Testing antidepressant compounds in a neuropsychological model of drug action

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Chapter 1

General Introduction

Background

The efficacy of antidepressants is tested in randomized controlled trials (RCTs). RCTs report rather modest remission rates when it comes to the efficacy of serotonin reuptake inhibitors (SSRIs) (Keller *et al.*, 1998; Trivedi *et al.*, 2006). For instance, an RCT with the SSRI sertraline in chronically depressed patients yielded a response rate of 22% (i.e. reduction of symptoms of at least 50%) and a remission rate of 36% (i.e. symptom reduction below the threshold of disorder) after 12 weeks (Keller *et al.*, 1998). The response rate of outpatients with major depressive disorder (MDD) to treatment with the SSRI citalopram in an observational study was 47%, while the remission rate was only 28-33% after 8 weeks of treatment (Trivedi *et al.*, 2006). Overall, this leaves ± 50% of MDD patients who do not respond to antidepressants (Keller *et al.*, 1998; Trivedi *et al.*, 2006). Meta-analysis of RCTs have also indicated that antidepressants improve the symptoms of depression, but the difference with placebo is small and may only be clinically worthwhile in severely depressed patients (where placebo is less effective) (Khan *et al.*, 2002; Kirsch *et al.* 2008; Fournier *et al.*, 2010).

A complementary tool to predict efficacy of novel antidepressant drug

Although much research effort has been put into the development of new antidepressant drugs, the process of developing a drug often fails at the stage of large RCTs in which an initially promising compound appears to lack efficacy after all (Harmer *et al.*, 2011). Several experimental medicine models have been proposed as preclinical tools in order to predict drug efficacy before the stage large RCTs (Dawson *et al.*, 2011). Experimental medicine models focusing on antidepressant drug development tap into a range of biomarkers underlying the defective processes seen in MDD patients including behavioural measures, electroencephalogram (EEG), Magnetic Resonance Imaging (MRI) and sleep physiology (Pangalos *et al.*, 2007; Dawson *et al.*, 2011).

Among the various experimental medicine models, the cognitive neuropsychological model has been proposed as a complementary tool to predict the efficacy of antidepressant drug even before the stage of large scale and expensive RCTs (Harmer *et al.*, 2009; 2011). Despite the fact that antidepressants may take weeks to show a clinical effect in depressed patients, they do have immediate effects on emotional information processing, even after single doses in healthy volunteers (Harmer *et al.*, 2003; Harmer *et al.*, 2008; Arnone *et al.*, 2009; Murphy *et al.*, 2009; Rawlings *et al.*, 2010). The neuropsychological model of drug action aims to measure early antidepressant effects by detecting the shift from a negative to a more positive bias in different domains of emotional information processing such as attention, memory and processing of emotional facial expressions (reviewed by Harmer *et al.*, 2009). Some markers of depression that are present in remitted patients are also detectable through pharmacological challenges in healthy individuals (Harmer *et al.*, 2003; Harmer *et al.*, 2008; Arnone *et al.*, 2009; Murphy *et al.*, 2009; Rawlings *et al.*, 2010). Furthermore, healthy individuals do not have a current or history of psychiatric disorders and their response to

antidepressants is not contaminated by earlier pharmacological treatment. These attributes of healthy individuals makes them suitable for assessing the effects of interventions in experimental psychological settings.

Cognitive neuropsychological model of drug action

Antidepressants may take 6-12 weeks to elicit a clinically observable reduction of depressive symptoms in MDD patients (Frazer and Benmansour 2002). Antidepressants do have immediate biological effects as several processes (e.g. increasing the amount of serotonin in the synaptic cleft by blockade of transporters) are directly initiated at the molecular level (Berton and Nestler 2006; Stahl *et al.*, 2013). Already in the early stage of antidepressant administration these direct biological effects lead to the initiation of downstream neuro-adaptive processes but also to a shift from a negative to a positive bias in information processing. According to the neuropsychological model of drug action, these induced changes in information processing interact with emotional and social cues from the environment, resulting over the course of time in an effect on mood states (Figure 1). This gives an alternative (complementary) explanation for the delay in clinically observable antidepressant effects. The early shift towards positive emotional processing is proposed to be associated with neural fine-tuning in subcortical and cortical circuits (Review, Harmer *et al.*, 2009), which will be discussed in the following paragraphs.

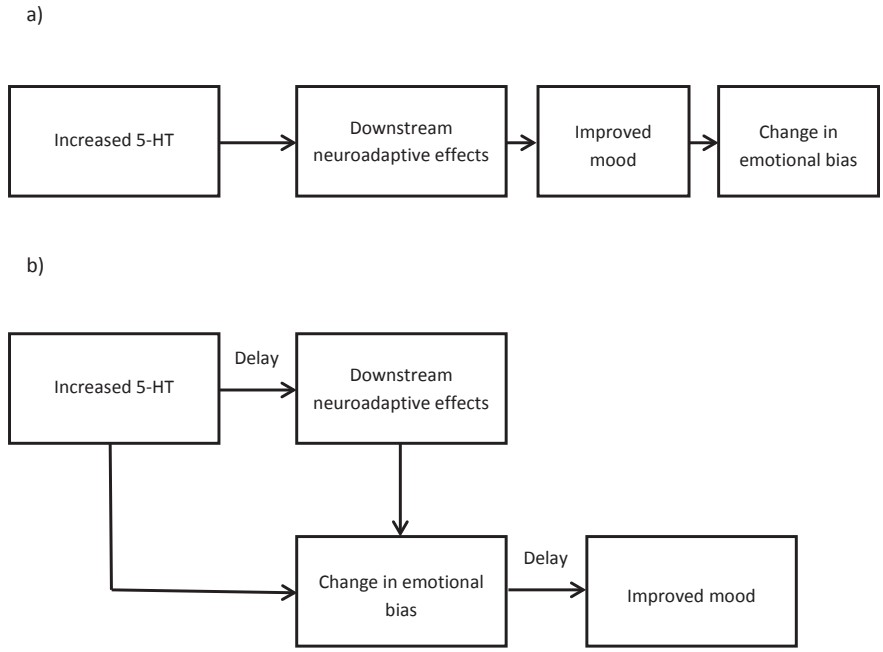

Figure 1. a) Mechanisms underlying the delay in antidepressant drug action as it is generally accepted; b) Mechanisms underlying the delay of drug action as it described in the neuropsychological model of drug action by Harmer et al., 2009.

Validation of the cognitive neuropsychological model of drug action

The cognitive neuropsychological model of drug action has been validated in healthy volunteers by means of behavioural tasks and neural measures, assessing different domains of emotional information processing following administration of conventional antidepressant drugs which act on different neurotransmitter systems (Harmer *et al.*, 2003; Harmer *et al.*, 2008; Arnone *et al.*, 2009; Norbury *et al.*, 2007; Murphy *et al.*, 2009; Rawlings *et al.*, 2010).

Drug administration and early behavioural changes

Two hours following a single dose of the tetracyclic antidepressant mirtazapine (15 mg), healthy volunteers were less accurate in recognizing fearful faces during a facial expression recognition task (FERT) without affecting reaction times (Arnone *et al.*, 2009). Mirtazapine increased reaction times to self-referential words in general, and specifically improved the recall of positive self-referential words during an Emotional Categorization and Memory Task, which is another component of the Emotional Test Battery (ETB) (Arnone *et al.*, 2009).

In another study a single dose of the noradrenergic antidepressant reboxetine (4 mg) improved the recall of happy facial expressions only 2 hours after administration in healthy volunteers (Harmer *et al.*, 2003). In addition, the reboxetine group had a faster reaction time towards positive *vs* negative self- referential words, while this difference was much smaller in the placebo group during the Emotional Categorization Task (Harmer *et al.*, 2003).

The ETB was also completed by healthy volunteers 6 hours after administration of a single dose of the serotonin-norepinephrine reuptake inhibitor duloxetine (60 mg) (Harmer *et al.*, 2008). Duloxetine improved the recognition of both disgust and happy facial expressions without affecting reaction times (Harmer *et al.*, 2008).

These findings provide evidence for early changes in the behavioural response to emotional stimuli following antidepressant drugs administration, however, the interpretation of the changes found on the behavioural measures may vary.

Drug administration and early neural changes

Next to the early detection of changes in emotional processing and biases by means of behavioural measures following antidepressant treatment, early changes at a neural level can also be detected by means of neuroimaging techniques measuring neuronal activity. Neuronal activity is accompanied by an increase in oxygenation metabolism, cerebral blood flow and volume. MRI is an imaging technique that measures these changes in blood flow and provides an indirect measure of neuronal activity in the brain.

A single dose of mirtazapine (15 mg) *vs* placebo was associated with a differential neural response to emotional faces in healthy volunteers two hours after administration (Rawlings *et al.*, 2010). Mirtazapine decreased activation to fearful faces and increased activation to happy

faces in brain regions involved in emotional processing such as the amygdala, hippocampus and fronto-striatal cortex (Rawlings *et al.*, 2010).

Reboxetine administration (8 mg daily for seven days) in healthy volunteers was associated with decreased neural response to subliminally presented fearful faces in the right amygdala and with increased activation in the right fusiform gyrus to subliminally presented happy faces (Norbury *et al.*, 2007).

Furthermore, a single dose of the SSRI citalopram (20 mg) reduced the neural response to fearful faces in the amygdala of healthy individuals, while it did not affect the response to happy and neutral facial expressions (Murphy *et al.*, 2009).

Thus, the findings on the neural response to emotional stimuli following antidepressant drugs administration seems to be more unequivocal compared to the findings on the behavioural response.

The majority of the aforementioned studies investigated the acute effect of various antidepressants on emotional information processing (i.e. on behaviour and neural responses) and reported differences between the antidepressant *vs* placebo groups in absence of changes in mood or subjective state (Harmer *et al.*, 2003; Norbury *et al.*, 2007; Murphy *et al.*, 2009; Rawlings *et al.*, 2010). The findings indicate a positive shift in emotional information processing. These observations may be interpreted as evidence for the cognitive neuropsychological model which hypothesizes that early changes in emotional bias precede the improvement in mood following antidepressant treatment (Harmer *et al.*, 2009). Since single or short-term administration of antidepressant drugs already have a detectable effect in healthy volunteers, the ETB has been applied as a tool to investigate whether new compounds such as GSK424887 (NK1 antagonist and serotonin reuptake inhibitor) (Harmer *et al.*, 2013) and erythropoietin (Miskowiak *et al.*, 2007a; Miskowiak *et al.*, 2007b) may have antidepressant effects. As mentioned above, this approach implements a potential new step in the development of new antidepressant drugs. This might improve the efficiency of the registration process, as initially promising compounds in animals often fail to be effective in depressed patients (Review, Harmer *et al.*, 2011).

In addition to the altered neural and cognitive responses to emotional information, altered resting-state connectivity is associated with MDD and may contribute to its pathophysiology. Several pharmacological studies have investigated the effect of conventional antidepressant drugs on connectivity within the affective networks associated with MDD (Anand *et al.*, 2005; McCabe and Mishor, 2011; van Wingen *et al.*, 2014). These studies have mainly focussed on abnormalities in the cortico-limbic mood regulating circuit (MRC), the default-mode network (DMN) and the task-positive network (TPN) as these have been reported to be altered in depressed patients (reviewed by Wang *et al.*, 2012). Pharmacological resting-state studies

conducted with conventional antidepressant drugs in healthy individuals reported reduced connectivity within the cortico-limbic network (McCabe and Mishor *et al.*, 2011) and reduced connectivity in DMN and TPN, the latter two networks are thought to be increased in MDD patients (Greicius *et al.*, 2007; Wang *et al.*, 2012).

Structure of this dissertation

The studies presented in this thesis concern two projects. In both projects we applied the cognitive neuropsychological model of drug action to test antidepressant effects of a compound in healthy volunteers. In the second project, concerning a well-known compound (L-tryptophan), we further investigated the model by tapping into HPA-axis reactivity and social decision making as additional outcomes, and investigated their interaction with a genetic marker. In the first project, concerning a novel compound (ARA290), we used not only behavioural/cognitive measures but also neuroimaging to detect antidepressant effects.

Pharmacological compound ARA290

Erythropoietin (EPO) is an endogenous protein of which the primary function is to regulate the proliferation of hematopoietic stem cells into red blood cells (erythropoiesis). Next to its role in erythropoiesis, EPO plays a role in tissue protection and repair through anti-inflammatory actions (Brines and Cerami, 2005). EPO administration in animal models of clinical conditions involving tissue damage such as experimental brain damage (Brines *et al.*, 2000) and neonatal stroke (Gonzalez *et al.*, 2009) have shown that EPO crosses the blood brain barrier (BBB) and exerts neurotrophic actions. In humans, administration of EPO improved cognitive performance of patients with multiple sclerosis and schizophrenia (Ehrenreich *et al.*, 2007a; 2007b).

Based on these beneficial effects of EPO on brain tissue and cognition, a series of studies have been conducted to investigate the antidepressant-like effects of EPO, using the neuropsychological model of drug action (Miskowiak *et al.*, 2007a; Miskowiak *et al.*, 2007b). The first studies were in healthy volunteers and later in patients with MDD (Miskowiak *et al.*, 2009; 2010). Although the results of these studies did suggest that a single dose of EPO administration may have antidepressant effects, its utilization as antidepressant drug is rather limited due to hematopoietic side effects of repetitive EPO administration (Wolf *et al.*, 1997; Stohlawets *et al.*, 2000). However, the hematopoietic and tissue protective roles of EPO are regulated by two distinct receptor systems (Figure 2). ARA290 is an 11-amino acid, linear peptide developed as an EPO analogue, which solely acts on a specific receptor (i.e. Innate Repair Receptor; IRR) that initiates tissue protective and anti-inflammatory actions. The IRR consist of a β-common receptor (βCR) subunit (CD131) coupled to an EPOR and its activation initiates multiple signalling pathways resulting tissue protective actions, without initiating hematopoietic effects (Figure 2) (Review, Brines and Cerami, 2012). ARA290 has an

elimination half-life of approximately 2 minutes following i.v. administration (Niesters *et al.*, 2013). Despite the short elimination half-life, ARA290 is suggested to elicit durable effects due to the activation of IRR which regulates the innate tissue-protective response in various stages over a period from hours to days (Brines *et al.*, 2008a; Brines and Cerami, 2012).

The lack of hematopoietic effects makes ARA290 suitable for repetitive administration and - if similar antidepressant-like effects are found as was reported with EPO administration – more promising to develop into an effective antidepressant drug. In Chapter 2 and 3, the first studies testing potential antidepressant-like effects of ARA290 in humans will be described. Specifically, effects of ARA290 on behavioural measures (emotional information processing) and neural measures (task-related and resting-state fMRI) will be presented.

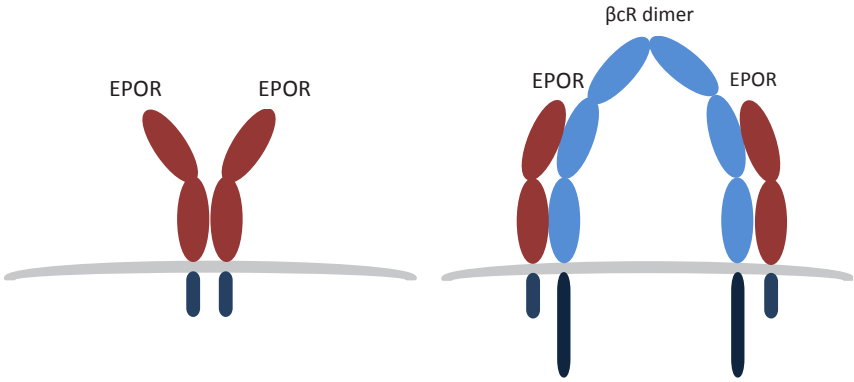

Figure 2. ARA290 is acting on the EPO-R-CD131 receptor to initiate tissue protection (adapted from Brines and Cerami, 2012, The receptor that tames the innate immune response).

Dietary compound: L-tryptophan

Reduced serotonergic activity is involved in the pathophysiology of depression (reviewed by Nestler *et al.*, 2002). Experimental manipulations of serotonin in humans, for instance by means of acute tryptophan depletion, have been extensively used in order to shed light on its role in depression (Review, Booij *et al.*, 2003). Acute Tryptophan Depletion (ATD) is an dietary intervention in which deprivation of L-tryptophan (the amino acid precursor of serotonin) causes a transient state of reduced serotonergic activity. ATD affects mood and cognition in a subgroup of remitted patients treated with SSRIs and recovered depressed patients in a manner that is consistent with a depressogenic effect (Review, Booij *et al.*, 2003). Since reducing levels of tryptophan may induce (transient) depressive symptoms, dietary interventions that increase tryptophan have been used in order investigate whether opposite effects on mood and cognition can be achieved (Booij *et al.*, 2006; Markus *et al.*, 2008). This approach has been applied in depression vulnerable populations defined by, for instance,

high neuroticism scores, high cognitive reactivity scores or s-carriers of the 5-HTTLPR genotype (Markus *et al.*, 1998;Firk and Markus, 2009; Markus and Firk, 2009). One of the multiple dietary interventions to increase plasma Tryptophan/Large Neutral Amino Acids ratio (TRP/LNAA) – an index of central serotonin availability – is to administer pure L-tryptophan (Tryptophan) capsules (Markus *et al.*, 2008). Tryptophan is an amino acid present in nutrients and is daily ingested as part of our diet (World Health Organization, 2002). Besides being the precursor of serotonin, tryptophan is metabolized in various tissues in the periphery and has multiple physiological functions (Le Floc'h *et al.*, 2011). Approximately 90% of ingested tryptophan found in blood and plasma is bound to albumin (Madras *et al.*, 1974; Pardridge 1979). The remaining 10% of free tryptophan in plasma is available for transport through the BBB into the extracellular fluid and cerebrospinal fluid of the central nervous system (CNS) (Le Floc'h *et al.*, 2011). Transport through the BBB is facilitated by transporters embedded in the capillaries of the BBB. These transporters are shared with Large Neutral Amino Acids (LNAA), therefore, the transportation of tryptophan into the CNS is under influence of the TRP/LNAA ratio in plasma (Le Floc'h *et al.*, 2011). Once tryptophan has crossed the BBB it is taken up by cells within the CNS, and is used for i.e. serotonin synthesis as depicted in Figure 3. The synthesis of serotonin is dependent on the amount of tryptophan entering the brain as the rate limiting enzyme, Tryptophan hydroxylase is not saturated at physiological levels of tryptophan in the brain (Le Floc'h 2011; Silber and Schmitt 2010).

Chapter 4 and 5 describes two studies based on the premise that increasing dietary tryptophan intake will increase TRP/LNAA ratio and as a consequence serotonin availability in the brain. The effect of increasing serotonin availability in a genetically vulnerable (i.e. 5-HTTLPR genotype) group was expected to attenuate the physiological response to social stress (i.e. HPA-axis response) and to attenuate the regulation of emotions in response to unfairness (i.e. social-emotional decision making).

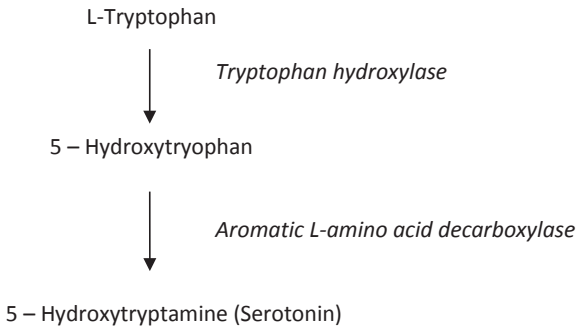

Figure 3. Serotonin synthesis with the precursor L-tryptophan (adapted from Le Floc'h *et al.*, 2011)

Serotonin transporter polymorphism and vulnerability to depression

One of the genetic variations found to be related to depression which has been studied extensively is an allelic variation in the promoter region of the serotonin transporter gene: the serotonin transporter polymorphism (Heils *et al.*, 1996). The serotonin transporter polymorphism is a repetitive sequence in the promoter region consisting of two alleles which can be either long or short. The long (L) variant is associated with higher, whereas the short (S) variant is associated with lower transcription activity. Depending on the combination of the two variants of alleles (i.e. L/L, L/S or S/S) more or less serotonin transporters are expressed in the membrane of the presynaptic neurons. The serotonin transporter regulates the availability of serotonin in the synaptic cleft by removing and recycling serotonin and therefore influences serotonin neurotransmission (Canli and Lesch, 2007).

The serotonin transporter polymorphism (5-HTTLPR) has been associated with personality traits related to anxiety and depression (Lesch *et al.*, 1996). Meta-analysis, however, has reported no consistent direct effects of 5-HTTLPR genotype on the anxiety-related personality traits such as harm avoidance and neuroticism (Munafo *et al.*, 2009).

Apart from the direct effect of 5-HTTLPR genotype, its interaction with adverse childhood events was associated with the probability of depression and with suicide risk (Caspi *et al.*, 2003). Despite the mixed results of meta-analyses and reviews on 5-HTTLPR as a vulnerability gene for depression (Munafo *et al.*, 2009; Risch *et al.*, 2009; Belsky *et al.*, 2009; Uher & McGriffin *et al.* 2010; Karg *et al.*, 2011) the literature on Gene x Environment (G x E) interactions and the modulation of vulnerability markers related to depression by 5-HTTLPR genotype continues to expand (Favaro *et al.*, 2014).

One of these vulnerability markers is the regulation of neuroendocrine responses to stress. The association between 5-HTTLPR genotype and HPA-axis reactivity has been of special interest due to the interaction of the serotonin system and HPA axis (Fuller, 1990; Porter *et al.*, 2004) in regulating neuroendocrine responses. The effect of 5-HTTLPR genotype on HPA-axis reactivity has been studied by inducing acute (social) stress through exposure to a public speaking task and assessing levels of the stress hormone cortisol (Gotlib *et al.*, 2008; Way and Taylor 2010). Some studies showed that homozygous S- allele carriers have an increased cortisol response to acute stress (Gotlib *et al.*, 2008; Way and Taylor., 2010), whereas Mueller *et al.* (2011) reported the opposite: homozygous L-carriers showed an increased cortisol response compared to individuals carrying at least one S allele. A few other studies did not find an effect of 5-HTTLPR genotype on HPA-axis reactivity (Wüst *et al.*, 2009; Verschoor and Markus, 2011). These inconsistent findings may have been caused by confounding effects of gender, type of stressor, time of testing during the day and the use of hormonal contraceptives. Despite the inconsistent findings, however, the association between 5-HTTLPR genotype and HPA-axis reactivity to stress, with homozygous s-carriers exhibiting an increased cortisol response has been confirmed in a meta-analysis (Miller *et al.*, 2013). However, the association had a rather small effect size (d = 0.27) (Miller *et al.*, 2013).

Chapter 4 of this dissertation describes a study in which the association between 5-HTTLPR genotype and cortisol response, as a measure of HPA-axis reactivity, has been investigated. We carefully identified and excluded methodological limitations of previous studies that investigated the association between 5-HTTLPR genotype and HPA-axis activity, but reported inconsistent findings. Specifically, we a) pre-selected participants based on genotype b) exposed every participant to the stressor only once to avoid anticipation) c) and excluded possible confounders of the cortisol response (e.g. use of hormonal contraceptives). The strict study design and in- and exclusion criteria were expected to facilitate the detection of the distinct effect of 5-HTTLPR genotype on the cortisol response. Additionally, we investigated whether the hypothesized elevated cortisol response in homozygous s-allele carriers can be treated by tryptophan supplementation.

Next to HPA-axis reactivity, the 5-HTTLPR genotype has also been associated with emotional processing as a behavioural endophenotype (Perez-Edgar *et al.*, 2010; Antypa *et al.*, 2011; Koizumi *et al.*, 2013). Furthermore, the association between serotonin availability and the regulation of emotions in social decision making has been shown by studies with serotonergic manipulations in healthy individuals (Crockett *et al.*, 2008; 2010). Since variation in 5-HTTLPR genotype is associated with altered processing of emotional and socially relevant information, and serotonin availability is involved in decision making processes in which people have to overcome an emotional reaction, we investigated whether 1) 5-HTTLPR genotype is associated with altered regulation of emotions in response to unfair offers made by others and whether 2) the regulation of emotions in response to unfair offers can be altered by tryptophan supplementation (Chapter 5).

Outline of this thesis

- Chapter 2 describes a placebo-controlled randomized clinical trial, testing the effect of a novel pharmacological intervention, ARA290, on the behavioural and neural measures related to emotional information processing in healthy individuals.
- Chapter 3 describes a study on the effect of ARA290 versus placebo on resting-state connectivity in the same healthy individuals.
- Chapter 4 describes a placebo-controlled randomized clinical trial, testing the effect of a dietary intervention, tryptophan supplementation, on HPA-axis reactivity in healthy carriers of the 5-HTTLPR genotype variants.
- Chapter 5 describes the effect of tryptophan versus placebo on social decision making in healthy carriers of the 5-HTTLPR genotype variants.

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