Universiteit Utrecht

Master Neuroscience and Cognition, track Cognitive Neuroscience

Thesis

Anxiety Disorders and Serotonin: Insights on Effects of rTMS

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3449769

1 December 2010

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Abstract

Anxiety disorder patients display chronic hypervigilance towards threatening stimuli, which could result from hyperexcitability of fear circuitry. Repetitive transcranial magnetic stimulation (rTMS) might normalize functioning of the fear circuitry by affecting serotonergic content, receptors, and transporters.

In panic disorder patients, increased sensitivity of inhibitory autoreceptors could underly increased sensitivity and hyperactivity. high-frequency stimulation of the frontal cortex should increase $5HT_{1A}$ receptor sensitivity, normalize serotonin turnover, and decrease activity in dorsal raphe nuclei. In social anxiety disorder, hyperactivity can be caused by decreased functioning of $5HT_{1A}$ inhibitory autoreceptor. Low-frequency rTMS stimulation of left frontal areas could be an effective treatment. Reduced functioning of the serotonin transporter may underly hyperactivity of fear circuitry in generalized anxiety and post-traumatic stress disorder. Low frequency stimulation of the frontal cortex should increase transporter mRNA, thereby decreasing activity. Obsessive-compulsive disorder is characterized by hyperactivity of basal ganglia, thalamus, orbitofrontal and medial frontal cortices, and is possibly caused by decreased serotonin transporter activity. Low-frequency stimulation of the frontopolar cortex could prove to be effective.

Thus, low-frequency rTMS could normalize HPA-axis functioning by increasing serotonin transporter functioning, eventually also affecting functioning of inhibitory autoreceptors. Hereby, feedback of the frontal cortex on the limbic system and HPA-axis is increased. Moreover, sensitivity to environmental factors of the HPA-axis might decrease. A functionally guided, well controlled PET study needs to be performed to compare patients to healthy controls, and to compare the effects of active stimulation versus sham to investigate the effects of frontal rTMS on the serotonergic system.

1. Introduction

1.1 Anxiety Disorders

Anxiety and fear are crucial and normal behavioural responses to dangerous situations. However, when anxiety is excessive, persistent or no longer serves to signal danger it is dysfunctional and could imply an anxiety disorder. Anxiety disorders (AD) are one of the most prevalent psychiatric disorders, roughly one out of three people in the United States will meet anxiety disorder criteria at some point in their life. The costs of AD are measured at 42 billion dollars per year (Anxiety Disorders Association of America; AADA.org).

Six AD have been described by DSM-IV: panic disorder (PD), generalized anxiety disorder (GAD), social anxiety disorder/social phobia (SAD), post-traumatic stress disorder (PTSD), obsessive-compulsive disorder (OCD) and specific phobia (DSM-IV-TR). Patients display chronic hypervigilance towards threatening stimuli, which could result from hyperexcitability of neural circuits responsible for adaptive fear states (Rosen and Schulkin, 1998). This is displayed by increased fearfulness and subjective manifestations, such as heightened sense of awareness and fear of impeding disaster, as well as objective changes, such as a racing heart, avoidance, restlessness, tremor, dry mouth and desire to escape (Kandel, Schwartz, and Jessell, 2000). Hypervigilance often results in panic attacks: recurrent, brief, unexpected, and discrete episodes of intense terror without a clearly identifiable cause. There are three types of panic attacks: spontaneous attacks, situational bound, and situational predisposed panic attacks. Spontaneous attacks refers to an absent environmental trigger, not to an absent neurobiological cause. Situationally predisposed panic attacks are triggered by some situations that increase the probability of a panic attack. Situational bound (cued) panic attacks are inevitably caused by a stimulus, as seen in specific phobias (Klein and Klein, 1989).

The amygdala is one of the important parts of the neural circuitry mediating fear and fear-related behaviour (Millan, 2003). Thalamic inputs to the amygdala mediate an associative fear circuitry, hippocampal inputs mediate a higher cognitive level in fear (Mineka and Öhman, 2002). Both hippocampus and amygdala receive processed input from the prefrontal cortex (PFC), which may be hyperactive in AD (Bear, Connors, and Paradiso, 2007). These and limbic structures interact with the bed nucleus of the stria terminalis, temporal auditory and perirhinal cortices, midbrain (periaqueductal gray, PAG), septum, raphe nuclei and hypothalamus. All of these areas are implicated in fear-related behaviour (Rosen and Schulkin, 1998; Millan, 2003). In AD, altered functioning of this fear circuitry results in an excessive stress response. Repetitive transcranial magnetic stimulation (rTMS) might normalize functioning of this circuitry; it has already shown effectiveness in

depression, and several rodent studies also indicate positive effects of rTMS on fear and anxiety.

1.2 The stress response

Fear and anxiety are mediated by the behavioural and autonomic 'stress' response (Rosen and Schulkin, 1998; Mineka and Öhman, 2002), which originates from an evolutionary defensive system to cope with threat and danger by escape and avoidance. The stress response is behaviourally characterized by freezing, autonomic changes, increased reflexive responses to sensory stimuli, increased urination and defecation, hypoalgesia, and defensive behaviour, such as fight and flight responses. These symptoms overlap with those seen in panic attacks, such as sweating, increased heart rate and muscle tension, chest pain, fear of losing control or dying, confusion etc.

The stress response results in neuroendocrine activation, especially of the hypothalamo-pituitary axis (HPA-axis); a stressor induces the release of corticotropin-releasing-hormone (CRH) by the hypothalamus, that in turn triggers the stress response by the release of cortisol from the adrenal gland (Bear, Connors, and Paradiso, 2007). CRH releasing neurons are regulated by the frontal cortex, amygdala, and hippocampus, allowing integration of sensory information and providing negative feedback via glucorticoid receptors (Bear, Connors, and Paradiso, 2007). Next to exerting inhibiting effects on the stress-response by providing negative feedback on the HPA-axis via glucocorticoids, the cortex (especially medial PFC) also regulates autonomic functions. The cortex can suppress a reflexive emotional response, as is the case in healthy individuals, for example after realizing the 'monster' is just a shadow (Kandel, Schwartz, and Jessell, 2000). Moreover, learning and experience can be integrated in stress responses due to the reciprocal connection between amygdala and neocortex. In AD, hyperactivity of the amygdala (responsible for conditioned responses) has been observed, and the hippocampus (responsible for context processing and fear memories) is hypoactive. Together, this results in an increased cortisol release. A high and prolonged increase in cortisol levels results in hippocampal cell death and an increased stress response (Bear, Connors, and Paradiso, 2007).

The stress-response could be modulated by the serotonergic system, for several reasons. Firstly, the serotonergic system has close interactions with cerebral arteries. Secondly, serotonergic neurons are primary chemosensory cells, and can thus translate cortisol release in action potentials. Thirdly, extensive serotonergic projections to the PAG are likely to play a comprehensive role in supressing panic behaviourally and autonomically. Further are the hypothalamus and pituitary strongly innervated by serotonergic neurons, and amygdalar activity has been related to polymorphisms of the

serotonin transporter (5HTT) and $5HT_{1A}$ genes (Freitas-Ferrari et al., 2010). Finally, it has been shown that depletion of tryptophan (a precursor of 5HT) increases stress in rodents, as measured by increases in plasma cortisol concentrations. This is possibly because 5HT inhibits HPA-axis activity (Hood et al., 2006). Thus, deficiencies in the 5HT system could be responsible for dysregulated HPA-axis activity in AD.

1.3 The serotonergic system

Tryptophan is the precursor of 5HT, it is converted to 5-hydroxytryptophan by tryptophan hydroxylase and is finally converted to 5HT by 5-hydroxytryptophan decarboxylase (Kandel, Schwartz, and Jessell, 2000). 5HT is taken up in transporter vesicles and released in the synaptic cleft on demand. After exerting its effect, reuptake to the presynaptic terminal via the 5HTT occurs and 5HT is either broken down by monoamine oxidase (MAO) or is taken up by a vesicle again (Kandel, Schwartz, and Jessell, 2000). 5HT can bind to one of seven 5HT receptors. The serotonergic system consists of several kinds of receptors, $5HT_{1-7}$, all of which are G-protein coupled receptors, except for $5HT_3$ (Kandel, Schwartz, and Jessell, 2000). The most commonly studied are the 5-HT_{1A}, 5-HT_{1B} (both inhibitory autoreceptors), 5-HT_{2A}, 5-HT_{2C}, and 5-HT_{3A} receptors.

Medications usually prescribed for AD work by inhibiting 5HT and/or norepinephrine reuptake from the synaptic cleft, inhibiting monoamine oxidase activity, such that monoamines like 5HT are more available in the synaptic cleft and inhibition of excessive activation in fear circuits (anticonvulsants) (Ravindran and Stein, 2010). Thus, most medications prescribed for AD exert their effects via the serotonergic system and result in an increase in overall 5HT levels (Kandel, Schwartz, and Jessell, 2000). Especially selective serotonin reuptake inhibitors (SSRI) treatment is used for AD. The underlying mechanism of this treatment has been mainly assessed in rodents and showed that SSRIs work by blocking the 5HTT, so that the serotonergic effect on postsynaptic receptors is prolonged (Kandel, Schwartz, and Jessell, 2000). This leads to an increase in 5HT release, and a decrease in sensitivity of $5HT_{1A}$ and $5HT_{1B}$ inhibitory autoreceptors (Kandel, Schwartz, and Jessell, 2000). Moreover, SSRIs may exert their anxiolytic effect by increasing inhibiting thalamic and cortical serotonergic input to the amygdala (Gorman et al., 2000). The serotonergic system and HPA-axis might therefore interact. Homberg and Contet (2009) have reviewed these interactions. They concluded, again mainly from rodent studies, that acute stress results in the release of CRH and activates CRH_1 receptors, thereby inhibiting serotonergic release in targets of the dorsal raphe nuclei, including amygdala, lateral septum, nucleus accumbens, and medial PFC. This results in active coping strategies in rodents, the behavioural impact of acute stress is probably regulated through inhibitory activity of the medial PFC. Prolonged, uncontrollable stress, however, inactivates medial PFC and gives

rise to internalization of CRH_1 receptors and activation of CRH_2 receptors. This results in stimulation of serotonergic neurons and passive coping strategies (Homberg and Contet, 2009). Thus, the effect of SSRIs could result in a shift of passive to active coping strategies by increasing 5HT levels, thereby increasing frontal inhibition on the limbic system.

Efficacy of SSRI treatment is not evidence for an underlying dysfunction in the 5HT system in AD, as these medications may affect downstream systems through an intact serotonergic system. However, SSRI efficacy is consistent with other findings implicating the serotonergic system in anxiety. For example, 5HT pathways mainly originate from the raphe nuclei, which might be important in anxiety. The caudal raphe nuclei project to the spinal cord and cerebellum, and are involved in motor behaviour and pain perception. Whereas the rostral nuclei project to virtually all forebrain structures, such as the hypothalamus, thalamus, limbic forebrain (amygdala, hippocampus, nucleus accumbens, cingulate), basal ganglia (globus pallidus, caudate nucleus, putamen), PAG, deep cerebellar nuclei, and olfactory, entorhinal and neocortices (Kandel, Schwartz, and Jessell, 2000). These projections are involved in anxiety, sleep-wake cycles, affective and sexual behaviour, thermoregulation and food intake (Kandel, Schwartz, and Jessell, 2000), behaviours affected in AD. The serotonergic (dorsal) raphe nuclei projection to the PAG might especially be important, as direct stimulation of PAG induces a freezing response in animals. Stimulation of the raphe nuclei decreases activity in the PAG, but also leads to anxious behaviour and behavioural inhibition, probably due to altered 5HT input to septohippocampal nuclei (Pratt, 1992) which are implicated in the control of fear (Millan, 2003).

Another important area in anxiety, the amygdala, is also affected by 5HT; a subset of dorsal raphe neurons that activate during uncontrollable stress projects to the basolateral amygdala (BLA). Anxiety expression after stress correlates with BLA 5HT in animals and is dependend on controlability of the stressful stimulus. Moreover, increased serotonergic output of the BLA correlates with increases in fear and $5HT_{2c}$ agonists increase expression of anxiety by activating projection regions of the BLA including limbic areas involved in fear and anxiety. Both antagonist administration and knock-out of the $5HT_{2c}$ receptors have an anxiolytic effect, correlating with decreased BLA activity in rodents (Christianson et al., 2010). The role of the $5HT_{1A}$ receptors in anxiety has also been established. Presynaptically, these inhibitory autoreceptors are desensitized during prolonged stress, resulting in increased 5HT transmission and activation of hypothalamus, and limbic areas, as well as increased ACTH and corticosteroid release (Millan, 2003). Thus, all forementioned areas contain serotonergic receptors (Millan, 2003; Vinkers et al.,

2010) thereby implicating the serotonergic system in anxiety. An overview is provided in figure 1.

1.4 Repetitive transcranial magnetic stimulation

Pharmacological treatment of AD is often combined with cognitive-behavioural therapy. However, according to Bystritsky (2006), only 30% of AD patients recovers after treatment and 30-40% improves, this still leaves up to 30% of all AD patients unaffected by standard treatments. Development of new treatment strategies could improve treatment resistance in AD. One of the options is rTMS which was first opted as a therapeutic tool (of depression and neurosis) in 1902 by Pollacsek and Beer. However, the first TMS device was only developed in 1985 by Anthony Barker and his colleagues. They induced finger and foot movements after stimulating the motor cortex with a coil. This coil is made out of wire, is electromagnetic, and has to be placed at the scalp, after which a high intensity current is passed through. This current is rapidly switched on and off, which produces a magnetic field with an electric current that passes through the scalp and depolarizes neurons 1.5 to 2 cm from the coil. In rTMS, the pulses are delivered repetitively and rythmically. Fast, high frequency (HF) rTMS reaches over 5 Hz (Chae, Nahas, Li, and George, 2001) and facilitates neuronal excitability and long-term potentiation bilaterally (Speer et al., 2000). Slow, low frequency (LF) rTMS (1 Hz or less) may decrease excitability and facilitate long-term depression (Zwanzger et al., 2009), especially contralaterally (Speer et al., 2000).

As most disorders, including AD, are associated with hyperactivity of several areas, LF rTMS has mostly been opted to be effective for treatments. Anxiolytic effects of rTMS treatment was indicated by several animal studies: LF rTMS in Wistar rats led to less anxious behaviour (Kanno et al., 2003), HF rTMS led to better coping strategies in high-anxious rats (Keck et al., 2001), but has also been shown to induce anxiety in animals (Isogawa et al., 2003; 2005). In healthy volunteers, LF rTMs of the left PFC resulted in withdrawal of selective attention from angry faces (D'Alfonso et al., 2000), where stimulation of the right PFC resulted in decreased attention to fearful faces (Van Honk et al., 2002), decreases in anxiety, and increases in left theta activity (Schutter et al., 2001).

rTMS shares some properties with pharmacological AD treatments, for example HF rTMS may have anticonvulsant effects in animals (Fleischmann et al., 1995) and could affect 5HT levels (Kanno et al., 2003) as the HPA-axis and PFC are both under strong influence of the serotonergic system. Indeed, next to affecting long term potentiation and long term depression, rTMS may also have effects on neuroendocrinological processes. 5 Hz lateral prefrontal stimulation has been shown to temporally increase cortisol levels. rTMS of midfrontal, cerebellar and occipital regions declines thyroid-stimulating hormone,

prolactin and cortisol levels (George et al., 1996). As these hormones are part of the stress response and involved in regulation of the HPA-axis, rTMS effects on these hormones may account for beneficial effects of rTMS in AD.

3.1 rTMS effects on fear-circuitry

As discussed above, changes in activity of several brain areas underlies AD pathology. Normalizing this activity could provide symptom relief in AD patients, who now have to cope with a strong treatment resistance. As well, effects of rTMS on the serotonergic system and deficits in the serotonergic system in AD patients indicate that rTMS may be a good treatment option for AD.

The use of LF rTMS to depress activity in hyperactive neural circuits seems to be more plausible and saver than the use of HF rTMS, as the use of the former has already been effective in epilepsy and auditory hallucinations (Wassermann and Lisanby, 2001). LF rTMS inhibits cortical excitability, which can spread to frontal cortices and is long lasting and robust (Wassermann and Lisanby, 2001). Acute LF rTMS of the dlPFC had anxiolytic effects on anxiety ratings and withdrawal of selective attention from aversive facial expressions in healthy volunteers (D'Alfonso et al., 2000; Van Honk et al., 2002; Schutter et al., 2001), indicating a potential beneficial effect in AD. However, differences in cortical excitability between patients and healthy controls might make it difficult to extend these findings. Anxiolytic effects of HF rTMS in humans have not been evidenced (Zwanzger et al., 2009), because of epileptogenic effects. HF rTMS can saturate the inhibitory capacity of the cortex, thereby producing increasing excitability as indicated by spreading of evoked activity, lasting for several minutes (Wassermann and Lisanby, 2001). A new stimulation protocol introduced recently may provide outcome; by using intermittent theta-burst pattern long-term potentiation in the human motor cortex was mimicked (Huang et al., 2005). Thus, although a shorter, low-intensity protocol was used, the effects were similar but longer lasting compared to HF rTMS.

Thus, in AD, the normal stress response is aberrant, possibly resulting from hyperexcitability of fear circuitry. rTMS might be a new upcoming therapeutic tool, as it could decrease hyperexcitability in the fear circuitry by affecting neuroendocrine systems. 5HT is apparent in areas related to fear and anxiety, is known to affect stress responses, and affect HPA-axis activity. Therefore, anxiolytic TMS effects could be mediated by this system. rTMS has not been studied extensively in AD; most studies combining rTMS and AD were researching OCD or PTSD. Moreover, as different mechanisms underly different AD, results of (rTMS) treatment are not consistent. Therefore, we will discuss neuroanatomical and serotonergic functioning underlying each specific AD, as well as results of rTMS studies. This will lead to a conclusion for future research possibilities.

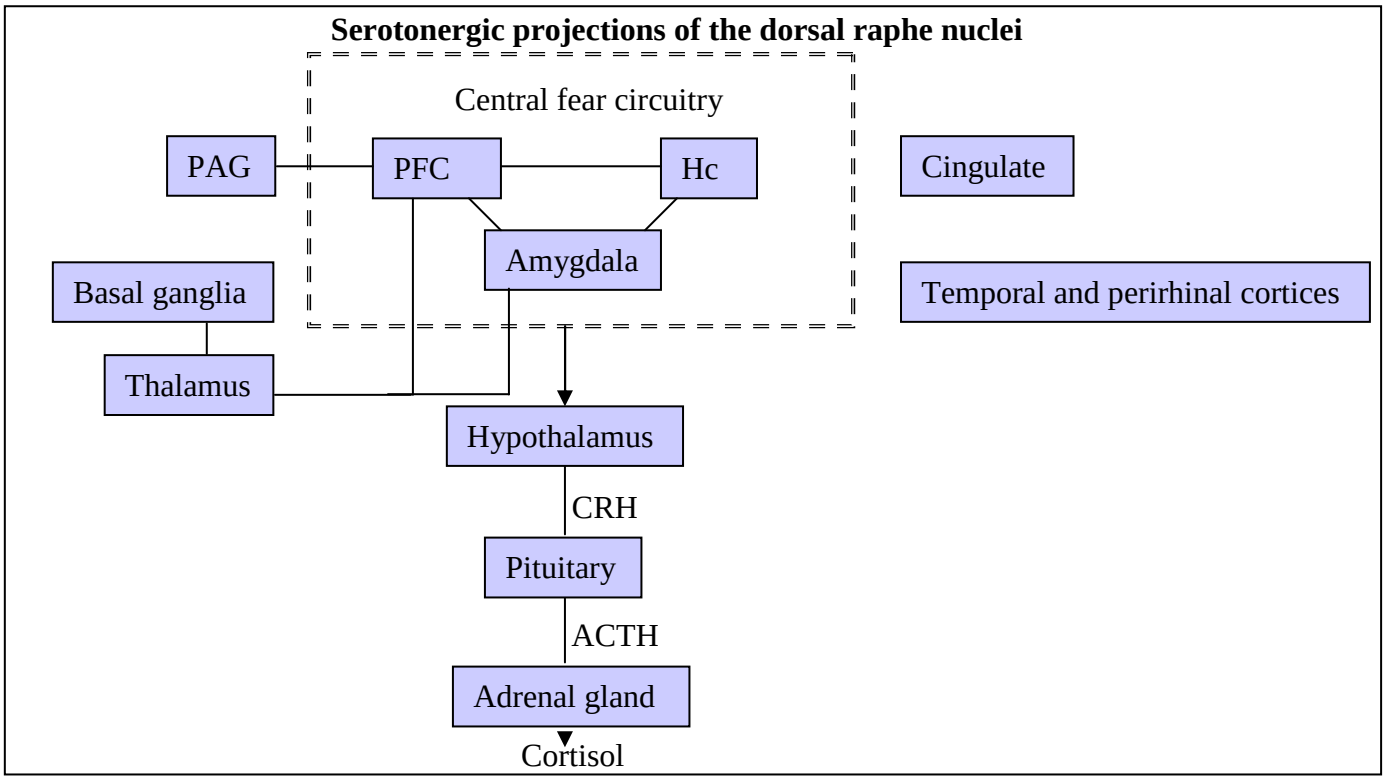

Figure 1. **Overview of serotonergic projections of the dorsal raphe nuclei to several areas involved in the fear response.** PFC: prefrontal cortex; Hc: hippocampus; CRH: corticotropin releasing hormone; ACTH: adrenocorticotropic hormone; PAG: periaqueductal gray.
In AD, deficits in this (inhibitory) serotonergic system are evident, usually resulting in increased activity of the amygdala, decreased frontal and hypothalamic activity, increased sensitivity of the HPA-axis, and an excessive fear response.

2. Effects of rTMS

2.1. Serotonergic system

There are indications that rTMS over the frontal cortex can affect activity in the limbic system, possibly through, thereby normalizing HPA-axis functioning, as the frontal cortex sends extensive inhibitory serotonergic projections to the limbic system and the HPA-axis. Thus, increases in serotonergic transmission between paralimbic and limbic regions after rTMS treatment could underly its effectiveness in the treatment of mood disorders.

Acute HF rTMS has been reported to affect monoamine content and turnover (Ben-shachar et al., 1997; Keck et al., 2000). More specifically, acute HF rTMS increased content of 5HT and its metabolite 5-hydroxyindolaceticacid in hippocampus, without affecting 5HT turnover, as assessed with HPLC (Ben-shachar et al., 1997). However, this was not replicated in an in vivo microdialysis study (Keck et al., 2000). In anothe rodent study, 60 Hz stimulation of the medial PFC resulted in current-dependent increases of 5HT levels in hippocampus and amygdala. The authors suggest that this is through activation of the closely connected raphe nuclei, as stimulation of other areas had no effect on 5HT levels (Juckel et al., 1999). A three day series of HF 25 Hz rTMS improved anxiety-related behaviours in rats, and was accompanied by a reduced increase in 5HT levels, normally associated with the performed task (elevated-plus maze) (Kanno et al., 2003). However, more intense stimulation (15 Hz, 10 days) showed anxiogenic effects in rodents in the same task (Isogawa et al., 2005). In other studies, chronic rTMS (10 sessions of 15 Hz) did not affect tissue 5HT levels (Ben-Shachar et al., 1999; Kanno et al., 2003), although the same stimulation procedure in another study decreased sensitivity of presynaptic $5HT_{1A}$ of the raphe nuclei and $5HT_{1B}$ receptors in nerve terminals, which control 5HT synthesis and output in the PFC via negative feedback (Gur et al., 2000). Conversely, Kole et al. (1999) found that acute 20 Hz rTMs increased $5HT_{1A}$ binding sites in anterior olfactory nucleus, cingulate and frontal cortices of rodents. Chronic stimulation has been found to reduce frontal and striatal $5HT_{2A}$ receptors (Ben-Shachar et al., 1999).

5HT modulation might be mediated by rTMS effects on gene expression levels, acute rTMS has been shown to increase *c-fos* mRNA in thalamus, frontal and cingulate cortices, where chronic rTMS has been shown to have this effect in the parietal cortex. A rodent study indicated that chronic rTMS (15 Hz, 20 sessions) downregulates 5HTT mRNA expression in cerebellum, brainstem and cerebrum, resulting in a functional change similar to that seen in SSRI treatment: a decrease in 5HT uptake and binding. Chronic HF rTMS did not affect trytophan hydroxylase mRNA expression (Ikeda et al., 2005). However, in healthy human subjects rTMS did affect 5HT synthesis: acute 10 Hz stimulation of the left

dlPFC at 90% of the motor threshold decreased ^{11}C-αMtrp trapping constant, which is indicative of 5HT metabolic conversion, in parahippocampus and insula, and increased in cingulate and cuneus. Thus, frontal rTMS in healthy controls affected 5HT turnover in limbic areas, but not in cortical areas or brainstem (Sibon et al., 2006).

It could be the case that dlPFC stimulation in AD patients needs more time to show effects. Decreases in 5HT turnover, could possibly need more time to show effects on serotonergic transmission, as is seen in SSRI treatment (Kandel, Schwartz, and Jessell, 2000). Moreover, response to rTMS by depressive patients depends on a polymorphism of the $5HT_{1a}$ receptor; patients that have the G allele respond worse to rTMS (and SSRI) treatment than patients having the C/C genotype; the S allele of the 5HTT did not affect responses to rTMS in these patients (Zanardi et al., 2007). This indicates that rTMS may indeed affect 5HT receptors by either increasing 5HT release or uptake by affecting the binding capacity of the receptor through magnetic current.

Thus, acute rTMS increases c-fos mRNA in areas involved in the stress response, it also increases 5HT content and increases $5HT_{1A}$ levels. Chronic rTMS does not affect 5HT levels, although decreases in 5HTT and limbic 5HT turnover have been observed. As well, decreases in $5HT_1$ and $5HT_{2A}$ receptors have been observed. rTMS might especially be a good treatment option for AD, as the S allele of the 5HTT gene has been related to AD, but does not affect responses to rTMS treatment. The effects or rTMS are summed up in table 1.

Table 1. Effects of rTMS on neuronal excitability, plasticity, anxiety, and the serotonergic system.
5HT: serotonin; 5HTT: serotonin transporter.

	High-frequency stimulation		Low-frequency stimulation	
	Acute	Chronic	Acute	Chronic
Intensity	> 5 Hz, once	> 5 Hz, repeated	< 1 Hz, once	< 1 Hz, repeated
Site of effect	bilateral	bilateral	contralateral	contralateral
Excitability	increased	increased	decreased	decreased
Neuronal plasticity	long-term potentiation	long-term potentiation	long-term depression	long-term depression
Anxiety	increased	increased	decreased	
mRNA	more c-fos			
5HT levels	increased / reduced increase during testing	decreased 5HTT mRNA		Effects are hypothesized to be opposite to chronic HF stimulation
Metabolite levels	increased			
Metabolism	decreased in parahippocampus, insula increased in cingulate, cuneus	no effect on tryptophan hydroxylase mRNA		
$5HT_{1A}$	increased binding	less sensitive		
$5HT_{1B}$		less sensitive		
$5HT_{2A}$		reduced		

2.2. Panic Disorder

According to DSM-IV-TR, PD is characterized by recurrent panic attacks and persistent concern about having additional attacks, worry about its implications and consequences (e.g. losing control, having a heart attack, "going crazy") and a change in behaviour following from this. PD often (one third of all cases) leads to agoraphobia, avoidance of the places or situations where an anxiety attack has occurred. Panic attacks and agoraphobia can occur separately from each other as well (ADAA.org)

Gorman et al. (2000) propose a neuroanatomical model of PD including a hyperactive, or more sensitive fear circuitry with the central nucleus of the amygdala (CeA) at its center and including the hippocampus, thalamus, hypothalamus, PAG, locus coeruleus, and other brainstem sites. These areas are rich in 5HT receptors, as discussed above. It has also been hypothesized that PD is characterized by a heightenend sensitivity to novelty, indicated by an increase in cortisol responses and thus enhanced HPA-axis sensitivity. This could decrease over time by sensitization (Abelson et al., 2007). The resulting hyporesponsiveness of the HPA-axis could be due to decreases in CRH or ACTH signalling (Petrowski et al., 2009). This partly contradicts the findings by Abelson et al. (2007) who found increased sensitivity for contextual factors, such as controllability. This

might be explained by baseline measures of HPA-axis activity. Abelson et al. (2007) measured 30 minutes before testing, Petrowski et al. (2009) only 10 minutes before testing. Shortly before testing, anxiety and stress levels could already be upregulated, resulting in less activation during testing.

Deficits in the serotonergic system could underly the symptomatology of PD. Esler et al. (2007), found an increase in central 5HT turnover rate (which could be compensated for by SSRIs) correlating with PD symptom severity, and caused by increased firing of raphe neurons. The effect of LF stimulation on 5HT levels are unknown, but this could be effective as it is opposite to HF stimulation, which is known to increase 5HT levels without affecting metabolism (Ben-Shachar et al., 1997). The serotonergic system has also been implicated by genetic studies. For example, reduced binding potential of the postsynaptic $5HT_{1A}$ receptor in amygdala, temporal and orbitofrontal cortices (OFC) in PET and neuroendocrine challenge tests has been found (Nash et al., 2008; Neumeister et al., 2004; Lesch et al., 1992). This decrease may signify decreased ability of the brain to control panic (Nash et al., 2008) and may involve presynaptic receptor modification, defective postreceptor signal transduction, abnormalities in 5HT synthesis and metabolism, or postsynaptic 5HT receptor-effector system related dysfunction (Lesch et al., 1992). However, Maron et al. (2010) found that $5HT_{1A}$ rs6295 G allele at chromosome 5 causes elevated $5HT_{1A}$ receptor levels in PD patients, resulting in inhibition of raphe nuclei activity, as well as decreased activity in right ventromedial, orbitofrontal and anterior cingulate cortices during processing of anxious facial expressions.

PD has been related to polymorphisms of the 5HTT gene as well, Maron et al. (2004) and Iny et al. (1994) found lower 5HTT binding in the midbrain raphe nuclei, in the temporal lobes and in the thalamus using PET techiques. Reduced binding may be induced by decreased synaptic 5HT levels. Indeed, a critical literature review by Maron et al. (2010) showed that studies have not found a clear linkage between 5HTT polymorphisms and PD. Decreased 5HTT binding might be improved by chronic LF stimulation, as chronic HF stimulation is known to decrease 5HTT binding (Ikeda et al., 2005).

Improvement was indeed accomplished by LF rTMS treatment; 14 trials of 1 Hz rTMS over the right dlPFC showed a significant, gradual and sustained improvement (up to 6 months) in over 80% of patients. Moreover, this improvement was related to decreased cortical excitability (Mantovani et al., 2007). However, right frontal LF rTMS was not effective in a case reported by Guaiana et al. (2005), although only nine sessions were performed. Here, 20 sessions of HF left frontal rTMS did improve symptoms of PD, which lasted up to 6 months. As the right hemisphere is more involved in processing of negative mood (George et al, 2002), effectiveness of left frontal HF stimulation can be explained by

decreasing $5HT_{1A}$ sensitivity (Gur et al., 2000) and by improved inhibitory capacity of the right PFC. This was shown by Dresler et al. (2009). They measured improved recruitment of the PFC using near-infrared spectroscopy after left dlPFC stimulation at 10 Hz and 110% of the motor threshold.

Thus, increased firing of the raphe nuclei results in increased 5HT synthesis. As 5HTT binding is decreased, synaptic 5HT levels are probably not elevated, this is in accordance with decreases in $5HT_{1A}$ receptors caused by sensitization. Increased sensitivity of inhibitory autoreceptors could underly increased sensitivity and hyperactivity found in PD patients. This can even be exaggerated due to decreased recruitment of frontal cortices. Therefore, normalizing sensitivity of the fear circuitry in PD patients could be accomplished by HF stimulation of the frontal cortex. This should increase $5HT_{1A}$ receptor levels, thereby normalizing 5HT turnover, and decreasing activity in dorsal raphe nuclei because of increased frontal inhibition.

None of the discussed rTMS studies incorporated sham stimulation to assess TMS effects, neither was comorbidity ruled out. This should be improved in future studies, that also need to include a larger patient group. If chronic HF stimulation of the frontal cortex does not prove effectiveness in all PD patients, individually defined stimulation sites can be addressed, as was done by Dresler et al. (2009).

2.3. Generalized Anxiety Disorder

GAD is defined by at least six months of excessive or unrealistic anxiety and worry about a variety of daily life events and situations, such as work and family, and is difficult to control. Several symptoms have to be present, such as feeling tense or restless, becoming easily fatigued, vigilance, concentration problems, irritability, motor tension, autonomic hyperactivity, and/or difficulty sleeping. The symptoms cause "clinically significant distress" or problems functioning in daily working and social life (DSM-IV-TR).

Etkin et al. (2009) found increased connectivity between BLA and CeA in GAD patients. The BLA contains fear memories and processes threat values. Furthermore, it is hypothesized to increase activation in CeA, which regulates defensive responses. The CeA was found to have increased gray matter volume in GAD patients. Etkin et al. (2009) also showed increased connectivity of the amygdala with frontal cortices, providing a compensatory network. This is in accordance with worrying in GAD, a function to regulate excessive anxiety too. Moreover, pre-stimulus amygdalar hyperactivity has been seen in GAD patients (Carter and Krug, 2009) and medial PFC remains more active in post-worry state in high-worrying patients, compared to low-worrying controls (Paulesu et al., 2010). These findings support the notion that the serotonergic system may be involved in GAD, as

the amygdala and frontal cortices receive extensive serotonergic connections and are connected to each other via serotonergic projections.

No studies found altered 5HT receptor activity in GAD to our knowledge, nor did tryptophan depletion combined with CO_2 inhalation have an anxiolytic effect in GAD. There are studies reporting reduced 5HTT functioning (Hernandez et al., 2002; Iny et al.,1994; You et al., 2005), although Maron et al. (2004) did not find any differences in 5HTT functioning in GAD patients compared to healthy controls. Thus, LF stimulation of the left frontal cortex might be an effective treatment of GAD, by increasing 5HTT mRNA levels. Studies by Bystritsky et al. (2008; 2009) showed that GAD patients remitted (decreases in clinician and patient rated anxiety scales) after 6 sessions of fMRI guided 1 Hz rTMS at 90% of the motor threshold over the right PFC, which remained significant after 6 months. Location of stimulation was determined using the most active area in a gambling task which was assumed to activate anxiety related areas. This could be the reason of effectiveness of right frontal stimulation. As well, LF stimulation could have some effects ipsilaterally, next to decreasing frontal activity.

Thus, GAD patients show hyperactive functioning of fear-circuitry. Increased connectivity within the amygdala indicates increased threat processing and more defensive responses to threats. However, amygdalar hyperactivity is compensated for by increased connectivity with and hyperactivity of the frontal cortex. Reduced functioning of the 5HTT may underly this pathology, as decreases in 5HTT functioning have been reported. Consequently, decreasing amygdalar and frontal activity by increasing 5HTT functioning could relieve GAD symptoms. To conclude, LF stimulation of the frontal cortex in GAD, should increase 5HTT mRNA. Thereby, activity in frontal areas and amygdala could be decreased. However, due to the lack of studies assessing the serotonergic system or effectiveness of rTMS in GAD, it is difficult to establish possible effective rTMS treatments.

2.4. Social Anxiety Disorder

SAD is marked by a persistent fear of social or performance situations in which the person is exposed to unfamiliar people or to possible scrutiny by others. The individual fears that he will humiliate or embarrass himself. The feared situation can be specific, but can also include a broad array (generalized social anxiety disorder). The feared social situation may provoke a situationally predisposed panic attack. Avoidance, anxious anticipation, or distress in the feared situations interferes with daily routine, occupational or social functioning (DSM-IV-TR). Physical symptoms include blushing, profuse sweating, trembling, nausea, rapid heartbeat, shortness of breath, headaches, and feelings of detachment and loss of self-control (ADAA.org), indicating involvement of fear circuitry.

A recent review of several imaging studies concluded that limbic and paralimbic regions may be hyperactive in SAD. This includes amygdala, hippocampus, insula and medial PFC (Freitas-Ferrari et al., 2010). Thus indicating changes in fear circuitry. Interestingly, the medial PFC is hyperactive during provoked anxiety, but hypoactive during anticipation of anxiety. This could be due to functional subdivision within the medial PFC. Ventral medial PFC is associated with self-referential/relevant processing, and could thus be hyperactive during provoked anxiety, the dorsal part is more associated with theory of mind, and could therefore be hypoactive in SAD patients during anticipation (Freitas-Ferrari et al., 2010).

The S allele of the 5HTT gene was related to decreased 5HT reuptake (Furmark et al., 2004), increased blushing propensity (Domschke et al., 2008), increased trait and state anxiety, and increased amygdalar activation in response to anxiety provocation in SAD patients (Furmark et al., 2004). A SPECT study found increased 5HTT binding in thalamus, but not midbrain or pons in SAD compared to controls. This can result from decreased 5HT availability and/or increased 5HTT levels (Van der Wee et al., 2008). However, static peripheral 5HT measurements did not differ between patients and healthy controls (Stein et al., 2010). Thus, dysfunctions could be related to decreased functioning of the 5HTT gene. This is not accompanied by increased 5HT levels, because decreased 5HTT functioning is compensated for by increased 5HTT binding. However, this was contradicted by Stein et al. (1998) who did not find an interaction between 5HTT polymorphisms and SAD. Functioning of the 5HTT cannot be improved by chronic LF stimulation, as has been discussed in PD and GAD, as 5HTT levels are already increased in SAD patients.

Some receptor dysfunctions have been found as well. Stein et al. (1998) did not find a link between SAD and excitatory $5HT_{2A}$ receptor dysfunction, and $5HT_{1A}$ agonist have not proven to be effective (Van Vliet et al., 1997). Still, dysfunctions of the inhibitory $5HT_{1A}$ receptor have been linked to SAD. Decreased $5HT_{1A}$ binding was found in amygdala, anterior cingulate and OFC. This indicated decreased inhibition of frontal cortical, amygdalar and insular activity (Lanzenberger et al., 2007). LF stimulation should also be able to improve $5HT_{1A}$ binding in this patient group. Effectiveness of LF stimulation in SAD has not been studied yet.

However, HF stimulation was assessed in SAD patients and showed that 20 sessions of 20 Hz rTMS over the left dlPFC did decreas scores on a social anxiety scale, but did not lead to remission. There was no comparison to a control group, and comorbidity with depression was not ruled out (Uzun et al., 2009). Thus improvements in mood could account for the improvement seen in SAD symptoms.

To conclude, SAD patients show increased activation of limbic and frontal structures. This can be caused by underlying serotonergic mechanisms, as the $5HT_{1A}$ inhibitory autoreceptor shows reduced binding potential and the 5HTT shows decreased functioning. Thus, normalizing frontal and amygdalar activity, possibly by enhancing $5HT_{1A}$ availability might ameliorate SAD. Therefore, LF rTMS stimulation of left frontal areas could cause normalization of activity by increasing $5HT_{1A}$ function in the affected areas through the spread of magnetic current (Speer et al., 2000).

More studies need to be done to assess the effects of rTMS on SAD. These studies should investigate serotonergic deficits, as well as possible positive effects of LF rTMS on the serotonergic system and SAD symptomatology. Moreover, comorbidity with depression was not ruled out in the study by Uzun et al. (2009), this could have affected their results and should be ruled out in future studies.

2.5. Post-traumatic stress disorder

PTSD develops after exposure to an extremely traumatic, stressful event in which there was (the possibility) of harm, accompanied by intense fear, helplessness, or horror. There might be also be psychological and physiological distress dduring reexperience and at exposure to cues related to the traumatic event. PTSD is also defined by emotional numbing, unability to recall important aspects of the trauma, and persistent symptoms of psychological and physiological hyperarousal (difficulty falling or staying asleep, irritability or outbursts of anger, difficulty concentrating, hypervigilance, and exaggerated startle response) (DSM-IV-TR). However, not everyone exposed to a traumatic event will develop PTSD, thus there must be psychobiological factors at work (Lee et al., 2005).

Neuroimaging studies of PTSD are showing quite consistent data suggesting that PTSD is accompanied by a hyperresponsive amygdala and a hyporesponsive PFC. Moreover, PTSD has been related to decreases in cortisol levels and greater sensitivity to feedback inhibition of the HPA-axis. Thus, frontal hypoactivity and HPA-axis hypoactivity may be associated in PTSD patients (Cohen et al., 2004). The frontal cortex exerts less inhibition on the amygdala, resulting in decreased regulation of emotional responses, hyperarousal and reexperience. The hyperactive amygdala also provides input to the hypothalamus which regulates neuroendocrine responses. Therefore, a traumatic experience can sensitize the CRH circuitry and responses to cortisol. Extreme or persistent (for example, when reexperiencing) hyperarousal during stress eventually results in hypocortisolism, 5HT depletion, and decreased inhibition of emotional responses by the PFC.

5HT depletion was evidenced by Choi, Kung and Choi (1999). This was not caused by metabolic changes, as indicated by a study by Maes et al. (1999), who found no differences in plasma tryptophan between PTSD patients and healthy controls. Maes et al. (1999) did

find decreased platelet [^{3}H]-paroxetine binding in PTSD patients that correlated with symptoms of depression and arousal-anxiety. This decreased binding indicates decreased central 5HTT activity. Using the same method, Arora et al. (1993) found decreased density of platelet 5HT-uptake sites correlating with state anxiety, and Spivak et al. (1999) found decreases in density of platelet 5HT uptake sites correlating negatively with scores on the Hamilton Anxiety Rating Scale in PTSD patients. This is in accordance with findings by Murtza et al. (2006), Lee et al. (2005), and Sayin et al. (2010) who report a link between the S allele of the 5HTT and vulnerability to and severity of PTSD. This connection might depend on the number of traumatic events experienced (Xie et al., 2009) and on high or low risk environment. The S allele was associated with decreased risk of PTSD in low-risk environments (low crime/unemployment rates), but increased risk of PTSD in high-risk environments (Koenen et al., 2009). However, Grabe et al. (2009) found that the L_A allele of the 5HTT predicted PTSD vulnerability in cases with three or more trauma exposures, this was not replicated by a later study (Mellman et al., 2009). The L_A allele was associated with increased hyperarousal, but not re-experience or avoidance (Grabe et al., 2009), although Sayin et al. (2010) found that the L allele may cause milder hyperarousal in PTSD patients. Overall, these results indicate that the L allele protects against development of PTSD, the S allele might increase risk of developing PTSD by increasing environmental influence and affect trauma responses, by decreasing 5HTT function. Thus, again, left frontal LF stimulation should be effective in treatment of this AD by increased 5HTT mRNA. This has not been studied yet, even though 17 sessions of LF right frontal rTMS at 80% of the motor threshold resulted in normalization of cerebral metabolism, correlating with symptom improvement, coming back to baseline after 1 month (this also holds for 30 sessions of rTMS) (McCann, 1998). Decreased cerebral activity can be caused by decreased in synaptic 5HT. Therefore, this finding implies increased 5HTT functioning after LF rTMS. However, improvement was not found after LF stimulation by Cohen et al. (2004). Improvement in PTSD core symptoms and a trend for improvement in anxiety and somatization was also shown by Grisaru et al. (1998) after acute TMS of 0.3 Hz at 100% of the motor threshold over bilateral motor cortex, with improvements being short and transient (24 hours). It is possible that effects of stimulation have spread to connected areas, thereby improving PTSD symptoms.

Although the $5HT_{1A}$ receptor is critically involved in mood and anxiety, Bonne et al. (2005) found no difference in $5HT_{1A}$ receptor expression between groups with or without PTSD. Possibly because density and mRNA levels of this receptor are insensitive to changes in 5HT levels (Krystal and Neumeister, 2009). Decreased expression of another 5HT receptor (2A) was associated with PTSD development (Mellman et al., 2009). The $5HT_{2c}$

receptor may in turn be hyperactive in PTSD, resulting in increased anxiety expression (Christianson et al., 2010). Effects of rTMS on $5HT_{2c}$ receptors are unknown. However, $5HT_{2A}$ expression is reduced after chronic HF rTMS. Therefore, LF rTMS could increase these levels.

In PTSD, hypoactivity of the serotonergic system is thus apparent. Due to 5HT depletion, the medial PFC can exert less inhibition on the amygdala, resulting in hyperactivation. This hyperactivity leads to increased sensitivity of the HPA-axis, as the amygdala is connected to the hypothalamus. This is in accordance with the environment-sensitive effect of the S alle of the 5HTT gene. Thus, increasing medial prefrontal inhibition on the amygdala possibly by increasing 5HTT and $5HT_{2A}$ expression might normalize sensitivity of the HPA-axis and could decrease PTSD core symptoms such as reexperience, avoidance and hyperarousal. This could be achieved by LF stimulation of the left frontal cortex. However, Rosenberg et al. (2002) found improvement in mood, anxiety, anger and sleep symptoms, but not in core PTSD symptoms, which did not differ between 1 and 5 Hz stimulation. This absent difference between LF and HF stimulation indicated effectiveness of HF stimulation as well. Indeed, HF 10 Hz rTMS over right dlPFC improved scores on the PTSD scale, as well as symptoms of reexperience and avoidance in a well controlled study (Cohen et al., 2004). It has been hypothesized that daily stimulation of the dlPFC reduces access to autobiographical memories and thereby reduces PTSD symptoms, as this area is involved in retrieval processes (Rossi et al., 2006). Thus, HF stimulation could have been effective by reducing access to traumatic memories. LF rTMS could adress the underlying deficits in the serotonergic system.

Not all studies discussed above ruled out comorbid depression, this could account for the positive results found after both LF and HF stimulation. LF stimulation did not improve PTSD core symptoms, but did improve mood. However, HF stimulation did improve core symptoms, but this could possibly be mediated by decreases in memory, not by improvement of serotonergic functioning. Thus, future studies should rule out comorbidity, especially with depression, before comparing HF and LF stimulation effects on PTSD symptoms.

2.6. Obsessive-Compulsive Disorder

OCD is defined by obsessions: recurrent unwanted, and distressing thoughts, images, or impulses. These thoughts trigger recurrent behaviours, e.g. compulsions. Compulsions are complex, repetitive, rule-governed behaviors that the patient feels driven to perform (DSM-IV-TR). The repetitive acts seen in OCD are mediated by the basal ganglia, an area involved in movement initiation, but also by other limbic and frontostriatal regions (Murphy, Frazier, and Kim, 2008). The caudate head and its pathway to the OFC and

cingulate gyrus are often hyperactive in OCD patients, and may show decreased volumes. The hyperactive caudate head provides input to the thalamus (Murphy, Frazier, and Kim, 2008) which subsequently innervates the OFC. SSRIs normalize activity in caudate head and OFC, indicating a role for 5HT in this AD. Not surprisingly, as the striatum (including caudate), and thalamus are highly innervated by 5HT, and the OFC and cingulate cortex are both involved in regulation of anxious behaviour (Gazzaniga, Irvy, and Mangun, 2002).

Accordingly, changes in the serotonergic system have been observed in OCD. Reduced 5HTT binding in midbrain/brainstem and thalamic/hypothalamic areas (Hasselbalch et al., 2007; Hesse et al., 2005; Pogarell et al., 2003; Reimold et al., 2007; Stengler-Wenzke et al., 2004) could result in diminished inhibitory regulation of 5HT on fronto-subcortical circuits (Hasselbalch et al., 2007). However, no differences in 5HTT binding were found in a study by Simpson et al. (2003) and Matsumoto (2009) found reduced insula 5HTT availability in OCD, but not in striatum, thalamus or midbrain. Differences in these findings could be caused by different ligands and/or by heterogeneity in the OCD patient groups. Genetic studies confirm the involvement of the 5HTT, as a meta-analysis of 13 independent case-control association studies showed an association with the S allele, but not the L allele of the 5HTT gene (Lin, 2007). However, in a later meta-analysis association with the S allele became non significant and association with the L allele became significant (Bloch et al., 2008). The association between OCD and the L_A allele of the 5HTT remains unclear, because of contradicotry findings (Dickel et al., 2007; Hu et al., 2006). Marazziti et al. (2000) found slower uptake of platelet 5HT, which increased significantly more after protein kinase C activation in OCD patients compared to healthy controls. This signifies an increased inhibitory activity of protein kinase C and increased activity of the phosphatidylinositol pathway or it could signify a hyperresponsive 5HT uptake system. This is in accordance with PTSD findings, where the S allele was related to increased sensitivity of the 5HT system. Thus, as was discussed in PTSD patients, LF stimulation could prove effectivenes by increasing 5HTT availability. Even though the OFC and anterior cingulate are not directly stimulable (Rauch et al., 2001), stimulation of frontal cortices could have remote effects on this regions, as shown by George et al. (1999).

Ruffini et al. (2009) tried to stimulate the left OFC by targeting the frontopolar cortex. They gave OCD patients 15 sessions of LF rTMS at 80% of the motor threshold which led to improvement at the Y-BOCS lasting up to 10 weeks. The procedure did not affect anxiety scores. Unsurprisingly, as the OFC is not implicated in other AD. Improvement was also found after stimulation over the right dlPFC by 1 Hz rTMS in refractory OCD (Chae et al., 2009). This was not replicated, although a non significant improvement in obsessions

was seen (Alonso et al., 2001). Combined LF stimulation over the right dlPFC and supplementary motor area did not improve OCD symptoms either (Kang et al., 2009). However, 10 sessions of LF 1 Hz rTMS over a functionally localized pre-supplementary motor area did improve OCD symptoms and decreased anxiety scores on the Hamilton Anxiety Rating Scale in two treatment-resistant OCD patients (Mantovani et al., 2010). Positive effects of HF stimulation are not always reported. It has shown positive effects on compulsions up to eight hours after stimulation of right lateral PFC (Greenberg et al., 1997), but stimulation of left lateral PFC has not shown effectiveness in OCD in most studies (Greenberg et al., 1997; Sachdev et al., 2001; 2007). Neither did continuous theta-burst stimulation (cTBS) over the left dlPFC affect OCD symptoms, although cTBS over the right dlPFC at 80% of the motor threshold did (Wu et al., 2010).

Changes in 5HT receptors might also predispose to OCD; Meira-Lima et al. (2004) found that the silent polymorphism C516 of the $5HT_{2A}$ gene was related to OCD. A review by Westenberg et al. (2007) concludes from several imaging studies, genetic studies and challenge tests that the $5HT_{2A}$ and $5HT_{2C}$ receptors are probably involved in OCD. Especially because antipsychotics with high affinity for these two receptors enhance the effect of SSRIs, indicating that the high SSRI dose needed to treat OCD patients might stem from desensitizing effects on these receptors (Westenberg et al., 2007), probably by enhancing 5HT transmission via adaptation of these receptors (Matsumoto et al., 2009). An in vivo PET study (Perani et al., 2008) showed that $5HT_{2A}$ receptors were reduced in drug-naïve OCD patients in frontal polar, dorsolateral and medial PFC, as well as in parietal and temporal associative cortices. Decreased $5HT_{2A}$ receptors in medial prefrontal and dorsolateral prefrontal cortices correlated with increased symptom severity. However, Dickel et al. (2007) failed to find associations between OCD and polymorphisms of the $5HT_{1B}$, or $5HT_{2A}$ genes using transmission-disequilibrium test of association. Neither the involvement of the HPA-axis nor changes in cortisol responses in OCD have been evidenced (Kluge et al., 2006; Lucey et al., 1992; Martinez et al., 1995). Thus, changes in 5HT receptors have not been evidenced in OCD.

To conclude, OCD is characterized by hyperactivity of basal ganglia, thalamus, orbitofrontal and medial frontal cortices, and is possibly caused by a hyperresponsive serotonergic system due to decreased 5HTT activity. Decreased 5HT inhibition has wide spread effects on the whole fear circuitry. Activity of the caudate head is increased and spreads to the PFC. This hyperactivity could be decreased by increasing 5HTT levels through LF stimulation of the frontopolar cortex. LF stimulation mainly has effects contralaterally and could therefore increase 5HTT function in the right hemisphere. HF stimulation of the right hemisphere has occasionally also been be effective, but this can be

explained by comorbid depression and improvement in mood after rTMS, apparent in both Greenberg et al. (1997) and Wu et al. (2010). Future studies should concentrate on rTMS treatment of OCD by LF stimulation of either the frontopolar cortex or supplementary motor area. These should be localized individually to ensure effective stimulation. Importantly, to assess real effectiveness of LF stimulation, depression should be ruled out. rTMS has been shown to improve mood and this could affect improvements in obsessions and compulsions.

3. Effects of rTMS on anxiety disorders: mechanisms and future applications

Thus, changes in the serotonergic system, which overlaps with areas implicated in fear and anxiety, are related to AD. We expected that rTMS might prove to be a promising therapeutic treatment for AD, because of its effects on the serotonergic system. Acute HF rTMS has been shown to increase content of 5HT and its metabolite (Ben-Shachar et al., 1997) and increase $5HT_{1A}$ binding sites (Kole et al., 1999), as well as affect 5HT metabolism (Sibon et al., 2006). Chronic HF rTMS showed anxiogenic effects in animals (Isogawa et al., 2005), decreased sensitivity of $5HT_{1a}$ and $5HT_{1B}$ receptors, reduced $5HT_{2A}$ receptors and reduced 5HTT mRNA (Ben-Shachar et al., 1999; Gur et al., 2000; Ikeda et al., 2005). LF stimulation has been shown to have opposing effects to HF stimulation on excitability and potentiation (Speer et al., 2000; Zwanzger et al., 2009) and could therefore also have opposing effects on the 5HT system.

AD could result from hyperexcitability of neural circuits responsible for adaptive fear states (Rosen and Schulkin, 1998) and hyperresponsivity of the HPA-axis gives rise to panic attacks and increased anxiety. Increased sensitivity of inhibitory autoreceptors could underly increased sensitivity and hyperactivity found in PD patients (Nash et al., 2008). HF stimulation of the frontal cortex should increase $5HT_{1A}$ receptor sensitivity (Gur et al., 2000), thereby normalizing 5HT turnover, and decreasing activity in dorsal raphe nuclei because of increased frontal inhibition. Both GAD and PTSD patients show hyperactive functioning of fear-circuitry caused by reduced functioning of the 5HTT (Sayin et al., 2010; You et al., 2005). Consequently, LF stimulation of the frontal cortex should increase 5HTT mRNA, thereby normalizing activity in frontal areas and amygdala. However, absent differences between LF and HF stimulation in PTSD indicated effectiveness of HF stimulation as well. HF stimulation could have been effective by reducing access to traumatic memories (Rossi et al., 2006). SAD patients show increased activation of limbic and frontal structures. This can be caused by decreased functioning of $5HT_{1A}$ inhibitory autoreceptor and 5HTT (Furmark et al., 2004; Lanzenberger et al., 2007). Therefore, LF rTMS stimulation of left frontal areas could cause normalization of activity by increasing

$5HT_{1A}$ function and increasing 5HTT mRNA. OCD is characterized by hyperactivity of basal ganglia, thalamus, orbitofrontal and medial frontal cortices, and is possibly caused by decreased 5HTT activity (Hasselbalch et al., 2007; Reimold et al., 2007). LF stimulation of the frontopolar cortex could prove to be effective.

PD, SAD, OCD, PTSD, and GAD patients indeed show increased activity of the fear circuitry. However, different serotonergic changes underlie this hyperactivity. It is important to note that the different AD may have overlapping but not similar mechanisms, for example, PD patients show hyperactivity of fear circuitry due to increased sensitivity of inhibitory autoreceptors, HF stimulation has been shown to decrease this sensitivity and could therefore be effective in treatment of PD. As well, PTSD symptoms could improve after both HF and LF stimulation, although HF rTMS propably is not effective because of serotonergic effects in this disorder. Moreover, a broader dysfunction could underly OCD. Therefore, not only stimulation of frontal cortices, but of motor cortices might have positive effects on OCD symptoms as well. An option for OCD rTMS treatment could also be stimulation of the frontopolar cortex, which lies in close contact with the OFC.

Although the effects of LF stimulation on the serotonergic system have not been studied yet, it can be expected to have effects on serotonergic transmission. LF stimulation has been shown to be effective in the treatment of several AD by normalizing activity in frontal and limbic regions (Juckel et al., 1999; Mantovani, 2007). HF and LF stimulation have been related to long term potentiation and long term depression, respectively. Accordingly, HF stimulation has been attributed excitatory effects, as opposed to LF stimulation (Speer et al., 2000; Wassermann and Lisanby, 2001; Zwanzger et al., 2009). Thus the effects of HF and LF rTMS could be opposite. Chronic HF stimulation decreases 5HTT, $5HT_1$, and $5HT_{2A}$ receptors (Ben-Shachar et al., 1999; Gur et al., 2000; Ikeda et al., 2005). Therefore, it might be the case that LF rTMS increases these levels, thereby increasing inhibitory 5HT activity, and possibly resulting in decreased activity and sensitivity of the HPA-axis. Moreover, HF stimulation has been shown to have effects bilaterally, as opposed to LF stimulation, that mainly has effects contralaterally (Speer et al., 2000). As the left hemisphere is associated with processing of positive moods, and the right hemisphere with negative moods; the right hemisphere might be hyperactive compared to the left hemisphere (George et al., 2002). LF stimulation might therefore be more effective in treating AD, especially if applied to the left PFC. Moreover, increases in regulation of the HPA-axis might decrease its sensitivity to environmental factors. It is interesting to explore cTBS as well, as it has been proposed to have similar effects to HF rTMS, which are longer lasting and cTBS is safer to use (Wu et al., 2010).

Several points have to be taken in consideration when assessing these results. Firstly, most AD are characterized by decreased functioning of the 5HTT, caused by an S allele. However, decreased 5HTT functioning might still be underestimated: two variants of the L allele (L_a and L_g) give different 5HTT expression levels, but have not been distinguished in most studies. It has been shown that the L_g variant gives nearly the same expression level of the 5HTT as the S allele does. The L_a allele provides increased levels compared to the S allele (Hu et al., 2006). Secondly, studies performed so far are mostly preliminary, using small patients groups, often with comorbid disorders such as depression, and without the use of proper controls. Thirdly, it has to be noted that rTMS has also been reported to affect dopamine levels (Keck et al, 2000) and neuroplasticity (Speer et al., 2000). Therefore, rTMS effects found in earlier studies could be mediated through other systems as well. This should be controlled for in future studies. Future research should also concern differences within AD pathologies and between genders that could account for inconsistent study results. Importantly, a proper site and manner of sham stimulation needs to be found, as any active stimulation has strong placebo effects (Wassermann and Lisanby, 2001), possibly because anxious states are not only affected by the serotonergic system, but also by (other) endocrine, neural, sensory, cardiovascular and motor systems (Millan, 2003). Finally, the effects of rTMS on mood have been demonstrated in depression already, but these results are doubtful as most studies did not us a procedure was used to vary the stimulation site or to incorporate individual neuroanatomical differences (Wassermann and Lisanby, 2001).

Therefore, a functionally guided PET study comparing patients of a particular AD to healthy controls and comparing the effects of active stimulation versus sham should be performed to investigate the effects of frontal rTMS on the serotonergic system. Comorbidity, especially comorbidity with depression, should be ruled out. As well, such a study could compare the effects of right versus left frontal stimulation. A proper functional stimulation site can be found using functional guided TMS, thereby defining the individual optimal stimulation site. Using this method, heterogeneity of patients suffering from the same AD could be partly ruled out. Results of such an study can be used to confirm the exact involvement of the 5HT system in AD, and the effect of rTMS on this system.

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