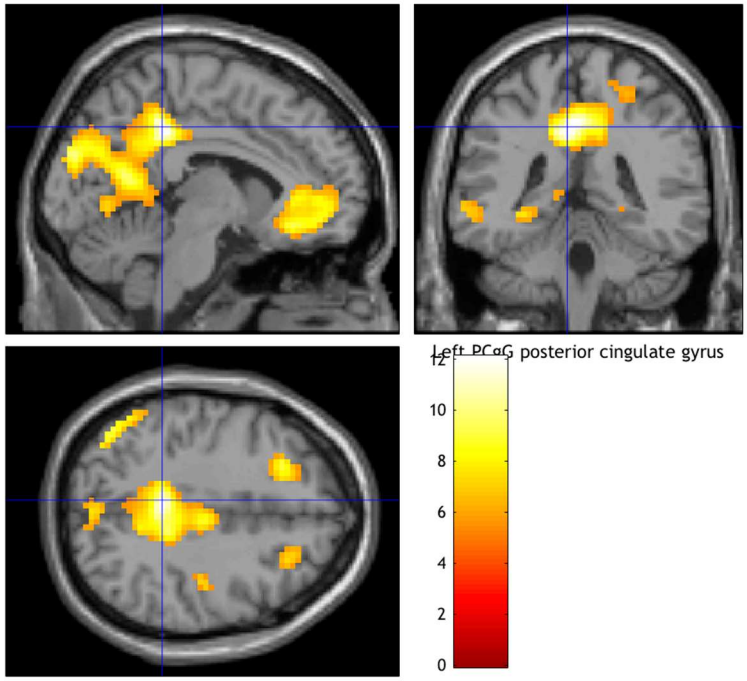

Figure 16: Left posterior cingulate gyrus (-6 -40 41), T: 12.09, KE: 2091; Peak activation (global maxima) in MDD patients after treatment during the presentation of neutral facial pictures compared to baseline.

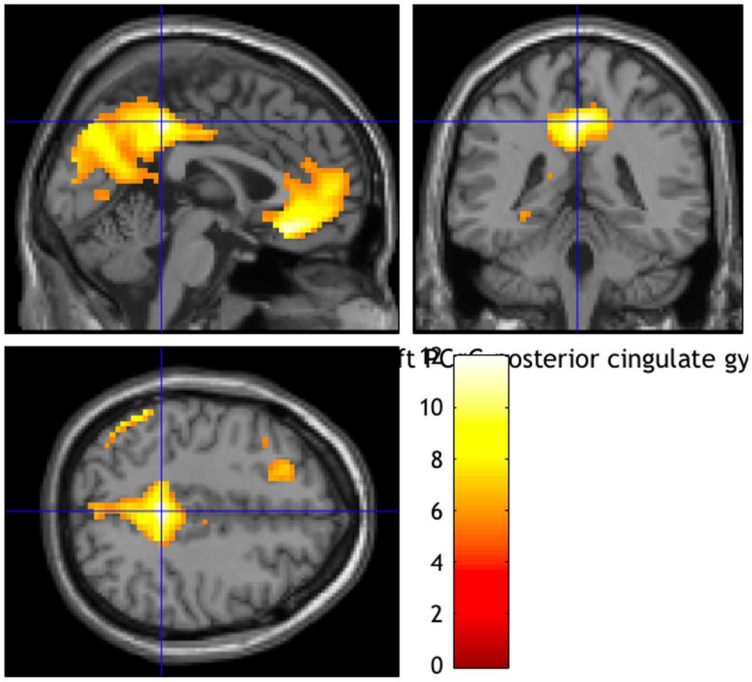

Figure 17: Left posterior cingulate gyrus (0 -40 44), T: 11.97, KE: 1868; Peak activation (global maxima) in MDD patients before treatment during the presentation of sad facial pictures compared to baseline.

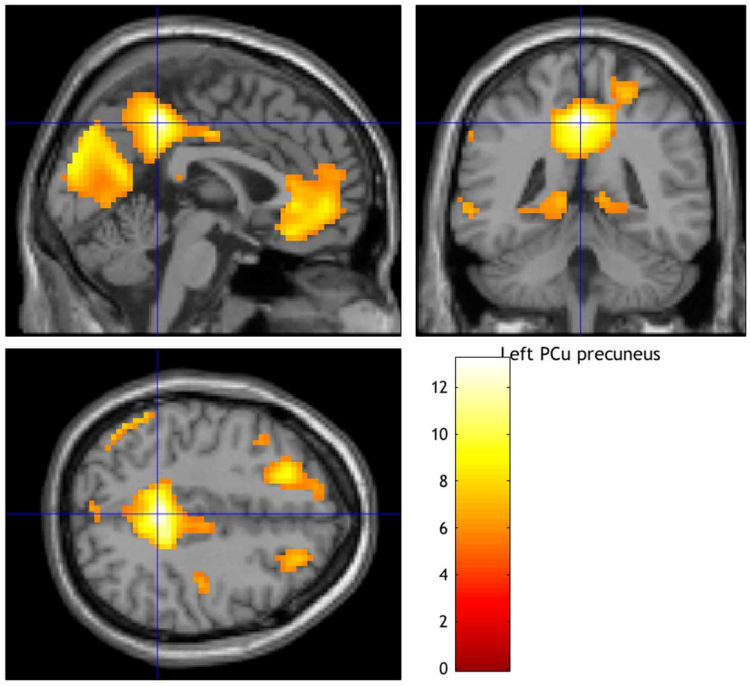

Figure 18: Left precuneus gyrus (0 -43 44), T: 13.20, KE: 2571; Peak activation (global maxima) in MDD patients after treatment during the presentation of sad facial pictures compared to baseline.

3.3. BOLD % signal change in MDD patients after short term treatment with antidepressants or placebo

3.3.1. BOLD % signal change in the different ROIs of MDD patients: comparison of two treatment groups receiving antidepressants in comparison to placebo

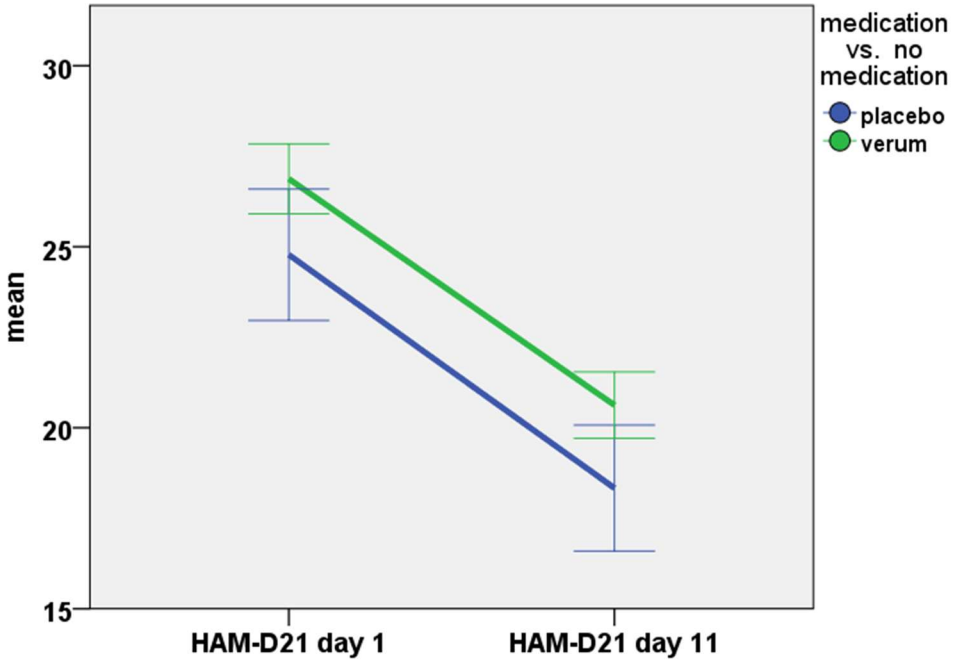

Figure 19: Clinical ratings (HAM-D21 scores), all medication (verum) treatment groups summarized (n = 24) vs. placebo (n = 9) before and after the fMRI scans (error bars ± SEM).

3.3.1.1. BOLD % signal change in the left and right amygdala

There was a statistically significant BOLD % signal change reduction after one week of short term treatment in the left amygdala during the presentation of angry (F [1, 32] = 4.73, p<0.037), happy (F [1, 32] = 4.76, p = 0.037), neutral (F [1, 32] = 5.89, p = 0.021), and sad (F [1, 32] = 11.36, p = 0.002) facial expressions compared to baseline. The same was true for the right amygdala during presentation of happy (F [1, 32] = 6.58, p = 0.015), neutral (F [1, 32] = 6.12, p = 0.019), and sad (F [1, 32] = 5.28, p = 0.029) faces compared to baseline in both patient groups, the patients treated with agomelatine, escitalopram or mirtazapine and the placebo treated patient. There were no statistically significant differences between both treatment groups.

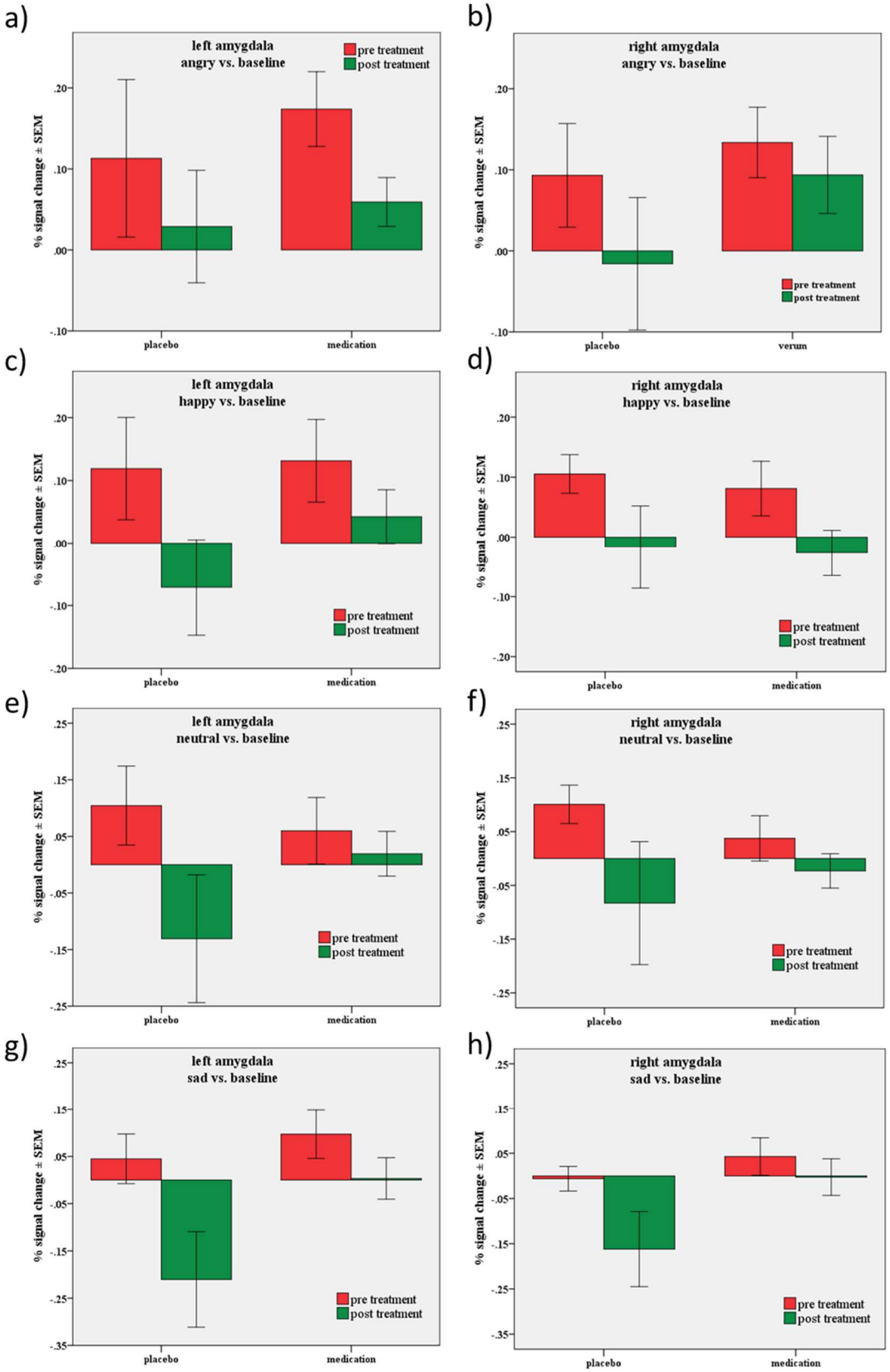

Figure 20: Statistically significant BOLD % signal change after treatment in the left and right amygdala during the presentation of emotional faces (a: angry; c, d: happy, e, f: neutral; g, h: sad) before and after treatment in both treatment groups (patients receiving antidepressants or placebo). In the right amygdala angry vs. baseline showed a nonsignificant trend (b).

The following statistically significant time effects were detected in the left amygdala after short term treatment with antidepressants or placebo during the presentation of afraid vs. neutral faces (rmANOVA: F [1, 32] = 5.56, p = 0.025), angry vs. neutral face (rmANOVA: F [1, 32] = 5.56, p = 0.025), happy vs. neutral faces (rmANOVA: F [1, 32] = 5.84, p = 0.022), sad vs. neutral faces (rmANOVA: F [1, 32] = 11.30, p = 0.002), sad faces vs. scrambled pictures (rmANOVA: F [1, 32] = 6.13, p = 0.019), angry vs. happy faces (rmANOVA: F [1, 32] = 6.67, p = 0.015), sad vs. happy faces (rmANOVA: F [1, 32] = 9.25, p = 0.005), and all faces vs. scrambled pictures (rmANOVA: F [1, 32] = 8.33, p = 0.007).

In addition, in the right amygdala the same was true for the contrasts between afraid and neutral faces (rmANOVA: F [1, 32] = 5.20, p = 0.030), angry and neutral faces (rmANOVA: F [1, 32] = 5.92, p = 0.021), angry faces and scrambled pictures (rmANOVA: F [1, 32] = 5.55, p = 0.041), happy and neutral faces (rmANOVA: F [1, 32] = 8.53, p = 0.006), happy faces and scrambled pictures (rmANOVA: F [1, 32] = 7.46, p = 0.010), neutral faces and scrambled pictures (rmANOVA: F [1, 32] = 5.95, p = 0.021), sad and neutral faces (rmANOVA: F [1, 32] = 7.83, p=0.009), sad faces and scrambled pictures (rmANOVA: F [1, 32] = 5.45, p = 0.026), angry and happy faces (rmANOVA: F [1, 32] = 7.44, p = 0.010), sad and happy faces (rmANOVA: F [1, 32] = 7.96, p = 0.008), and all faces together compared to scrambled pictures (rmANOVA: F [1, 32] = 8.76, p = 0.006). But there were no significant group effects between the patients using antidepressant medication vs. the placebo treated patients in any one of the contrast.

3.3.1.2. BOLD % signal change in the left and right DLPFC

In the right DLPFC the BOLD % signal change was significantly reduced during the presentation of angry facial pictures (rmANOVA: F [1, 32] = 10.35, p = 0.003) after one week treatment (medication and placebo), but again, significant differences between antidepressant treated patients and the placebo group could not be detected.

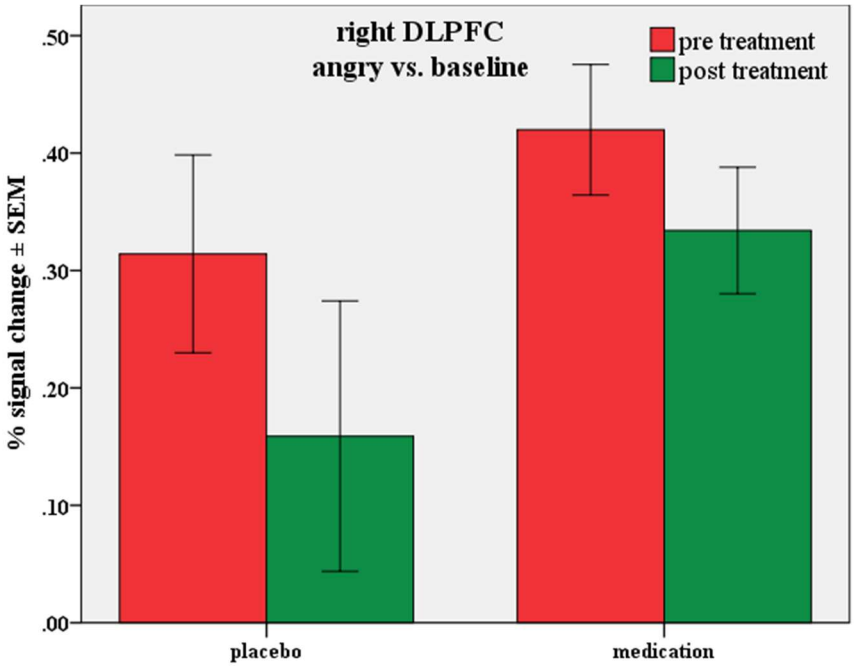

Figure 21: Statistically significant BOLD % signal change after treatment in the right DLPFC during the presentation of angry faces before and after treatment in both treatment groups (patients receiving antidepressants or placebo).

3.3.1.3. BOLD % signal change in the left and right fusiform gyrus

In the left fusiform gyrus the BOLD % signal change in contrast to baseline significantly was significantly different during the presentation of sad faces (rmANOVA: F [1, 32] = 4.25, p = 0.048) after the treatment, the same change could be detected in the right fusiform gyrus during the presentation of neutral faces (rmANOVA: F [1, 32] = 4.25, p = 0.048) and after sad facial expressions (rmANOVA: F [1, 32] = 4.69, p = 0.038) after one week of short term treatment. Here, we could detect a statistically significant difference between the two treatment groups (rmANOVA time * medication: F [1, 32] = 4.62, p = 0.040), because only in the placebo group a reduced BOLD % signal change was detected.

Again, there was no statistically significant difference in other ROIs.

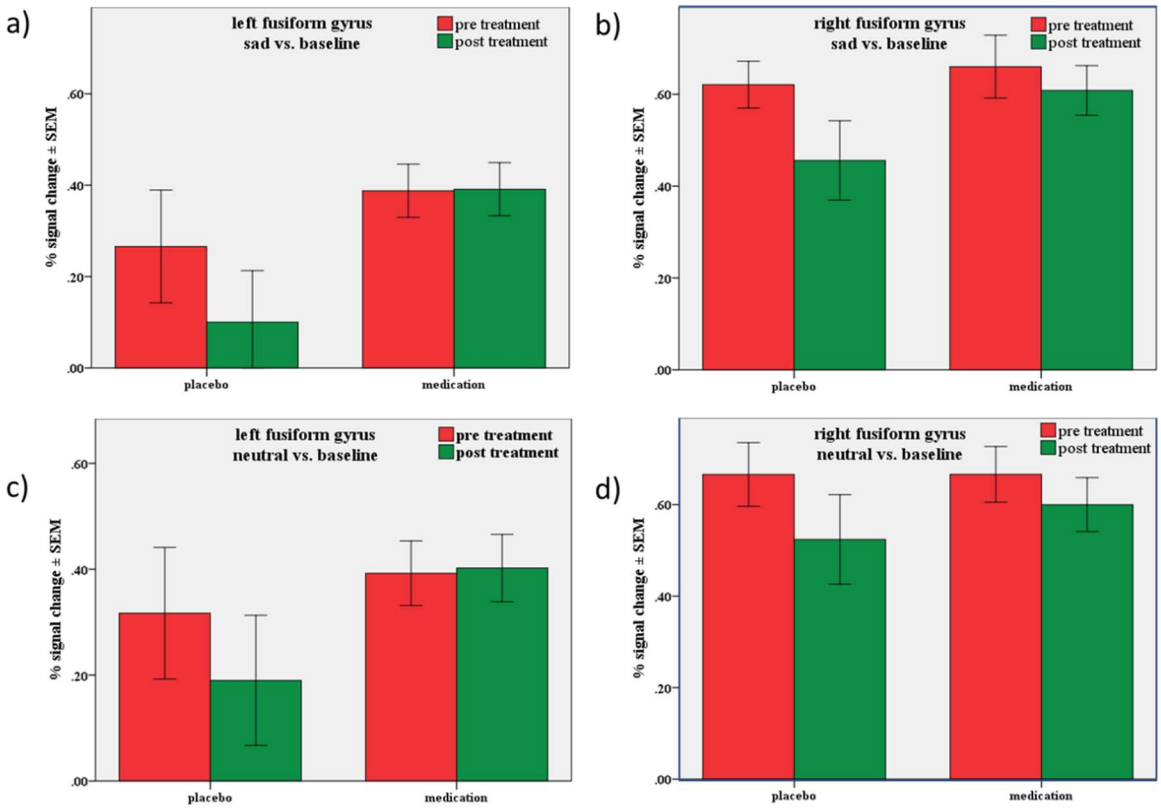

Figure 22: Statistically significant BOLD % signal change after treatment in the fusiform gyrus on both sides during the presentation of sad (a, b) and on the left side during the presentation of neutral faces (c) before and after treatment in placebo treated patients. All other differences were not statistically significant (a-d, right side, and d).

In the left fusiform gyrus the following statistically significant time effects after short term treatment were detected during the presentation of afraid vs. neutral faces (rmANOVA: F [1, 32] = 26.86, p = 0.000013), neutral faces vs. scrambled pictures (rmANOVA: F [1, 32] = 4.18, p = 0.050), sad vs. neutral faces (rmANOVA: F [1, 32] = 5.08, p = 0.031), sad faces vs. scrambled pictures (rmANOVA: F [1, 32] = 4.20, p = 0.049, and all faces together vs. scrambled pictures (rmANOVA: F [1, 32] = 21.23, p = 0.000006). No significant effects due to antidepressant medication or placebo could be detected.

3.3.1.4. BOLD % signal change in the left and right insula

In the left insula of MDD patients statistically significant time effects in the BOLD % signal change was detected during the presentation of angry vs neutral faces (rmANOVA: F [1, 32] = 4.56, p = 0.041) and neutral faces in contrast to scrambled pictures (rmANOVA: F [1, 32] = 4.56, p = 0.041). No significant effects of medication or placebo were detected.

3.3.2. BOLD % signal change in different ROIs of MDD patients: comparison of four treatment groups receiving agomelatine, escitalopram, mirtazapine or placebo

In the next step the patients of all four treatment groups were analyzed separately to detect significant group effects of the three medication groups or the placebo group. Figure 23 is demonstrating the clinical development of all four treatment groups showing an overall significant reduction of the HAM-D21 sum scores (rmANOVA: $F\ [1, 32] = 48.4$, $p < 0.001$) without distinguishable significant differences between the treatment groups.

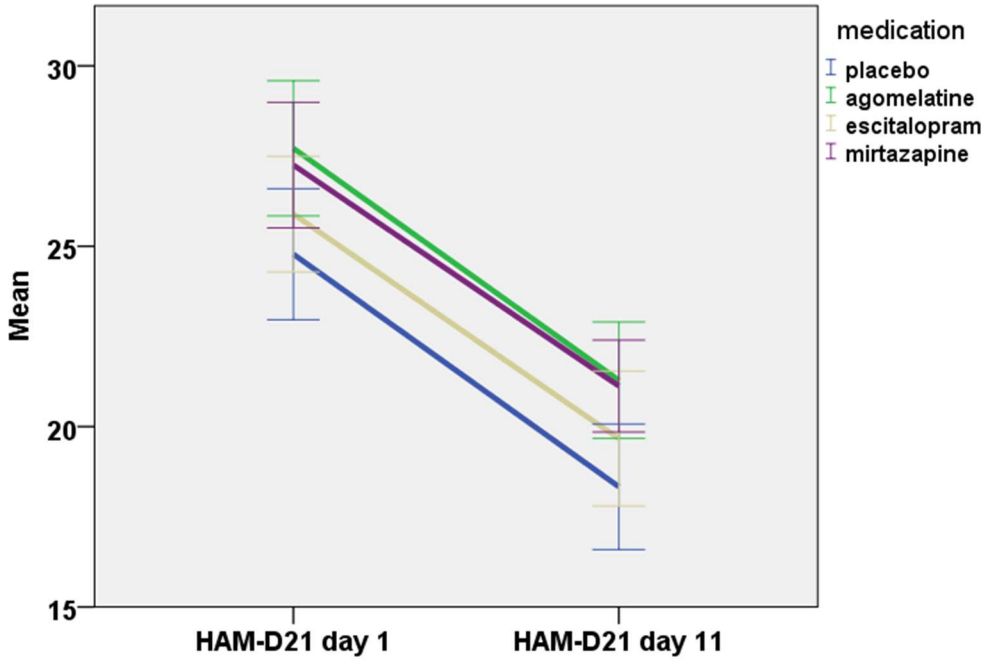

Figure 23: Clinical ratings (HAM-D21 scores) in all four treatment groups before and after the fMRI scans (error bars ± SEM)

3.3.2.1. BOLD % signal change in the left and right amygdala

In the next chapter the BOLD % signal change in response to facial expressions and scrambled pictures after the subdivision of 33 MDD patients in four treatment groups (agomelatine, escitalopram, mirtazapine, or placebo) is illustrated. In all four groups the BOLD % signal change was significantly reduced in the left amygdala during the presentation of angry (rmANOVA: $F\ [1, 32] = 6.33$, $p = 0.018$) and sad faces (rmANOVA: $F\ [1, 32] = 7.72$, $p = 0.009$). In addition, the BOLD % signal change was significantly reduced in the right amygdala during the presentation of happy facial pictures (rmANOVA: $F\ [1, 32] = 7.11$, $p = 0.012$). Amygdala BOLD response (mean ± standard error) to different pictures with emotional facial expressions in different conditions (emotional contrasts to baseline) in MDD patients. The conditions angry and sad were significantly different in the left amygdala and condition happy

in the right amygdala after short-term treatment with antidepressants or placebo. The medication group had no significant influence

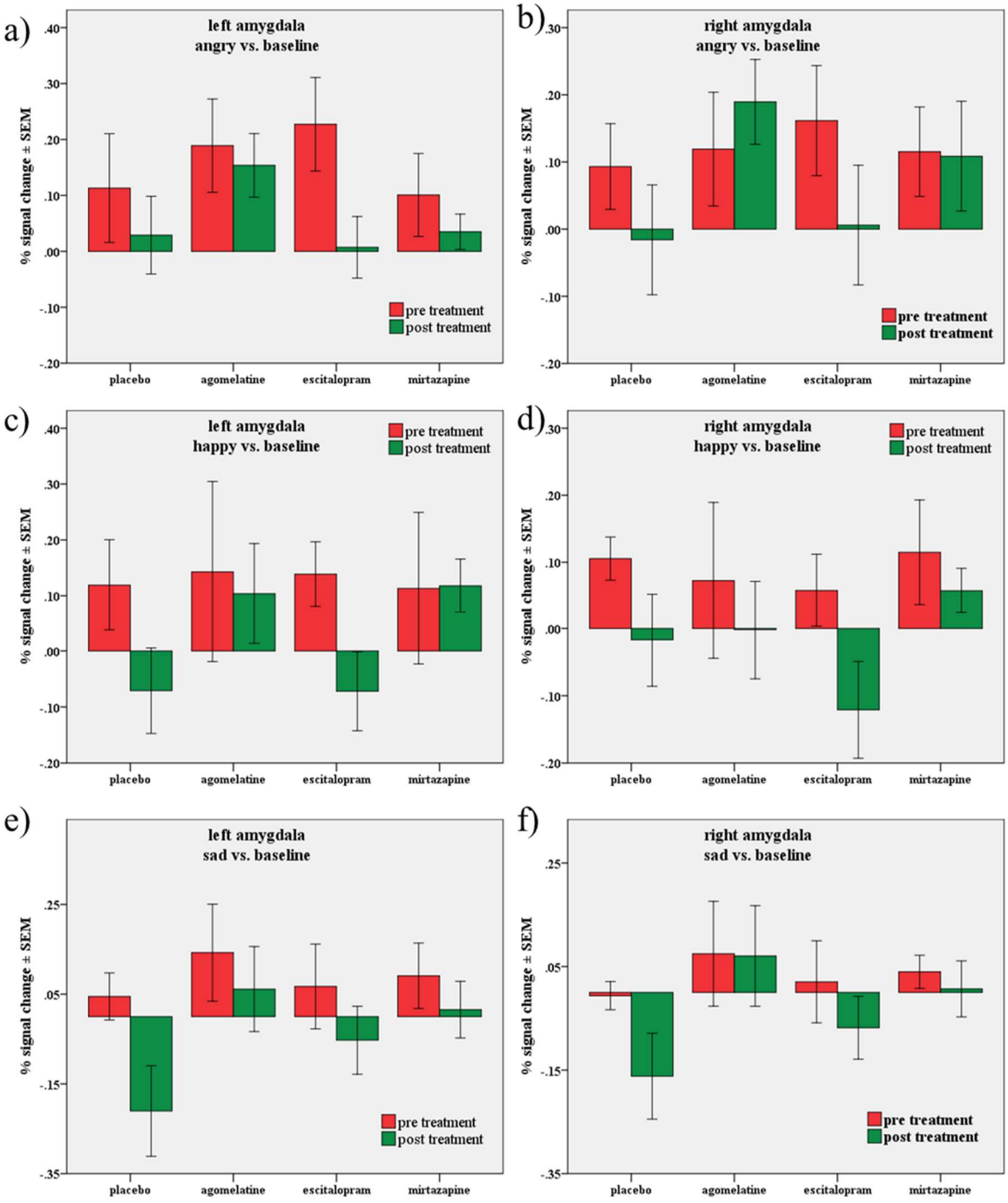

Figure 24: Amygdala BOLD response (mean ± standard error) to different pictures with emotional facial expressions in different conditions (emotional contrasts to baseline) in MDD patients. The conditions angry (a) and sad (e) were significantly different in the left amygdala and the condition happy (d) in the right amygdala after short-term treatment with antidepressants or placebo. The conditions displayed on b, c, f showed no statistically significant differences. Also, the medication group had no significant influence.

In the right amygdala of all four treatment groups during the presentation of angry compared to neutral faces (rmANOVA: F [1, 32] = 3.17, p = 0.086), angry faces in contrast to scrambled pictures (rmANOVA F [1, 32] = 4.52, p = 0.042), happy versus neutral faces (rmANOVA: F [1, 32] = 7.11, p = 0.012), happy faces compared to scrambled pictures (rmANOVA: F [1, 32] = 6.95, p = 0.013), and neutral faces in contrast to scrambled pictures (rmANOVA: F [1, 32] = 4.31, p = 0.047) significant time effects after one week of antidepressant treatment could be detected. The same was true in the left amygdala during presentation of sad faces versus scrambled pictures (rmANOVA: F [1, 32] = 4.31, p = 0.047). No statistically significant group effects due to three different antidepressants or placebo could be calculated.

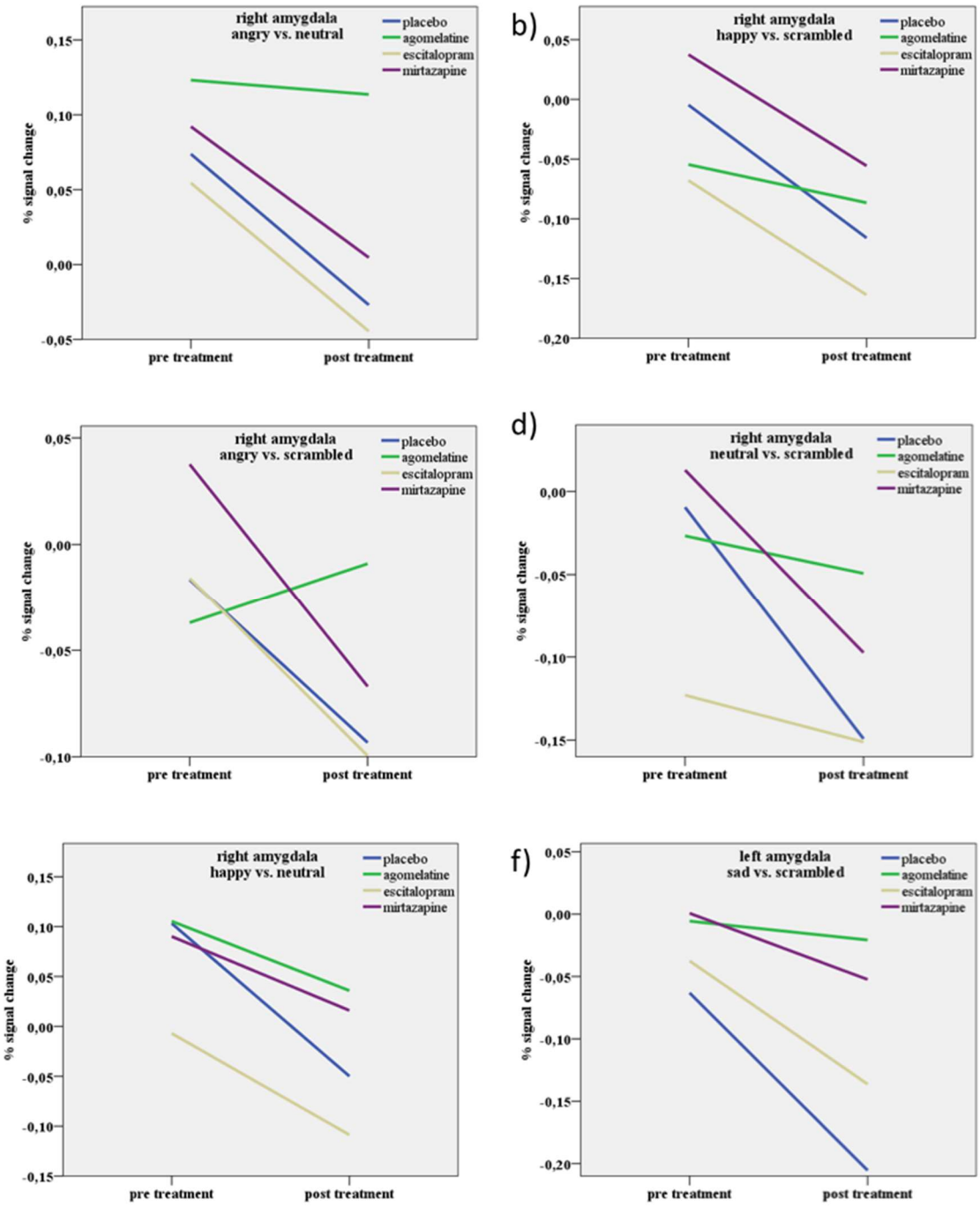

Figure 25: Statistically significant BOLD % signal change after treatment in the right amygdala during the presentation of angry vs. neutral faces (a), angry faces vs. scrambled pictures (b), happy vs. neutral faces (c), happy faces vs. scrambled pictures (d), and neutral faces vs. scrambled pictures could be detected. In the left amygdala there were significant changes during the presentation of sad faces vs. scrambled pictures.

In addition, there are significant time effects in the left and right amygdala of all four MDD patient groups during the presentation of sad versus neutral faces (left amygdala: rmANOVA: F [1, 32] = 6.46, p = 0.017; right amygdala: rmANOVA: F [1, 32] = 4.50, p = 0.043), angry compared to happy faces (left amygdala: rmANOVA: F [1, 32] = 5.50, p = 0.026; right

amygdala: rmANOVA: F [1, 32] = 8.71, p = 0.006), sad in contrast to happy faces (left amygdala: rmANOVA: F [1,32] = 6.33, p = 0.018, right amygdala: rmANOVA: F [1, 32] = 6.52, p = 0.016) and all facial expressions compared to scrambled pictures (left amygdala: rmANOVA: F [1, 32] = 5.30, p=0.029; right amygdala: rmANOVA: F [d1, 32] = 7.13, p = 0.012). In all contrasts no significant group effects (three different antidepressants or placebo) could be detected.

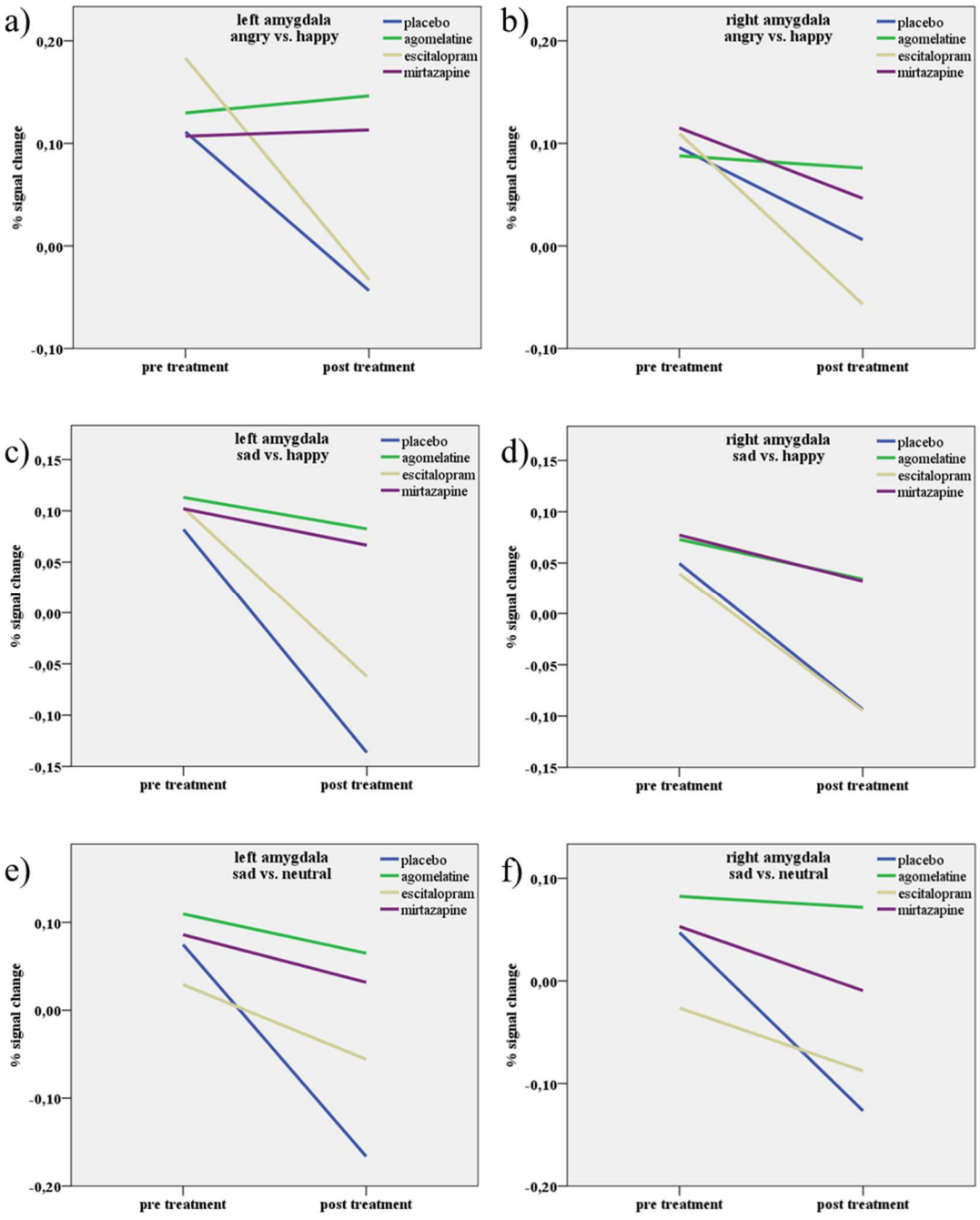

Figure 26: Statistically significant BOLD % signal change after treatment in the left and right amygdala during the presentation of angry vs. happy faces (a, b), sad vs. happy faces (c, d), and sad vs. neutral faces (e, f).

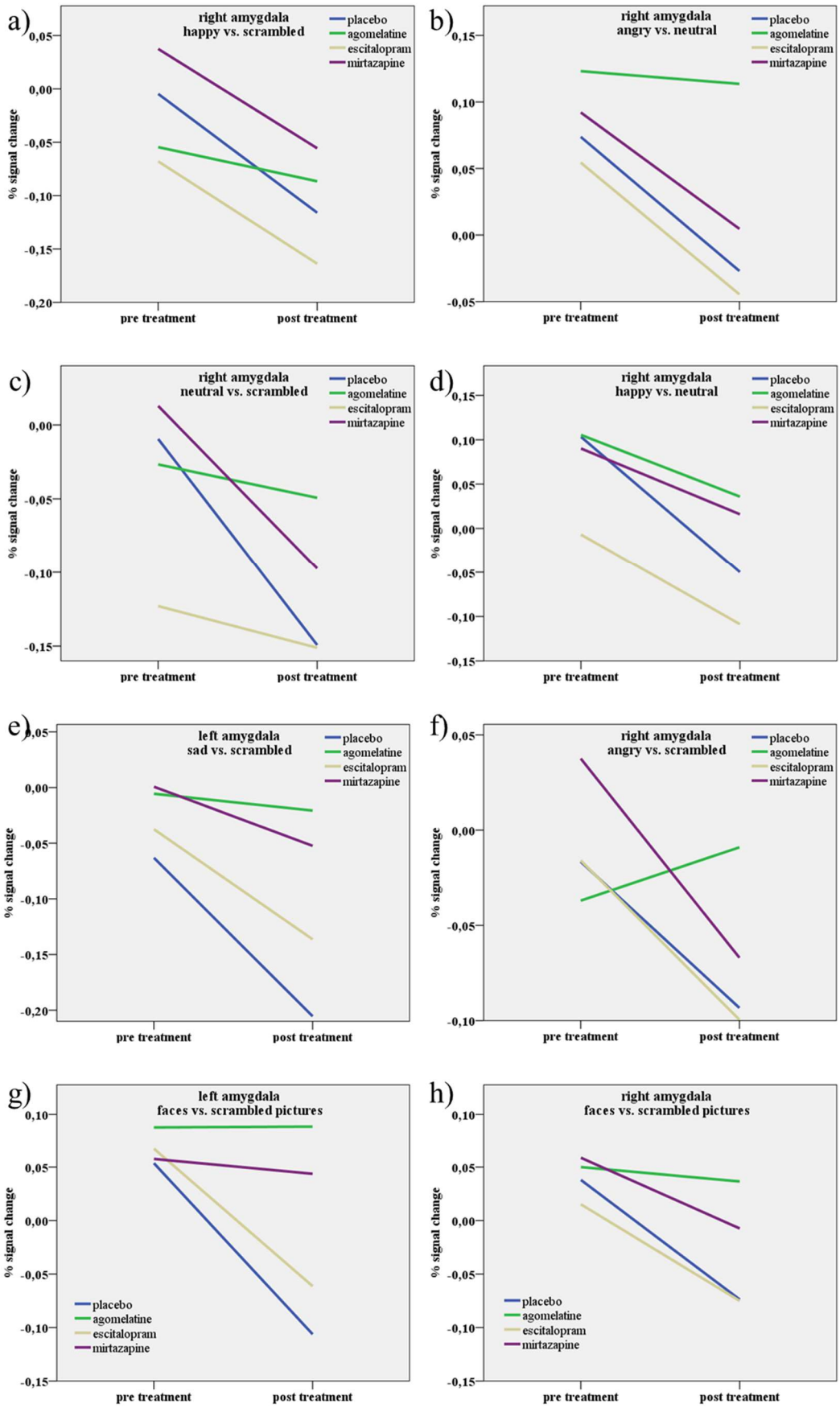

Figure 27: Statistically significant BOLD % signal change after treatment in the right amygdala during the presentation of happy (a), neutral (c), sad (e) and all (g) faces vs. scrambled pictures, angry (b) or happy (d) vs. neutral faces. In the left amygdala all faces vs. scrambled pictures showed a significant change (h).

3.3.2.2. *BOLD % signal change in the left and right DLPFC*

In the right DLPFC significantly reduced % signal change during the presentation of angry faces compared to baseline (rmANOVA: F [1, 32] = 7.11, p = 0.012) were detected. No further significant differences in any contrasts or on the left side could be detected, neither any significant differences between the four different medication groups.

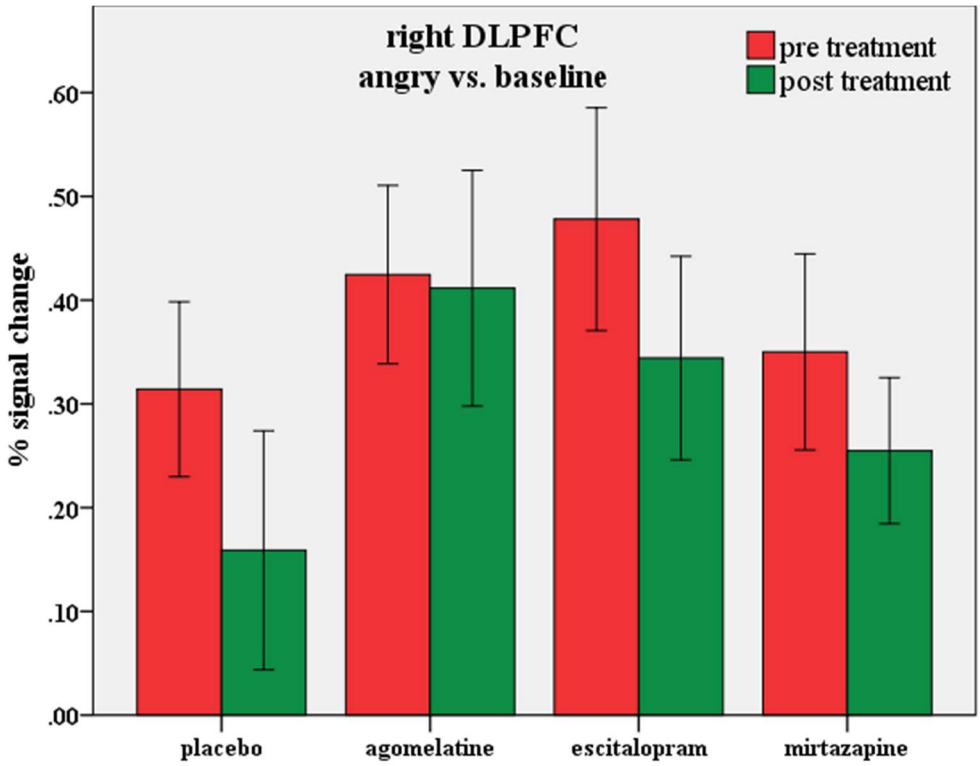

Figure 28: Statistically significant BOLD % signal change after treatment in the right DLPFC during the presentation of angry faces in contrast to baseline.

In the left DLPFC during the presentation of afraid compared to neutral faces (rmANOVA: F [1, 32] = 16.09, p = 0.00039) and during all emotional faces vs. scrambled pictures significant time effects were detected (rmANOVA: F [1, 32] = 31.38, p = 0.000081). Also in the right DLPFC during the presentation of angry in contrast to neutral faces (rmANOVA: F [1, 32] = 7.21, p = 0.012), angry vs. happy faces (rmANOVA: F [1, 32] = 9.58, p = 0.004) and sad vs. happy faces significant time effects were confirmed (rmANOVA: F [1, 32] = 6.20, p = 0.019). But again, no significant effects of the treatment with either one of the antidepressants or placebo could be seen.

3.3.2.3. BOLD % signal change in the left and right fusiform gyrus

In the left fusiform gyrus during presentation of afraid vs. neutral faces (rmANOVA: F [1, 32] = 35.74, p = 0.000002) and during the presentation of all faces in contrast to scrambled pictures significant time effects (rmANOVA: F [1, 32] = 39.16, p < 0.001) were confirmed. No significant effect of one of the four treatments were detected.

Notably, there are no statistically significant effects in the hippocampus and in the insula in all four tretment groups could be detected after short-term antidepressant treatment.

3.3.3. Correlation of the BOLD % signal change with the severity of depression (HAM-D21) in all ROIs and during all contrasts

A significant decrease in the sum score of the HAM-D21 depression rating scale could be detected in the mean values of all 33 investigated patients already after one week of treatment (figure 2). This was also true for the comparison between all medicated patients with the placebo treated patients (figure 19). Also after the subdivision in all three medicated treatment groups and the placebo treated patients similarly a progression was evident in all groups (figure 23). No clinically meaningful or statistically significant differentiation between the four treatment groups was possible after only one week of treatment. Possible correlations of the BOLD % signal change in different ROIs with the severity of depression measured using the HAM-D21 scale, were investigated using nonparametric and parametric correlations. First, correlations of the HAM-D21 sum score on day 1 with fMRI results (1st contrasts to baseline in all ROIs, 2nd contrasts between facial and scrambled pictures in all ROIs) of the first pre-treatment fMRI session were calculated, then correlations on day 11 with fMRI results of the second post-treatment fMRI session were calculated.

In the first step of this exploratory analysis the nonparametric Spearman's rank correlation coefficient (Spearman's rho) was calculated. Statistically significant correlations were described in the following section. All other correlations did not reach statistical significance ($p<0.05$).

The pre-treatment HAM-D21 sum score on day 1 was correlated with the BOLD % signal change on the first pretreatment fMRI in the right DLPFC during the presentation of angry faces vs. baseline (Spearman's correlation coefficient $\rho = 0.452$, p (two sided) = 0.008), in the right DLPFC during the presentation of neutral faces vs. baseline ($\rho = 0.392$, p = 0.024), and in the right fusiform gyrus during sad faces vs. baseline ($\rho = 0.353$, p = 0.044).

The HAM-D21 sum score after one week of treatment on day 11 was correlated with the BOLD % signal change on the second fMRI after one week of treatment in the left amygdala during the presentation of afraid faces vs. scrambled pictures ($\rho = 0.435$, $p = 0.011$).

In the second step of the exploratory analysis the Kolmogorov-Smirnov (K-S)-test was used to test for normal distribution (the significance level was set to $p < 0.05$). Not normally distributed were the following variables (BOLD % signal change) before treatment: right DLPFC (afraid faces vs. baseline), right insula (afraid faces vs. baseline), left DLPFC (angry faces vs. baseline), left DLPFC (neutral faces vs. baseline), and left DLPFC (scrambled vs. baseline). Also not normally distributed were the following variables after one week of treatment: right amygdala (happy faces vs. baseline), left DLPFC (happy faces vs. baseline), left hippocampus (happy faces vs. baseline), right hippocampus (happy faces vs. baseline), left DLPFC (sad faces vs. baseline), left hippocampus (sad faces vs. baseline), right hippocampus (sad faces vs. baseline), and left DLPFC (scrambled pictures vs. baseline).

These variables were excluded from the following analysis using the parametric Pearson correlation coefficient (Pearson's r) which can only be used with normally distributed variables. Statistically significant positive correlations of the BOLD % signal change with the HAM-D21 score pre-treatment could be identified in the following normally distributed variables: right DLPFC angry faces vs. baseline (correlation coefficient $r = 0.366$, $p = 0.036$), right DLPFC neutral faces vs. baseline ($r = 0.367$, $p = 0.036$) and in the left amygdala scrambled pictures vs. baseline ($r = 0.354$, $p = 0.043$). The same was true for the positive correlation of the HAM-D21 score with the BOLD % signal change in the left amygdala afraid faces vs. baseline ($r = 0.378$, $p = 0.030$). In these regions higher BOLD % signal changes were correlated with more severe depression.

Investigating the correlations of 13 differential contrasts with the severity of depression using the nonparametric Spearman's rank correlation coefficient revealed the following positive and statistically significant correlations with the first pretreatment fMRI results: left DLPFC afraid vs. neutral faces (correlation coefficient $\rho = 0.383$, $p = 0.028$), right DLPFC afraid- vs. neutral faces ($\rho = 0.370$, $p = 0.034$, left DLPFC angry vs. neutral faces ($\rho = 0.420$, $p = 0.015$), right DLPFC angry vs. neutral faces ($\rho = 0.429$, $p = 0.013$), right DLPFC angry faces vs. scrambled pictures ($\rho = 0.434$, $p = 0.012$), left DLPFC happy vs. neutral faces ($\rho = 0.365$, $p = 0.037$), right DLPFC happy vs. neutral faces ($\rho = 0.385$, $p = 0.027$), right DLPFC happy faces vs. scrambled pictures ($\rho = 0.363$, $p = 0.038$), right DLPFC neutral faces vs. scrambled pictures ($\rho = 0.400$, p

= 0.021), left DLPFC sad vs. neutral faces (ρ = 0.412, p = 0.017), right DLPFC sad vs. neutral faces (ρ = 0.394, p = 0.023), right DLPFC sad faces vs. scrambled pictures (ρ = 0.412, p = 0.017), left DLPFC afraid- vs. happy faces (ρ = 0.370, p = 0.034), left DLPFC angry vs. happy faces (ρ = 0.413, p = 0.017), right DLPFC angry vs. happy faces (ρ = 0.452, p = 0.008), left DLPFC sad vs. happy faces (ρ = 0.396, p = 0.022), right DLPFC sad vs. happy faces (ρ = 0.390, p = 0.025), and the left DLPFC faces vs. scrambled pictures (ρ = 0.408, p = 0.018).

The HAM-D21 sum score on the post-treatment day 11 was positively correlated with the BOLD % signal change on the second fMRI after one week of treatment in the following ROIs and during the following contrasts: left amygdala (afraid faces vs. scrambled pictures (correlation coefficient ρ = 0.384, p (two sided) = 0.027), and left amygdala afraid vs. happy faces (ρ = 0.349, p = 0.047).

The K-S test for normal distribution confirmed that the following variables (ROIs and contrasts) were not normally distributed before treatment and therefore excluded from the parametric correlative analysis: right amygdala (afraid vs. neutral faces), left DLPFC (afraid vs. neutral faces), left amygdala (angry vs. neutral faces), left DLPFC (angry vs. neutral faces), left amygdala (angry vs. neutral faces), left DLPFC (angry faces vs. scrambled pictures), left DLPFC (happy vs. neutral faces), left DLPFC (happy faces vs. scrambled pictures), left DLPFC (neutral faces vs. scrambled pictures), left DLPFC (sad vs. neutral faces), left hippocampus (sad vs. neutral faces), left DLPFC (sad faces vs. scrambled pictures), left hippocampus (sad faces vs. scrambled pictures), right hippocampus (afraid vs. happy faces), left hippocampus (afraid vs. happy faces), left amygdala (sad vs. happy faces), right amygdala (sad vs. happy faces), right DLPFC (sad vs. happy faces), and the left amygdala (faces vs. scrambled pictures) The same was true for the following post-treatment variables: right amygdala (afraid vs. neutral faces), left DLPFC (angry vs. neutral faces), right hippocampus (angry faces vs. scrambled pictures), left insula (angry faces vs. scrambled pictures), left DLPFC (happy vs. neutral faces), right DLPFC (happy vs. neutral faces), left hippocampus (happy vs. neutral faces), left DLPFC (happy faces vs. scrambled pictures), right hippocampus (happy faces vs. scrambled pictures), left DLPFC (neutral faces vs. scrambled pictures), right amygdala (sad vs. neutral faces), left hippocampus (sad vs. neutral faces), right hippocampus (sad vs. neutral faces), left DLPFC (sad faces vs. scrambled pictures), right hippocampus (sad faces vs. scrambled pictures), left DLPFC (afraid vs. happy faces), left hippocampus (afraid vs. happy faces), left DLPFC (angry vs. happy faces), left hippocampus (angry vs. happy faces), left DLPFC (sad vs. happy faces), right

DLPFC (sad vs. happy faces), left hippocampus (sad vs. happy faces), left DLPFC (faces vs. scrambled pictures), and the right DLPFC (faces vs. scrambled pictures).

Using Pearson's r in the remaining normally distributed variables the positive and statistically significant correlations of the BOD % signal change with the HAM-D21 score could be identified on the pre-treatment day 1 in the following ROIs and contrasts: right DLPFC afraid vs. neutral faces (correlation coefficient r = 0.366, p = 0.036), right DLPFC angry vs. neutral faces (r = 0.429, p = 0.013), right DLPFC angry faces vs. scrambled pictures (r = 0.390, p = 0.025), right DLPFC happy vs. neutral faces (r = 0.391, p = 0.025), right DLPFC happy faces vs. scrambled pictures (r = 0.366, p = 0.036), right DLPFC neutral faces vs. scrambled pictures (r = 0.388, p = 0.026), right DLPFC sad vs. neutral faces (r = 0.368, p = 0.035), right DLPFC sad faces vs. scrambled pictures (r = 0.356, p = 0.042), right DLPFC angry vs. happy faces (r = 0.401, p = 0.021), and the left DLPFC faces vs. scrambled pictures (r = 0.387, p = 0.026).

3.3.4. Correlation of the BOLD % signal change with the HPA-axis activity (cortisol levels) in all ROIs and during all contrasts

The subgrup of ten depressed patients investigated with the combined Dex/CRH-test under resting conditions on day 2 of the study before starting the antidepressant pharmacotherapy showed the typical profile of a marked cortisol stimulation after administration of CRH at 3:30 pm. After one week of treatment, on day 10 of the study, the cortisol secretion was blunted slightly without showing statistically significant differences in comparison to the secretion profile on day 2 (figure 29).

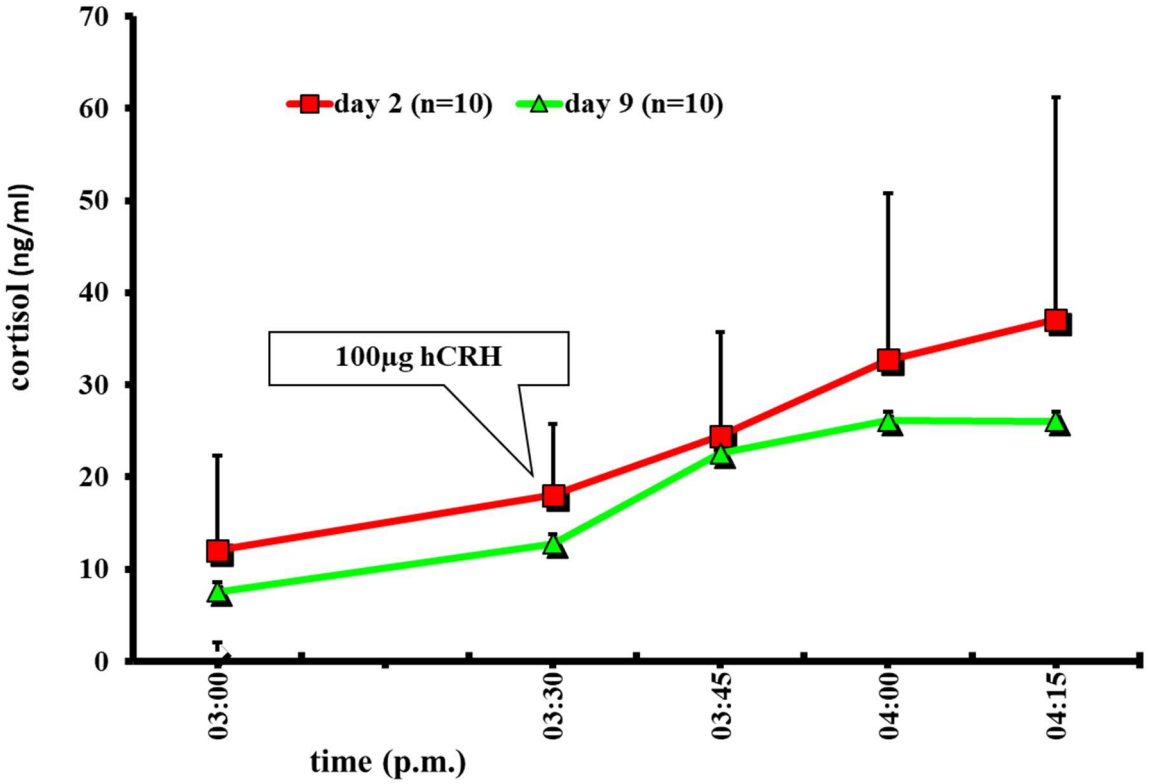

Figure 29: Cortisol secretion (mean ± SEM in ng/ml) during the combined Dex/CRH-test before and after one week of antidepressant short-term treatment with agomelatine, escitalopram, mirtazapine or placebo. Slightly reduced cortisol secretion post-treatment (not significant, rmANOVA $F [1, 32] = 0.39$, $p > 0.05$). No detectable differences between the treatment groups.

For the correlative analyses first the AUCs representing the quantity of the cortisol secretion during the 75 minutes lasting combined Dex/CRH test, wer calculated. Comparing the total cortisol secretion before treatment and after one week of antidepressant treatment with three antidepressants or placebo, the descriptive analysis showed a cortisol reduction which did not reach statistically significance (figure 30).

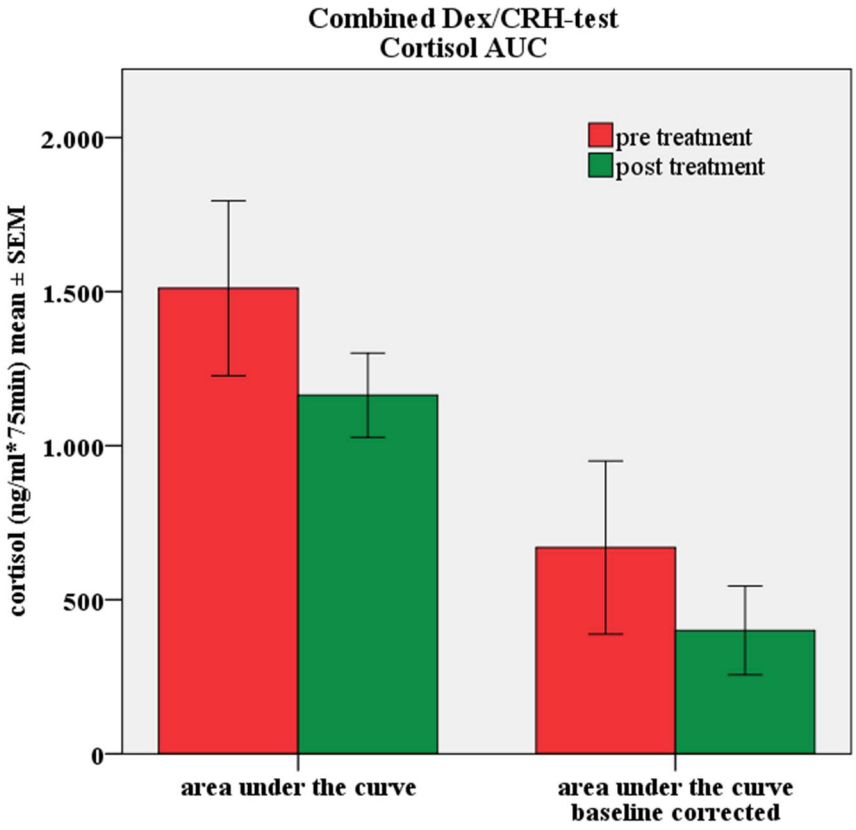

Figure 30: Cortisol secretion during the combined Dex/CRH-test (mean AUC ± SEM in ng/ml*75min) before and after one week of antidepressant short-term treatment with agomelatine, escitalopram, mirtazapine or placebo. AUC and baseline corrected AUC. Slightly reduced cortisol secretion post-treatment (not statistically significant, n.s.).

Interestingly, in spite of the lack of statistical significance, the following figures (figure 31, figure 32, and figure 33) are showing differential reactions of the cortisol secretion which were consistent in all three analyses. Analyzing the peak cortisol levels and the AUCs with and without baseline correction an increase of the cortisol secretion after one week in the escitalopram group and decreasing cortisol secretions in all other groups (mirtazapine, agomelatine, and placebo) could be detected.

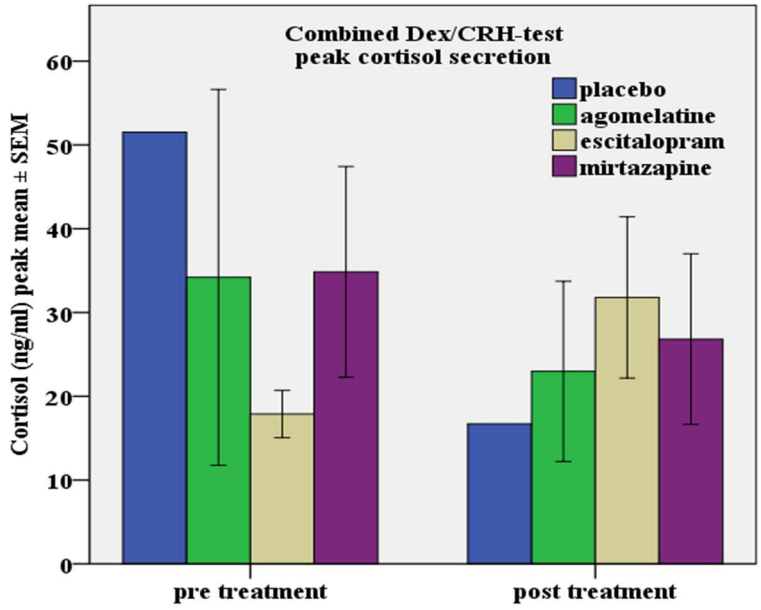

Figure 31: Cortisol secretion during the combined Dex/CRH-test (mean peak ± SEM in ng/ml) before and after one week of antidepressant short-term treatment with agomelatine, escitalopram, mirtazapine or placebo. No statistically significant differences before and after treatment and between different medication groups. Differential influence on cortisol in different treatment groups (n.s.).

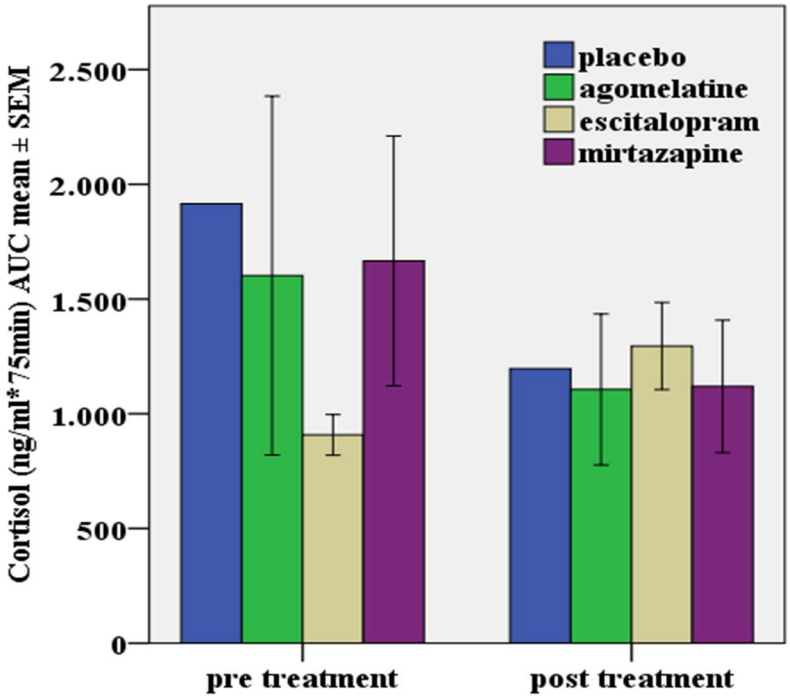

Figure 32: Cortisol secretion during the combined Dex/CRH-test (mean AUC ± SEM in ng/ml*75min) before and after one week of antidepressant treatment with agomelatine, escitalopram, mirtazapine or placebo. No statistically significant differences before and after treatment and between different medication groups. Differential influence on cortisol in different treatment groups (n.s.).

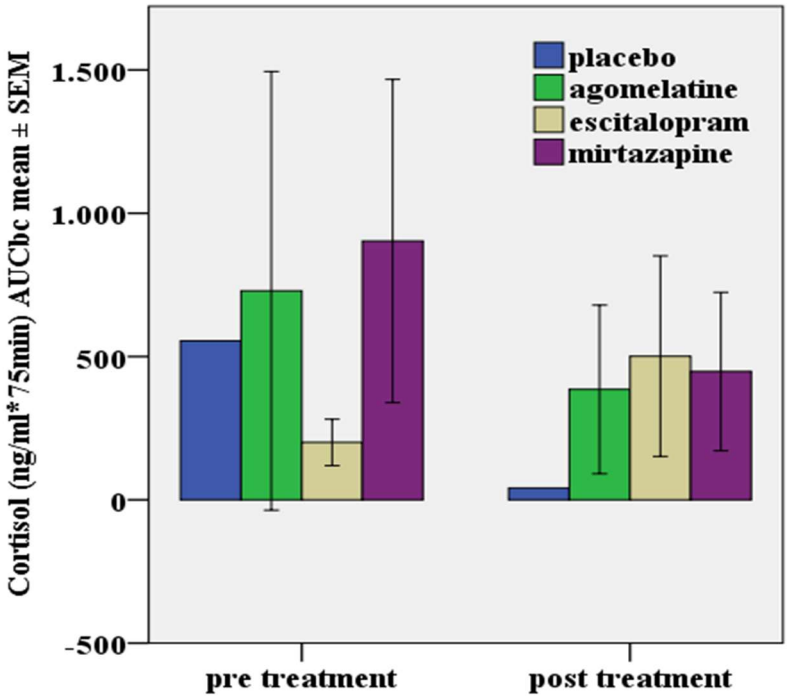

Figure33: Cortisol secretion during the combined Dex/CRH-test (mean AUC_{bc} ± SEM in ng/ml*75min) before and after one week of antidepressant treatment with agomelatine, escitalopram, mirtazapine or placebo. No statistically significant differences before and after treatment and between different medication groups. Differential influence on cortisol in different treatment groups (n.s.).

Because some of the investigated variables were not normally distributed, for the correlative analysis again first the nonparametric Spearman's rank correlation coefficient (Spearman's rho) was calculated. Statistically significant correlations were described in the following section. The nonparametric Spearman's rank correlation coefficient (Spearman's rho) was calculated. Significant correlations could be found for the variables described in the following paragraph. All other correlations did not reach statistical significance ($p < 0.05$).

No pretreatment Dex/CRH test results were correlated with the BOLD % signal change in any ROI and any contrast vs. baseline. The results of the second combined Dex/CRH test after one week of antidepressant treatment (AUC, AUC_{bc}, peak) were correlated with the BOLD % signal change on the second posttreatment fMRI in the following ROIs and contrasts: right amygdala (afraid faces vs. baseline) was correlated with cortisol AUC (correlation coefficient $\rho = -0.745$, p (two sided) = 0.021) and with the cortisol peak ($\rho = -0.695$, $p = 0.038$). The right hippocampus (afraid faces vs. baseline) was correlated with cortisol AUC ($\rho = -0.867$, $p = 0.002$), AUC_{bc} ($\rho = -0.750$, $p = 0.020$), and cortisol peak values ($\rho = -0.900$, $p = 0.001$). The left hippocampus (neutral faces vs. baseline) was correlated with cortisol AUC_{bc} ($\rho = -0.783$, $p = 0.013$), and the

right hippocampus (neutral faces vs. baseline) was correlated with cortisol AUC_{bc} ($\rho = -0.667$, $p = 0.050$).

Calculating the contrasts between different emotional and neutral faces and scrambled pictures in the predefined ROIs and calculating the nonparametric Spearman's rank correlation coefficients with the first pre-treatment Dex/CRH test, only a single significant correlation of the BOLD % signal change in the right DLPFC after the presentation of afraid vs. happy faces with cortisol AUC_{bc} ($\rho = -0.703$, $p = 0.035$) could be detected.

The second Dex/CRH test showed more correlations with fMRI results after one week of treatment. The following nonparametric correlations of the combined Dex/CRH test after treatment (AUC, AUC_{bc}, peak) with the BOLD % signal change could be found: right fusiform (afraid vs. neutral faces) was correlated with cortisol AUC_{bc} ($\rho = -0.683$, $p = 0.042$); the left hippocampus (afraid vs. neutral faces) was significantly correlated with cortisol AUC ($\rho = -0.700$, $p = 0.036$), AUC_{bc} ($\rho = -0.783$, $p = 0.013$), and the cortisol peak values ($\rho = -0.750$, $p = 0.020$); the right hippocampus (afraid vs. neutral faces) was significantly correlated with cortisol AUC ($\rho = -0.767$, $p = 0.016$), AUC_{bc} ($\rho = -0.667$, $p = 0.050$), and the cortisol peak values ($\rho = -0.783$, $p = 0.013$); the right DLPFC (angry vs. neutral faces) was significantly correlated with cortisol AUC ($\rho = -0.783$, $p = 0.013$), AUC_{bc} ($\rho = -0.683$, $p = 0.042$), and the cortisol peak values ($\rho = -0.733$, $p = 0.025$); the right DLPFC (happy vs. neutral faces) was significantly correlated with cortisol AUC ($\rho = 0.817$, $p = 0.007$), and the cortisol peak values ($\rho = 0.733$, $p = 0.025$); the right hippocampus (happy vs. neutral faces) was significantly correlated with cortisol AUC ($\rho = -0.678$, $p = 0.045$), AUC_{bc} ($\rho = -0.703$, $p = 0.035$), and the cortisol peak values ($\rho = -0.695$, $p = 0.038$); the right DLPFC (happy faces vs. scrambled pictures) was significantly correlated with the cortisol AUC ($\rho = -0.667$, $p =0 .050$); the right DLPFC (neutral faces vs. scrambled pictures) was significantly correlated with the cortisol AUC ($\rho = -0.733$, $p = 0.025$); the right DLPFC (sad vs. neutral faces) was significantly correlated with the cortisol AUC ($\rho = -0.733$, $p = 0.025$); the right DLPFC (sad faces vs. scrambled pictures) was significantly correlated with cortisol AUC ($\rho = -0.667$, $p = 0.050$), and the AUC_{bc} ($\rho = -0.667$, $p = 0.050$), the right fusiform (afraid vs. happy faces) was significantly correlated with cortisol AUC_{bc} ($\rho = -0.717$, $p = 0.030$); the left hippocampus (afraid vs. happy faces) was significantly correlated with cortisol AUC ($\rho = -0.711$, $p = 0.032$); the right DLPFC (angry vs. happy faces) was significantly correlated with cortisol AUC ($\rho = 0.800$, $p = 0.010$), and the cortisol peak values ($\rho = 0.733$, $p = 0.025$), the right DLPFC (sad vs. happy faces) was

significantly correlated with cortisol AUC ($\rho = 0.717$, $p = 0.030$); and the right DLPFC (faces vs. scrambled pictures) was significantly correlated with cortisol AUC ($\rho = 0.717$, $p = 0.030$).

To prevent from information loss due to the nonparametric procedure in a further analysis it was calculated whether some of our investigated variables were not normally distributed to be able to calculate the parametric Pearson correlation coefficient for the detection of interdependencies between the HPA-axis activity and the fMRI results.

The test for normal distribution (Kolmogorov-Smirnov (K-S)-test, $p < 0.05$) revealed, that the results of the Dex/CRH test before treatment are not normally distributed: cortisol AUC and AUC_{bc} (ng/ml*75min). The pre-treatment fMRI results showed no normal distribution in the following ROIs and contrasts: right DLPFC (afraid faces vs. baseline), right insula (afraid faces vs. baseline), left DLPFC (angry faces vs. baseline), left DLPFC (happy faces vs. baseline), left DLPFC (neutral faces vs. baseline), left DLPFC (scrambled pictures vs. baseline), right amygdala (afraid vs. neutral faces), left DLPFC (afraid vs. neutral faces), left amygdala (angry vs. neutral faces), left DLPFC (angry vs. neutral faces), left DLPFC (angry faces vs. scrambled pictures), left DLPFC (happy vs. neutral faces), left DLPFC (neutral faces vs. scrambled pictures), left DLPFC (sad vs. neutral faces), left hippocampus (sad vs. neutral faces), left hippocampus (sad faces vs. scrambled pictures), right hippocampus (afraid vs. happy faces), left hippocampus (afraid vs. happy faces), left amygdala (sad vs. happy faces), right amygdala (sad vs. happy faces), right DLPFC (sad vs. happy faces), and left amygdala (faces vs. scrambled pictures).

After one week of treatment the following fMRI activity levels were not normally distributed: right amygdala (happy vs. baseline), left DLPFC post (happy vs. baseline), left hippocampus post (happy vs. baseline), right hippocampus post (happy vs. baseline), left hippocampus post (sad vs. baseline), right hippocampus post (sad vs. baseline), left insula post (sad vs. baseline), and left DLPFC post (scrambled pictures vs. baseline), left DLPFC (angry vs. neutral faces), left DLPFC (angry faces vs. scrambled pictures), right hippocampus (angry faces vs. scrambled pictures), left insula (angry faces vs. scrambled pictures), left DLPFC (happy vs. neutral faces), right DLPFC (happy vs. neutral faces), left hippocampus (happy vs. neutral faces), left DLPFC (happy faces vs. scrambled pictures), left hippocampus (happy faces vs. scrambled pictures), right hippocampus (happy faces vs. scrambled pictures), left DLPFC (neutral faces vs. scrambled pictures), right amygdala (sad vs. neutral faces), left DLPFC (sad vs. neutral faces), left hippocampus (sad vs. neutral faces), right hippocampus (sad vs. neutral faces), left DLPFC

(sad faces vs. scrambled pictures), right hippocampus (sad faces vs. scrambled pictures), left DLPFC (afraid vs. happy faces), left hippocampus (afraid vs. happy faces), left DLPFC (angry vs. happy faces), left hippocampus (angry vs. happy faces), left DLPFC (sad vs. happy faces), right DLPFC (sad vs. happy faces), left hippocampus (sad vs. happy faces), left DLPFC (faces vs. scrambled pictures), and right DLPFC (faces-scrambled pictures).

Using the Pearson correlation coefficient (Pearson's r) for the remaining normally distributed variables the following statistically significnat correlations could be identified before treatment: left amygdala (happy faces vs. baseline) was correlated significantly with the cortisol AUC_{bc} ($r = -0.702$, $p = 0.035$); right insula (scrambled pictures vs. baseline) was correlated significantly with the cortisol AUC ($r = -0.685$, $p = 0.042$) and the cortisol peak value ($r = -0.670$, $p = 0.048$); left amygdala (afraid faces vs. scrambled pictures) was correlated significantly with the cortisol AUC ($r = -0.699$, $p = 0.036$); left amygdala (happy faces vs. scrambled pictures) was correlated significantly with the cortisol AUC ($r = -0.690$, $p = 0.040$) and AUC_{bc} ($r = -0.739$, $p = 0.023$); left amygdala (angry vs. happy faces) was correlated significantly with the cortisol AUC_{bc} ($r = -0.680$, $p = 0.044$); the left amygdala (faces vs. scrambled pictures) was correlated significantly with the cortisol AUC_{bc} ($r = -0.695$, $p = 0.038$). After one week of treatment the following BOLD % signal changes of the second fMRI were correlated significantly with the results of the Dex/CRH-test: the right hippocampus (afraid vs. neutral faces) was correlated significantly with the cortisol AUC ($r = -0.712$, $p = 0.031$), the AUC_{bc} ($r = -0.746$, $p = 0.021$), and the cortisol peak values ($r = -0.767$, $p = 0.016$); the right DLPFC (angry vs. neutral faces) was correlated significantly with the cortisol AUC ($r = -0.676$, $p = 0.046$), the AUC_{bc} ($r = -0.678$, $p = 0.045$), and the cortisol peak values ($r = -0.674$, $p = 0.046$); the right hippocampus (happy vs. neutral faces) was correlated significantly with the cortisol AUC_{bc} ($r = -0.702$, $p = 0.035$); the right DLPFC post (happy faces vs. scrambled pictures) was correlated significantly with the cortisol AUC ($r = -0.721$, $p = 0.029$), the AUC_{bc} ($r = -0.673$, $p = 0.047$), and the cortisol peak values ($r = -0.720$, $p = 0.029$); the right DLPFC post (neutral faces vs. scrambled pictures) was correlated significantly with the cortisol AUC_{bc} ($r = -0.673$, $p = 0.047$), and the cortisol peak values ($r = -0.676$, $p = 0.045$); the right DLPFC (sad vs. neutral faces) was correlated significantly with the cortisol AUC_{bc} ($r = -0.744$, $p = 0.022$), and the cortisol peak values ($r = -0.671$, $p = 0.048$); the right hippocampus post-treatment (sad vs. neutral faces) was correlated significantly with the cortisol AUC_{bc} ($r = -0.681$, $p = 0.044$); the right DLPFC (sad faces vs. scrambled pictures) was correlated significantly with the cortisol AUC_{bc} ($r = -0.693$, $p = 0.038$); the right DLPFC (angry vs. happy faces) was correlated significantly with the cortisol AUC ($r = -0.727$, $p = 0.026$), and the cortisol peak values ($r = -0.702$, $p = 0.035$); right DLPFC (sad vs. happy faces) was

correlated significantly with the cortisol AUC ($r = -0.698$, $p = 0.037$), the AUC_{bc} ($r = -0.732$, $p = 0.025$), and the cortisol peak values ($r = -0.698$, $p = 0.036$); and the right DLPFC (faces vs. scrambled pictures) was correlated significantly with the cortisol AUC_{bc} ($r = -0.705$, $p = 0.034$), and the cortisol peak values ($r = -0.687$, $p = 0.041$).

4. Discussion

4.1. Facial processing in major depressed patients

In principal, this pharmaco-fMRI study focused on the effects of antidepressant short term treatment on the processing of facial emotional pictures in major depressed patients. This represents a neurocognitive assessment target because a huge amount of evidence has shown that major depressed patients exhibited an impaired facial processing. They show bias in interpreting facial expressions, which may cause interrelationship difficulties (Fu, Williams et al. 2004). There are several published studies targeting facial processing, but the role of facial processing impairment in MDD patients in relation to HPA axis hyperactivity and to the severity of depression and it´s amelioration in an early stage of the treatment so far is not clear. Therefore, the research presented here first considers neurological aspects of the central nervous processing of emotional expressions, later it focuses on the combination of biological and clinical assessments to elucidate early effects of antidepressant treatment on the investigated systems. Thus, it bridges the gap between biological, behavioral and neurological aspects of depression to provide a better understanding of mechanisms facilitating pathophysiological changes due to MDD and during the first reduction of depressive symptoms. This may be helpful for the development of treatment options such as antidepressant medication or nonpharmacological treatments such as transcranial magnetic stimulation or neurofeedback. Specifically, this study investigated the effects of antidepressant treatments on BOLD % signal change in the amygdala, DLPFC, hippocampus, fusiform gyrus and insula as brain regions of interest during the presentation of afraid, angry, happy, neutral, and sad faces or scrambled pictures at the baseline before treatment and after one week of antidepressant treatment.

During the recent years with further development of neuroimaging techniques a strong support was provided for a critical role of the amygdala in emotional processing. Haxby and Gobbini mentioned in their review (Rhodes, Calder et al. 2012) that the amygdala is critical for fear conditioning, but also it seems to involve more than only mirroring the emotion of fear. The amygdala can be engaged by positive emotions and positive experiences. Originally Whalen et al. suggested, that the amygdala is part of a vigilance system that is activated in ambiguous situations with biological relevance (Whalen, Rauch et al. 1998). Therefore, the ambiguity, not the emotion of fear, is the essential factor and can be positive or negative.

The results of the study show that the BOLD % signal change in the left amygdala was significantly reduced during the presentation of angry, happy and sad facial expressions in contrast to baseline after one week of antidepressant treatment (figure 3a, b).It was also

significantly reduced during the presentation of emotional faces (afraid, angry, happy and sad) in contrast to neutral faces or scrambled pictures and during the processing of negative facial expressions (afraid, angry and sad) in contrast to happy faces (figure 4a). In addition, the BOLD % signal change in the right amygdala was significantly decreased during the presentation of happy and neutral facial expressions in contrast to baseline (figure 3c). The same was true during the processing of emotional facial expressions (afraid, angry, happy, and sad) in contrast to neutral faces or to scrambled pictures and during the processing of negative facial expressions (afraid, angry, and sad) in contrast to happy faces (figure 4b).

As described above, it seems that the amygdala BOLD response to all facial emotional and neutral expressions in the post fMRI sessions was reduced. Therefore, this study suggests that hyperactivities in the amygdala of depressed patients even after one week of short term antidepressant treatment show a trend to normalize and to be significantly reduced during emotional and neutral facial expression processing. The current findings are consistent with other pharmaco-fMRI studies in emotional face processing in MDD patients (Sheline, Barch et al. 2001, Sergerie, Chochol et al. 2008). For example, Sheline et al. applied in their fMRI study the masked faces paradigm to MDD patients and matched control subjects to compare the amygdala activation in response to masked emotional faces before and after antidepressant treatment. At baseline, MDD patients showed exaggerated left amygdala activation to all faces, and even a greater activity during the presentation of fearful faces, while the right amygdala was not different between the patients and the placebo control group. After eight weeks of antidepressants treatment, MDD patients had bilateral reduced amygdala BOLD response to masked fearful faces and bilateral reduced amygdala BOLD response to all faces. Moreover, Sergerie et al. confirm 2008 in their metanalysis of fMRI studies of visual emotional perception, that the amygdala is associated to both, positive and negative stimuli with a preference for faces depicting emotional expressions (Sergerie, Chochol et al. 2008).

The role of the prefrontal cortex and pattern activity of the left and right DLPFC in MDD patients were discussed in several fMRI investigations (Liotti, Mayberg et al. 2002, Keedwell, Andrew et al. 2005, Grimm, Beck et al. 2008). Those studies provide a consistent hypothesis in which MDD is associated with hypoactivity in the left DLPFC and hyperactivity in the right DLPFC. In their study especially Grimm et al. point to an imbalance of the neural activities in the right and left DLPFC (Grimm, Beck et al. 2008), which demonstrates that the left DLPFC hypoactivity is related to emotional judgement with abnormal modulation by positive and negative emotional valence. Furthermore, in this study hyperactivity in the right DLPFC was

associated with attentional modulation of emotional judgement. Mostly, the hypothesis of an imbalance of the activity in the left and right DLPF in MDD was based on repetitive transcranial magnetic stimulation (rTMS) studies. In these studies, stimulating rTMS (high frequency 10 Hz rTMS) activates the hypoactive left DLPFC, whereas suppressing TMS (low frequency 1 Hz rTMS) decreases neural activity in the hyperactive right DLPFC (Sackeim, Prohovnik et al. 1993, Mottaghy, Keller et al. 2002). The imbalance of neural activities between the right and left DLPFC was also registered during the procession of facial expressions in our MDD patient sample. The analysis of the BOLD % signal change in the left DLPFC was significantly enhanced during the presentation of afraid faces compared to neutral faces after short term treatment. Also, in the right DLPFC the BOLD % signal change was significantly reduced during the presentation of afraid faces versus baseline and angry compared to neutral faces or to scrambled pictures, as well as during the presentation of happy versus neutral faces. In addition, in right DLPFC the BOLD % signal change was significantly reduced during the presentation of angry and sad compared to happy faces. In fact, our results show that neural hyperactivities in the right DLPFC of MDD patients tend to normalize and are reduced even after only one week of antidepressant treatment. Because the activity (BOLD % signal change) is also reduced in the placebo treated patient group (figure 5b, figure 6b), this effect seems to be independent from the kind of pharmacological or supportive treatment.

This result is consistent with other neuroimaging and rTMS studies showing therapeutic effects in the right DLPFC associated with reduced neural activities in this brain region (Mottaghy, Keller et al. 2002, Grimm, Beck et al. 2008).

According to Haxby et al. the fusiform gyrus is the brain region with the most replicated findings in facial expression studies (Haxby, Hoffman et al. 2002). The perception of faces has consistently been found to stimulate activity in the lateral fusiform gyrus usually bilateral, but more consistently on the right side (Haxby, Ungerleider et al. 1999). Our results show that the BOLD % signal change was significantly reduced in the right fusiform gyrus (figure 7) during the presentation of neutral faces versus baseline. The same was true in the left fusiform during presentation of afraid faces versus neutral after one week of treatment.

Phan et al. reviewed 55 PET and fMRI activation studies and observed in a meta-analyses of neuroimaging studies on emotion that the medial prefrontal cortex had a main role in emotional processing (Phan, Wager et al. 2002). Sad condition induced activity in the subcallosal cingulate. Emotional induction by a visual task activated the occipital cortex and the amygdala.

Emotional recall/imagery were associated with the anterior cingulate and the insula, emotional stimuli with cognitive demand also involved the same regions (Phan, Wager et al. 2002).

In our study a visual task including gender recognition targeted emotional processing and focused on predefined brain regions of interest. In addition, with a whole brain analysis we investigated the peak activation in brain regions associated with emotional facial processing during the presentation of facial emotional picture stimuli (table 3). The results show the most significantly activated region (BOLD signal) was located in the left posterior cingulate gyrus as a highest BOLD response to different facial emotional conditions in contrasts to baseline before and after treatment. But also in the left angular gyrus a significant BOLD signal was detected and in the left anterior cingulate gyrus and bilateral precuneus we registered the greatest BOLD response to emotional facial expression before and after short term treatment (figures 9-18).

These study results are in line of other neuroimaging studies demonstrating the critical role of the posterior cingulate gyrus (Phan, Wager et al. 2002, Leech, Braga et al. 2012, Yang, Deng et al. 2015). Also Leech et al. suggest that the posterior cingulate cortex is a main node within the default mode network (DMN) and has great metabolic activity and dense structural connectivity to all other brain regions. Moreover, they have reported that the region appears to be induced by internally directed thought, for example, memory recollection. Even though, they suggest that the posterior cingulate has a complex function collecting and processing information from multiple other brain networks in overlapping regions (Leech, Braga et al. 2012). In fact, mostly ventral regions show strong functional connectivity to the other parts of the DMN, while subregions in the dorsal posterior cingulate cortex are connected to frontoparietal networks which are involved in cognitive control. Therefore, it was suggested, that “parts of the dorsal posterior cingulate cortex are interacting with frontoparietal networks to regulate the balance between internally and externally directed cognition” (Leech, Braga et al. 2012). In addition, Hamani et al. reported in their review published 2011 that the subcallosal cingulate gyrus (SCG), including Brodmann area 25 (area in cerebral cortex), parts of area 24 (part of anterior cingulate gyrus) and area 32 (also known as the dorsal anterior cingulate), is the portion of the cingulum that lies ventral to the corpus callosum (Hamani, Mayberg et al. 2011). It involved a critical node in a network that includes cortical structures, the limbic system, thalamus, hypothalamus, and brainstem nuclei. Also researchers armed with a body of functional neuroimaging have reported abnormal SCG metabolic activities in depressed patients, which are changed and show a reversed pattern activation in this regions after

antidepressant therapies (Hamani, Mayberg et al. 2011). Also Mayberg et al. observed in a positron emission tomography (PET) study a relationship between changes in metabolism in the SCC and the response to antidepressant medications (Mayberg, Lozano et al. 2005).

Table 4: BOLD % signal change after one week of treatment in predefined ROIs after emotional or neutral faces or scrambled pictures vs. baseline. Summary of results (* significant changes (rmANOVA, $p<0.05$); ↓ = reduced BOLD % signal change, ↑ = increased BOLD% signal change).

Post session ROI / contrast	Amygdala L	Amygdala R	DLPFC L	DLPFC R	Fusiform gyrus L	Fusiform gyrus R	Hippocampus L	Hippocampus R	Insula L	Insula R
afraid vs. baseline	↓	↓	↑	↓	↓	↓	↑	↑	↑	↑
angry vs. baseline	↓*	↓	↑	↓*	↓	↓	↑	↓	↓	↓
happy vs. baseline	↓*	↓*	↓	↓	↓	↓	↓	↑	↓	↓
neutral vs. baseline	↓	↓*	↑	↓	↓	↓*	↑	↓	↓	↓
sad vs. baseline	↓*	↓	↓	↓	↓	↓	↓	↑	↓	↓
scrambled pictures vs. baseline	↓	↓	↓	↓	↑	↓	↑	↑	↓	↓

In summary, our results demonstrate a reduced BOLD response to emotional and neutral visual stimuli in the bilateral amygdala, right dorsolateral and right fusiform gyrus while the clinical outcome measured with the Hamilton rating scale for depression indicated even after a short treatment period of only one week beneficial effects of the treatment independent of the treatment group including also placebo treated patients.

Also, a meta-analysis of neuroimaging studies using fMRI and PET in MDD patients employing emotional stimuli provocation reported an enhanced activation of dorsolateral, dorsomedial and ventrolateral prefrontal cortices (Delaveau, Jabourian et al. 2011). In addition, the activation of the amygdala, hippocampus, parahippocampal region, ventral anterior cingulate cortex, orbitofrontal cortex, and insula associated with emotional stimuli was decreased after antidepressant treatment. Furthermore, they reported a decreased BOLD signal in the anterior

and posterior cingulate cortices, as well as in the precuneus and inferior parietal lobe, which could possibly reflect a restored deactivation of the DMN (Delaveau, Jabourian et al. 2011).

4.2. Effects of antidepressant medication versus placebo treatment

Imaging effects of antidepressants in comparison to placebo treatment were replicated in pharmaco fMRI studies and a variety of other imaging techniques quite consistently (Miskowiak, Favaron et al. 2009, Harmer, de Bodinat et al. 2011, Godlewska, Norbury et al. 2012). Studies in healthy volunteers predominantly used a single dose stimulation design with antidepressant medication compared to placebo to explore mechanisms of action of the medication within the CNS (Maron, Wall et al. 2016). Also designs using a treatment duration of one week or even longer have been published (Maron, Wall et al. 2016). The crucial question is, whether results obtained in healthy volunteers can be translated to patients suffering from MDD, because some pharmaco-fMRI studies report evidence for differential effects of antidepressants on the CNS of healthy subjects or depressed patients. For example, Victor et al. investigated the amygdala response to sad, happy, and neutral faces in unmedicated participants suffering from MDD and in healthy controls (Victor, Furey et al. 2010). In this study major depressed patients showed a greater amygdala response than heathy controls to masked sad faces, whereas heathy controls showed a greater amygdala response to masked happy faces (Victor, Furey et al. 2010). Notably, by the time of the second fMRI, most of the patients showed clinically significant symptomatic improvement. This results also was confirmed by other authors (Fu, Williams et al. 2004). Furthermore, Lisiecka et al. studied the neural correlates of emotion processing and attention shifting in four groups; two groups were MDD patients with and without family history of depression and two groups were healthy controls with and without family history of depression (Lisiecka, Carballedo et al. 2013). They found that depressed patients with family history of MDD have stronger neural activation in subcortical areas during shifting attention from negative stimuli compared to healthy controls and depressed patients without family history of MDD who had less activation in the paralimbic regions and greater activation in core limbic areas, especially during emotion processing. Moreover, healthy controls with first-degree MDD relatives overactivated the somatosensory cortex and the attention controlling areas during both, emotion processing and attention shifting.

There is also a fMRI study showing effects of erythropoietin in comparison to placebo on the neural processing of emotion in depressed patients after 3 days of treatment. In addition, erythropoietin caused memory improvement distinguishable from placebo treated patients (Miskowiak, Favaron et al. 2009).

In our study we compared patients treated with one of three antidepressants with placebo treated patients without specific medication. The results show a significant time effect and also reduced BOLD signal change after one week of treatment in the left amygdala during the presentation of angry, happy, neutral and sad faces (each condition in contrast to baseline). In the right amygdala the same was true during the presentation of happy, neutral, and sad faces (each condition again versus baseline) (figure 20a-h). We could detect significant treatment effects in the right DLPFC (figure 21) in the fMRI session during the presentation of angry faces after one week of treatment. Interestingly, significant treatment effects in the left and right fusiform gyrus were detected during the presentation of sad faces and in the left fusiform gyrus during the presentation of neutral faces only in the placebo treated patient group (figure 22a-c).

Using pharmaco-fMRI we could demonstrate statistically significant pre-/post-treatment effects as described above, but we could not distinguish between medicated and unmedicated (placebo treated) patients. This may be due to the relatively small sample size or due to unspecific treatment effects which were similar in all treatment groups. Depression symptoms improved significantly after only one week of treatment also in the placebo treated patient group. Due to ethical reasons all patients received a similar amount of counseling and psychotherapy which contributed together with the unspecific relief of depressive symptoms due to the admission to the hospital. The changes seen in our fMRI investigation may also be more a state marker reflecting this reduction of depressive symptoms than a specific effect caused only in the group of medicated patients.

4.3. Effects of agomelatine, escitalopram, mirtazapine or placebo treatment

Harmer et al. studied the effects of administration of the antidepressant agomelatine or placebo over seven days on the emotional processing in healthy volunteers. The results show that agomelatine decreased subjective ratings of sadness together with the recognition of sad facial expressions. In addition, it improved positive affective memory compared to the placebo group (Harmer, de Bodinat et al. 2011).

There is a pharmaco- fMRI study report from Maron et al. (2016) employing facial expression stimuli on healthy volunteers before and after one week of treatment using the SSRI escitalopram in comparison to an unmedicated group of healthy controls. The results show a significant activation reduction to fearful, but not to happy facial expressions bilaterally in the amygdala, the cingulate and the right medial frontal gyrus following escitalopram medication (Maron, Wall et al. 2016). There are similarities and differences between this study from Maron

et al. and our findings in depressed patients. There was a BOLD % signal reduction in our study during both conditions, afraid and happy faces in the left and right amygdala after short-term treatment.

Studies in healthy subjects using one week of SSRI treatment revealed the same results of decreased amygdala activation during negative facial stimuli (Harmer, Mackay et al. 2006, Anderson, Del-Ben et al. 2007, Windischberger, Lanzenberger et al. 2010). Moreover, the amygdala activation was enhanced during the presentation of positive facial stimuli. These results could be replicated also another study in healthy volunteers published by Norbury et al. 2009. In an pharmaco fMRI study a group of healthy volunteers showed increased amygdala activation during the stimulation with happy faces without changes in the levels of mood or anxiety after a short term treatment with citalopram (in comparison to placebo) (Norbury, Taylor et al. 2009).

Therefore, it was not expected that in our study MDD patients treated with the SSRI escitalopram would reveal a reduced BOLD % signal change after one week of treatment in the amygdala response to happy facial expression stimuli. But our result is in line with other pharmaco-fMRI studies in MDD showing a decreased amygdala activation during the presentation of both, positive and negative conditions (which is different from studies in healthy controls) (Godlewska, Norbury et al. 2012). Godlewska et al. investigated the effects of short-term SSRI treatment on the neural response to fearful faces in depressed patients (Godlewska, Norbury et al. 2012). MDD patients received SSRI treatment or placebo. The neural response to fearful and happy faces was investigated after 7 days of treatment. They could demonstrate, that amygdala responses to fearful facial expressions were significantly greater in depressed patients in contrast to healthy controls. Even though, exaggerated amygdala response to fearful faces was normalized in MDD patients after short treatment. Interestingly, after 7 days there was no significant difference in clinical depression ratings between patient treated with placebo or escitalopram.

Whether the neural activation in response to facial expression stimuli changes in the depressed patients after treatment with longer duration was not demonstrated up to now, but a behavioral study in depressed patients receiving the SSRI citalopram or the NARI reboxetine has reported significant increases in recognition accuracy of disgust, happiness and surprise. This occurred after two weeks of antidepressant treatment, after six weeks the results were the same. Interestingly the fMRI results showed a significant correlation with the clinical improvement after six weeks of treatment (Tranter, Bell et al. 2009).

Pharmaco-fMRI studies considering the effects of the NaSSA Mirtazapine in healthy volunteers have shown an altered facial emotion processing even after the administration of a single-dose (Rawlings, Norbury et al. 2010, Komulainen, Heikkila et al. 2016). Rawlings et al. applied facial emotional stimuli after a single dose of mirtazapine or placebo in healthy volunteers. Finally, the mirtazapine treated group have shown significantly reduced BOLD response to fearful or angry faces and an increased BOLD response to happy facial expressions (Rawlings, Norbury et al. 2010).

Another fMRI study investigated the effects of a single dose of mirtazapine on processing of self-referential emotional information on healthy volunteers in comparison to a healthy control group. During the fMRI the participants categorized positive and negative self-referential adjectives. Mirtazapine reduced the BOLD response to positive self-referential processing in the posterior cingulate cortex and in the parietal cortex (Komulainen, Heikkila et al. 2016).

In contrast, a pharmaco-fMRI study investigated effects of emotional processing in depressed patients before and after 4 weeks of antidepressants treatments with mirtazapine or venlafaxine compared to healthy controls. Patients have shown an enhanced activation in the anterior cingulate cortex, the dorsomedial prefrontal cortex, the dorsolateral prefrontal cortex, and basal ganglia. In addition, a significant decrease of BOLD responses was seen in the hippocampus, basal ganglia, thalamus, and the cerebellum of venlafaxine-treated patients, and a significant enhanced BOLD responses was seen in the middle cingulate gyrus and supplementary motor area of patients treated with mirtazapine (Frodl, Scheuerecker et al. 2011).

Another pharmaco-fMRI study investigated effects of agomelatine or placebo in depressed patients in contrast to healthy controls during an emotional self-referential task before and after short term (one week) and long term (seven weeks) treatments (Delaveau, Jabourian et al. 2016) Patients treated with agomelatine exhibited significant deactivations in the ventrolateral prefrontal cortex during self-referential processing after one week. Interestingly, after seven weeks, depressed patients showed significant increases in the activation of the ventral anterior cingulate cortex. This shows that agomelatine had short- and long-term effects on brain structures involved in anhedonia and emotional regulation of depressed patients.

As described in the previous section, we could demonstrate statistically significant treatment effects (figure 24a, d, e and figure 28), but we could also not distinguish between the patient groups receiving agomelatine, escitalopram, mirtazapine or placebo. Again, the cause of this lack of differential results may be the small sample sizes in our four treatment groups or due to

unspecific treatment effects which were similar in all treatment groups. As described before, depressive symptoms deceased markedly during the first treatment week in all four treatment groups. The clinical course of placebo treated patients was not significantly different from patients receiving medication. Therefore, the changes seen in our fMRI investigation again may be interpreted as state markers reflecting the severity of the depressive disorder more than reflecting the specific modes of action of the used antidepressants.

4.4. Correlation BOLD % signal change with severity of depression

Exploratory correlations between the activations in our ROIs during specific contrasts and the clinical characteristics of the investigated major depressed patients were calculated using a two-tailed test with the Spearman's rank correlation coefficient.

Untreated depressed patients showed a significant positive correlation of their depression status measured with the HAM-D21 scale with the activity in the right DLPFC as a reaction to angry emotional faces and to neutral faces.

Because neurons located in the prefrontal cortex and especially in the DLPFC seem to selectively process face-specific information (SP, Wilson et al. 1997) and are involved in face perception (Dekowska, Kuniecki et al. 2008), the expected activation of the DLPFC in our face paradigm may be influenced by MDD and the resulting affective state. We are not able to confirm the reported association of MDD with a reduced activation in the DLPFC (MacNamara, Klumpp et al. 2017) which seem to be detectable especially in response to negative stimuli (Hamilton, Etkin et al. 2012) because we investigated only depressed patients. In addition, we could show a significantly increasing activation due to negative emotions (afraid vs. neutral faces) on the left side (figure 6a), while on the right side the activation after angry faces vs. baseline declines after one week of antidepressant treatment (figure 5). Functional abnormalities in the DLPFC representing possibly the neurophysiologic correlate of psychomotor slowing in MDD (Remijnse, Nielen et al. 2009) were confirmed also by other publications (Filkowski, Haas et al. 2017). Our investigation could confirm, that the activity in the DLPFC in response to negative facial stimuli was correlated significantly with the severity of the MDD measured with the HAM-D21 scale. After one week of treatment, we could confirm at least in some contrasts an increasing activity, but a significant correlation of the BOLD signal in the right DLPFC with the severity of the depressive syndrome was no longer detectable.

Our patients showed a positive correlation of the activity in the right fusiform gyrus during the presentation of sad faces in comparison to baseline with the severity of depression before

starting the treatment. After one week of antidepressant treatment we could demonstrate a declining activity in the right fusiform gyrus (figure 7, figure 8). After one week of treatment the significant correlation could no longer be detected.

The fusiform gyrus represents an important area in the ventral visual processing stream. In prior studies in adolescents suffering from MDD a bilateral hypoactivation in comparison to healthy controls was demonstrated (Ho, Zhang et al. 2016). Because more severe depression causes more activity in the fusiform gyrus in our study and due to the fact, that with lower HAM-D21 scores after one week of antidepressant treatment also reduced activities in the fusiform gyrus were detected, these findings contradict the finding of Ho et al. demonstrating lower activation in depression. On the other hand, Robertson et al. reported a reduction in the fMRI BOLD signal in the right fusiform gyrus after successful treatment with the dopaminergic and noradrenergic antidepressant bupropione, which was even correlated with the HAM-D improvement pointing in same direction as our correlative analysis (Robertson, Wang et al. 2007). Also, another study in adult MDD patients suggested larger BOLD responses in the fusiform gyrus (here at the left side) as predictors of antidepressant treatment response to the SNRI venlafaxine (Frodl, Scheuerecker et al. 2011).

In the left amygdala a reduction of the BOLD response to afraid faces vs. scrambled pictures was registered (figure 4a). In addition, this contrast was associated only after, but not before treatment with the severity of the depression.

The amygdala activity in MDD has been reported to be increased after negative stimuli (Jaworska, Yang et al. 2015). This was confirmed especially in the left amygdala also in another study comparing MDD patients with healthy controls (Jenkins, Kassel et al. 2016). It was even reported, that more pronounced symptoms of the MDD are associated with more distinct decreases of amygdala activation (Anderson, Juhasz et al. 2011). Moreover, positive correlations of the amygdala activity with the severity of an MDD were reported from a study in both, adolescents and older adults (Mingtian, Shuqiao et al. 2012). Both findings confirm both our results of the reduced amygdala activity after short term treatment and the association with the severity of the MDD. In contrast, a more pronounced illness, represented by higher HAM-D scores on the day of our first fMRI scan, was not correlated with the level of amygdala activity.

It can be summarized therefore, that the BOLD signal change during presentation of emotional and neutral faces in divergent ROIs such as the amygdala, the fusiform gyrus, and the DLPFC

are associated with the severity of MDD and may be state markers for the disease. The fact that these correlations are not consistently registered before and after short-term treatment may result from the limited power of the presented study or from a state dependent relationship before and after any treatment affecting the depressed state.

4.5. Correlation BOLD % signal change with HPA-axis activity

Brain areas corresponding to ROIs investigated in this study such as the hippocampus, the amygdala and the prefrontal cortex represent parts of the limbic system which may have a crucial impact on the regulation of the HPA axis. The hippocampus contains high levels of glucocorticoid receptors, is structurally connected with emotion-related brain regions such as the prefrontal cortex and amygdala, and regulates the HPA axis (Miller and Cohen 2001). Exposure to stress and activation of the HPA axis are associated with decreased activity in some of these regions (Pruessner, Dedovic et al. 2010). Therefore, MDD as a stress related disorder and the relief of depressive symptoms may be associated with the activity in these areas. The hippocampus contains glucocorticoid receptors and seems to provide a gateway to regulation mechanisms including also other interconnected brain regions such as the amygdala and the PFC (McEwen, Nasca et al. 2016). The amygdala stimulates CRH production and interacts also with the prefrontal cortex (Hakamata, Komi et al. 2017). One part of the PFC, the DLPFC mediates cognitive functions, including attentional control (Liu, Ge et al. 2017).

Exploratory correlations between the activations in the predefined ROIs during specific contrasts and the activity of the HPA-axis determined using AUCs and peak values of combined Dex/CRH test before and after short term treatment with antidepressants or placebo were calculated using the two-tailed test with the Spearman's rank correlation coefficient. The pre-treatment Dex/CRH-test revealed only a significant negative correlation of the activity in the right DLPFC calculating the contrast between responses to afraid and happy facial pictures: the smaller the registered BOLD % signal change, the higher was the HPA-axis activity. Because MDD represents a chronic exposure to stress activating the HPA axis due to functional loss of negative feedback loops, it seems plausible, that we could register a decreased activity in the DLPFC (Pruessner, Dedovic et al. 2010) with more severe depression ratings, even if we could not provide the linking data of strong and direct correlations between the HPA axis activity and the HAM-D21 scores as a direct link.

The post-treatment Dex/CRH-test results confirmed an even more pronounced relationship to the activation patterns in the predefined ROIs: Activity in the left hippocampus was inversely

correlated with cortisol secretion during contrasts between afraid vs. neutral as well as afraid vs. happy faces. The same was true for the right side during the contrasts between afraid and happy vs. neutral faces. On a structural level, these results are in line with a study describing the consequences of a loss of hippocampal function due to structural changes: hippocampus atrophy was related to both, HPA axis dysregulation and depression (O'Brien, Ames et al. 1996). Also a study in patients suffering from structural brain damage including an uni- or bilateral involvement of the hippocampus suffered from disturbed HPA axis regulation with an abolished cortisol response to awakening (Buchanan, Kern et al. 2004). Also, hippocampal shape abnormalities which have been found in patients suffering from MDD were associated with changes in cortisol levels (Watanabe, Kakeda et al. 2017). Moreover, more sophisticated structural MRI investigations in MDD patients confirmed a negative association between cortisol levels and the volume of a hippocampal subfield (cornu ammonis 1-3, horn of ammon, hippocampus major) on the left side (Travis, Coupland et al. 2016). On a functional level our results are in line with an investigation confirming that not only structure, but also the activity of the hippocampus plays a major role in adaptive processes to stress: individuals reacting to acute stress with cortisol secretion showed higher hippocampal deactivation patterns (Khalili-Mahani, Dedovic et al. 2010). Therefore, it seems plausible, that also the chronic stress of our MDD patients which includes chronic HPA axis hyperactivity is connected with reduced hippocampal activity. This was confirmed in our investigation with the negative correlation between cortisol secretion and hippocampal activity during the emotional faces task for the first time.

The post-treatment activity of right DLPFC was negatively correlated with the BOLD % signal change in contrasts between happy, neutral and sad faces vs. scrambled pictures and due to contrasts between angry and neutral faces. This negative correlation of the activity during the first four contrasts with cortisol secretion seems to be in line with the regulatory function of the DLPFC receiving downstream signals from the hippocampus for cognitive and attentional functions described above (Liu, Ge et al. 2017). Surprisingly, the contrasts of angry and sad vs. happy faces, happy vs. neutral faces, and the contrasts between faces and scrambled pictures showed statistically significant positive correlations with the HPA axis activity. Notwithstanding, these positive correlations of the DLPFC activity during the latter described contrasts cannot be interpreted easily. No published studies can be found that are in line of this result, in addition, it contradicts in part the above described regulatory functions which were in line to the higher cortisol secretion associated with lower DLPFC BOLD signal during face

processing. Therefore, also statistical artifacts or accidental correlations independent of regulatory processes cannot be ruled out.

The HPA axis activity during the post-treatment Dex/CRH-test was related and inversely correlated to the BOLD response during the presentation of afraid vs. happy or neutral faces in the right fusiform gyrus.

The fusiform gyrus is considered as a brain region specialized for face perception (Weiner and Zilles 2016). Moreover, a microanatomical asymmetry seems to provide the anatomical correlate for a hemispheric specialization which supports the predominance of the right hemisphere for face processing (Chance, Sawyer et al. 2013). Because the amygdala influences the function of the fusiform gyrus during the perception of faces (Herrington, Taylor et al. 2011) and is itself influenced by projections from the hypothalamus which is regulating the HPA axis activity as described above, the negative correlation of the BOLD response of the right fusiform gyrus during the perception of emotional faces with the status of the HPA axis activity seems to be plausible, even if this finding has not been published elsewhere up to now. The amygdala mediates the HPA axis activity especially during the perception of threat-related stimuli such as afraid faces and during anxiety symptoms, but up to now the neural mechanisms facilitating these reactions were considered to be unclear (Di Iorio, Carey et al. 2017). Therefore, it cannot be ruled out that the regulating cascade from the processing of emotional faces in the fusiform gyrus may interact with amygdala and hippocampus in the processes of stress and HPA axis activity regulation.

4.6. Limitations and strengths of the study

Some limitations of the present study exist. First of all, the power of the study was calculated to detect changes of the fMRI BOLD signals before and after one week of antidepressant short-term treatment. Further subdivisions of the patient sample, such as subgroup analyses of the four different treatment groups after randomization to investigate the differential developments of the BOLD signals, the clinical variables, and the results of the combined Dex/CRH tests may not have enough power to proceed beyond exploratory analyses.

The second main limitation of the study was the use of three different scanner models due to the replacement of the old research scanner, the reconstruction, installation, and setup-time for the new research scanner, during which time we used a clinical scanner at the Universitätsklinikum Regensburg, and the new research scanner which was used to produce most of the fMRI data presented here.

A third limitation may be the occasional use of concomitant medication which was limited to lorazepam for agitation and zopiclone for insomnia and given only in standardized dosages. The short treatment time of only one week before the 2nd fMRI and Dex/CRH test may be viewed either as a weakness or as a strength of this study. It may be a weakness due to the limited therapeutic proceedings during antidepressant treatments. Full antidepressant effects usually can be reached after four to six weeks. At the same time it was planned as an advantage, because besides the clinical changes the fMRI and Dex/CRH data may possibly be used in later analyses to predict the later response to the divergent antidepressant treatments.

A further weakness may be then the changes in therapeutic regimes which were possible after the first two weeks of treatment. These may induce a higher variability in the data, but were inevitable for the treated patients to avoid clinical disadvantages due to study participation.

Clear strengths of the study were the randomization to the treatment groups, the placebo control group, the standardized treatment during the first two weeks of treatment including both fMRI investigations and both Dex/CRH tests. Moreover, it is a strength to follow up the clinical data of all patients up to remission and discharge from the hospital.

The main advantage of the presented study is the combination of investigations including fMRI data using the emotional faces paradigm with high sensitivity tests for the HPA axis activity and clinical data. Further investigations not presented here will include also structural MRI scans, long resting state investigations, diffusor tensor imaging data, a battery of neuroinflammatory markers together with the status of Omega-3 polyunsaturated fatty acids, and the gut microbiome compositions.

5. Summary

Background: Major Depressive Disorder (MDD) is a chronic stress related disorder characterized by depressed mood, by vegetative and cognitive symptoms. Imaging biomarkers may help to predict the impaired processing and regulation of emotions related to MDD and to antidepressant treatment response. Pharmaco neuroimaging and behavioral studies have shown that antidepressants can affect emotional processing very early after starting the treatment and independently from changes in subjective mood. Moreover, normalization of hypothalamus-pituitary-adrenal (HPA) axis regulation, which is usually disturbed in MDD, is often associated with successful recovery from depression. Therefore, we investigated the relationship between neural activation before and after short term treatment with antidepressants and HPA axis activity in relation to clinical outcome in MDD patients.

Methods: We investigated 33 in-patients admitted to the Department of Psychiatry and Psychotherapy, University of Regensburg, for the treatment of MDD. Firstly, we considered the evidence for a cognitive neuropsychological model of antidepressant drug action by employing pharmaco-fMRI (3T) in a double-blind randomized placebo-controlled design to investigate the effect of short-term treatment with escitalopram, mirtazapine, agomelatine or placebo on the BOLD signal change in predefined brain regions associated with a visual facial emotional and neutral stimulation task. Additionally, all patients received the same amount of psychotherapeutic support.

Results: After one week of short-term treatment we detected a statistically significant reduction in the BOLD % signal change in the bilateral amygdala, right dorsolateral prefrontal cortex and the right fusiform gyrus during the presentation of facial emotional and neutral expressions. In a second evaluation, we compared medicated patients with unmedicated (placebo treated) patients. Here we could see significant effects in the described regions but could not detect significant differences between verum and placebo groups. After that, each treatment group was investigated separately and compared together. The results showed again statistically significant effects in the above described regions, but no significant differences between the treatment groups. The clinical outcome after one week of treatment showed a partial recovery of the patients with reduced scores in the Hamilton rating scale for depression together with and correlated with BOLD % signal change in some specific regions. Moreover, the activity of the HPA axis was reduced slightly. In addition, this reduction showed significant correlations with the BOLD % signal change in some of the regions of interest.

Discussion: The purpose of this study was to provide a better understanding of the interface between neural systems during antidepressant treatment, the short term effects of antidepressants on emotional processing, to bridge the gap between defined brain regions (amygdala, DLPFC, fusiform gyrus, hippocampus and insula) the fMRI BOLD signal, HPA axis hyperactivity, and the clinical status of major depressed patients. We could show that bilateral amygdala hyperactivities in depressed patients were reduced even after short term treatment. A trend to reduce and normalize also the activity in the right dorsolateral prefrontal cortex and in the right fusiform gyrus was consistent also with other fMRI studies. In addition, we could demonstrate a probable association between the HPA axis regulation and the activity in the brain regions of interest investigated in our study. We could demonstrate the onset of a normalization of the HPA axis activity, as well as the onset of clinical improvement after one week of treatment, but our study lacked statistic power to differentiate between our four treatment groups. In addition, it is possible that unspecific effects of counseling and the in-patient treatment regimen outweigh any specific pharmacological treatment effects after only one week of antidepressant treatment.

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7. Abbreviations

3T	3 Tesla
5HT	5 hydroxytryptamine, serotonin
5HT2a, 2c, 3	serotonin 2a, 2c, 3 (receptors)
AC	anterior commissure
ACC	anterior cingulate cortex
ACTH	adrenocorticotropic hormone
AKDEF	Averaged Karolinska directed emotional faces
ANOVA	analysis of variance
ASF	a simple framework (toolbox)
AUC	area under the curve
BA	Brodmann's area
Bc	baseline corrected
BfArM	Bundesinstitut für Arzneimittel und Medizinprodukte
BMBF	Bundesministerium für Bildung und Forschung
BOLD	blood oxygenation level-dependent
CBT	cognitive behavioral therapy
CNS	central nervous system
CRH	corticotropin releasing hormone
Dex	dexamethasone
Df	degrees of freedom
DGPPN	Deutsche Gesellschaft für Psychiatrie und Psychotherapie, Psychosomatik und Nervenheilkunde
DLPFC	dorsolateral prefrontal cortex
DMN	default mode network
DST	dexamethasone suppression test
DTI	diffusion tensor imaging
ECG	electrocardiogram
ELISA	enzyme linked immunosorbent assay

fMRI	functional magnetic resonance imaging
FWE	family wise error
GLM	general linear model
H_1	histamine 1 (receptor)
HAM-D	Hamilton rating scale for depression
HPA	hypothalamus-pituitary-adrenal
HRF	hemodynamic response function
ICD	international classification of diseases
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
K-S	Kolmogorov-Smirnov
LPDF	lateral prefrontal cortex
M.I.N.I.	Mini-International Neuropsychiatric Interview
MarsBaR	MARSeille Boîte À Région d'Intérêt (name of an SPM toolbox)
MATLAB	MATrix LABoratory
MDD	major depressive disorder
MED	medication
min	minute
MNI	Montreal Neurological Institute
MPFC	medial prefrontal cortex
MRI	magnetic resonance imaging
MT1, 2	melatonin 1, 2 (receptors)
NARI	noradrenaline reuptake inhibitor
n.s.	not statistically significant
OFC	orbitofrontal cortex
PCu	precuneus
PET	positron emission tomography
PFC	prefrontal cortex
PHG	parahippocampal gyrus

PHG	parahippocampal gyrus
phMRI	pharmacological MRI
ReML	restricted maximum likelihood
rmANOVA	repeated measures ANOVA
rTMS	repetitive transcranial magnetic stimulation
ROI	region of interest
s	second
SCG	subcallosal cingulate gyrus
SEM	standard error of the mean
SNRI	selective noradrenalin and serotonin reuptake inhibitor
SPM	statistical parametrical mapping
SPSS	Statistical Package for the Social Sciences
SSRI	selective serotonin reuptake inhibitor
STD	standard deviation
TR	time repetition
Vs.	Versus
WHO	world health organization

8. List of tables

9. List of figures

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