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REVIEW ARTICLE

Amino acid challenge and depletion techniques in human functional neuroimaging studies: an overview

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Abstract Imbalances of neurotransmitter systems, particularly serotonin (5-HT) and dopamine (DA), are known to play an essential role in many neuropsychiatric disorders. The transient manipulation of such systems through the alteration of their amino acid precursors is a well-known research tool. Among these methods are alterations of tryptophan, the essential amino acid (AA) precursor of 5-HT, as well as manipulations of tyrosine and phenylalanine, the AA precursors of DA, which can be metabolized into norepinephrine and subsequently into epinephrine. These systems can be loaded by applying a large dose of these AAs or depleted by applying an amino acid mixture lacking the respective AAs serving as precursors. Functional neuroimaging has given insights into differential brain activation patterns and functions depending on the tasks performed, pharmacological treatments or specific disorders. Such research has shed light on the function of many brain areas as well as their interactions. The combination of AA challenge approaches with neuroimaging techniques has been subject of numerous studies. Overall, the studies conducted in this particular field of research have shown that AA challenge techniques are valid and effective research tools that allow the investigation of serotonergic and dopaminergic systems without causing serious side effects or long-term damage to the subjects. In this review, we will present an overview of the results obtained so far and discuss the implications of these findings as well as open questions that remain to be answered.

Keywords Amino acids · fMRI · PET · Tryptophan · Tyrosine · Phenylalanine

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Introduction

The modulation of neurotransmission through the ingestion of amino acids (AA) that serve as precursors for neurotransmitters has been a valuable research tool for more than 40 years (Biggio et al. 1974). Since its discovery, many variations and alterations of such AA-related loading and depletion techniques have been used. Today, with regard to AA-related challenge techniques, serotonergic and catecholaminergic modulations as well as loading and depletion paradigms are commonly used. The present paper aims to review and summarize amino acid challenge and depletion techniques in human functional neuroimaging studies.

The neurotransmitter serotonin (5-HT) is a key contributor to the underlying pathophysiology of many

neuropsychiatric disorders such as anxiety and depression. Despite being the subject of many of studies, many facets of this particular neurotransmitter system remain to be uncovered. One well-known concept of studying a temporarily impaired 5-HT system (Walters et al. 1979) is the reduction of its essential AA precursor tryptophan (TRP) through acute tryptophan depletion (ATD). This particular method has been widely used for investigating a variety of aspects, such as endurance of physical training, impulsivity and neural correlates of reward evaluation (Demoto et al. 2012; Fikke et al. 2013; Hobson et al. 2013), in drug-free patients with depressive symptoms (Delgado et al. 1994; Neumeister et al. 1997; Price et al. 1998) as well as in different animal models (Taffe et al. 2003; Biskup et al. 2012; Sánchez et al. 2014). When using ATD, a mixture of AAs is administered that subsequently compete with endogenous TRP for transport across the blood–brain barrier. Thus, less TRP can be transported across the blood–brain barrier (BBB) (Dingerkus et al. 2012). Therefore, less TRP reaches the brain, which in turn reduces 5-HT synthesis (Biskup et al. 2012) and subsequently 5-HT function, as marked by decreases of in 5-hydroxyindolacetic acid (5-HIAA), 5-HT's primary metabolite. ATD can also be safely studied in children and adolescents by using the body weight-adapted modified AA mixture ATD Moja-De. ATD Moja-De has proven to be less nauseating than conventional mixtures and efficiently decreases central nervous serotonin synthesis (Zimmermann et al. 2009; Biskup et al. 2012; Dingerkus et al. 2012; Grabemann et al. 2013; Kötting et al. 2013; Mette et al. 2013; Zepf et al. 2013; Helmbold et al. 2013; von Polier et al. 2014) (Fig. 1).

Another possibility of challenging neurotransmitter systems consists of AA loading. To challenge the serotonergic system, a large dose of TRP is administered. 5-HT synthesis has been reported to be increased due to increased precursor supply, and therefore the serotonin function should be increased (Gál et al. 1978). This particular method has also been studied (Rizza et al. 1983; Glass et al. 1995), but to our knowledge has not been the subject of any imaging studies.

Catecholamines are a group of neurotransmitters comprising dopamine (DA) and its metabolites, norepinephrine (NE) and epinephrine. DA imbalances have been implicated in a variety of psychiatric disorders such as depression, attention deficit-hyperactivity disorder (ADHD) and psychotic syndromes (Hamilton et al. 2012; Seeman 2013; Sharma and Couture 2014). Increased levels of DA are thought to cause hallucinations and delusions, whereas decreased levels of DA are thought to be the cause of apathetic and lethargic symptoms. The catecholaminergic system can be challenged by using phenylalanine (PHE)/tyrosine (TYR) depletion (APTD) (Barratt et al. 1976). PHE is an essential AA and the precursor of the AA TYR. TYR crosses the BBB and is converted into DA, which can subsequently be converted into NE in some neurons, which in turn is the precursor of epinephrine (see Fig. 2). The precursor AAs can be depleted using a method paralleling ATD, in which an AA mixture lacking TYR and PHE is administered (Barratt et al. 1976). This method has been refined and elaborated over the years, most recently by introducing a new form of depletion, the combined monoamine depletion (CMD), which depletes 5-HT and DA simultaneously (Sánchez et al. 2014) (Fig. 2).

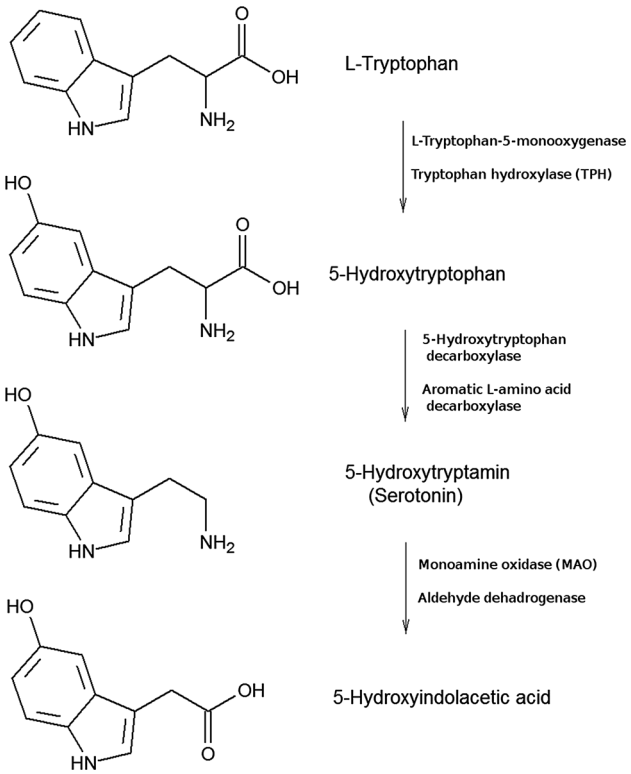

Fig. 1 5-HT synthesis pathway. TRP crosses the BBB and is subsequently synthesized to 5-hydroxytryptophan (5-HTP) via TPH 2. This is the rate-limiting step of serotonin synthesis. 5-HTP is then converted to 5-HT. After release into the synaptic cleft there is some reuptake into the presynapse, remaining 5-HT can be metabolized into 5-homoindoleacetic acid (5-HIAA)

As with 5-HT, the catecholamine system can be challenged with a large dose of TYR, increasing dopaminergic function (Chance et al. 1990). However, as Meyers (2000) suggested, there seems to be less scope for increasing NE synthesis with AA loading than is the case for 5-HT, because under normal conditions, TYR hydroxylase is 75 % saturated (Meyers 2000); whereas, TRP-hydroxylase 2 (TPH 2) is only 50 % saturated (Carlsson and Carlsson 1988). This method has been studied in humans and animals (Alonso et al. 1980; Chance et al. 1990). However, the TYR-challenge has not been used in imaging studies and will therefore not be discussed in the present paper.

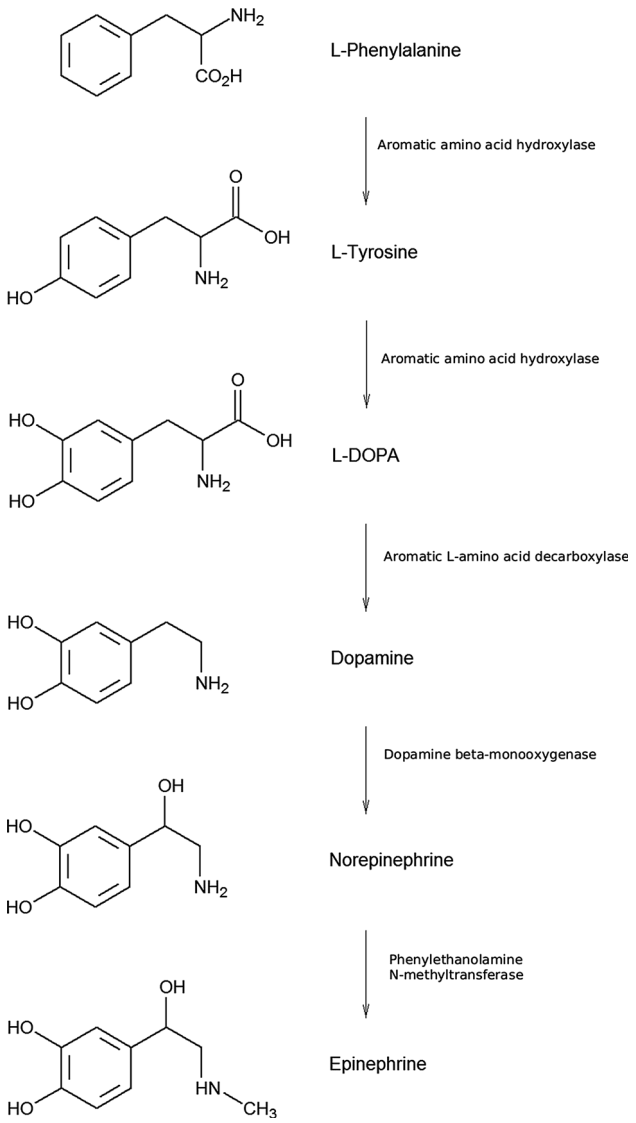

Fig. 2 DA synthesis. The AA phenylalanine is converted to the amino acid tyrosine, which in turn in converted to L-Dopa by the aromatic amino acid hydroxylase. L-Dopa is then converted to DA by the aromatic L-amino acid decarboxylase. DA can then be released as a neurotransmitter and subsequently metabolized to 3-methoxytyramine (3-MT); DA can also be converted to norepinephrine (NE) by the dopamine-β-hydroxylase. NE can in turn be converted to epinephrine

Neuroimaging

A commonly used technique is functional magnetic resonance imaging (fMRI). It is an important tool in translational brain research and is used for human and animal research alike. By measuring the blood oxygen level-dependent (BOLD) contrast, changes in regional blood flow can be measured, which are usually interpreted to reflect changes in the activation of the particular brain region (Ogawa et al. 1993). Another useful tool for functional neuroimaging is positron emission tomography (PET). A positron-emitting radionuclide tracer is introduced into the subject as part of a biologically active molecule. The emitted gamma-rays are detected by the PET, which can then be reconstructed into a three dimensional image of tracer concentrations by computer analysis. Almost any protein can be marked with a positron-emitting label, therefore becoming a tracer. Depending on the tracer, many different characteristics and effects can be measured. Pharmacological fMRI or PET combine AA challenges and functional neuroimaging and allow the detection of brain areas involved in or affected by specific neurotransmitters, which gives a deeper insight into the underlying neurobiology of certain brain functions (Table 1).

For a complete list of the studies reviewed please refer to Table 1.

Serotonergic system

fMRI and ATD

Behavior and social communication in humans are based on detecting, interpreting and responding to facial expressions. The lack of such skills can cause severe limitations to an individual, as seen in patients with autism spectrum disorders. In contrast, patients suffering from other psychiatric disorders, such as depression, show deficits in cognitive flexibility. Both of these areas, facial recognition and cognitive flexibility, are modulated by serotonergic function (Meneses and Liy-Salmeron 2012; Cowen and Sherwood 2013). One possibility of modulating serotonergic neurotransmission is by ATD.

Emotional processing

The influence of ATD on neural activation patterns has been studied under numerous different aspects using fMRI. One frequently studied topic is that of emotional processing. These particular studies apply two different types of paradigms, which either use emotional faces or words of emotional valance as stimuli. First, we will discuss studies on emotional processing of faces viewed during a scan, and we will then turn our attention to the emotional processing of words.

Facial emotional processing

In healthy individuals, a variety of brain regions are involved during face perception. Notably, the core visual analysis regions of the striate and extrastriatal cortices are activated when viewing faces (Haxby et al. 2002). The region that is specific to face recognition, regardless of

Table 1 Studies using amino acid depletion or challenge techniques and neuroimaging

Study	Participants	Task	Increased activation after ATPD	Decreased activation after ATPD	Findings at baseline	Other findings	Conclusion
fMRI and ATD							
Facial processing							
Fusar-Poli et al. (2007)	Healthy males and females (mean age 26 ± 4 years)	Facial emotional processing (happy vs. neutral, sad vs. neutral in blocks of ten, presentation for 2 s)	Left inferior frontal gyrus; response to all emotional faces in right amygdala	Right medial/inferior gyrus, posterior cingulate cortex, occipital and parietal cortex bilaterally, right hippocampus, claustrum insula; response to emotional faces in left amygdala	Processing happy faces compared to sad faces: activation in left anterior and posterior cingulate gyri	–	5-HT modulates the neural correlates of facial emotion processing
Daly et al. (2010)	Healthy males (mean age 28 ± 10 years)	Facial emotional processing (fearful, happy, sad, disgusted, neutral expressions with 50 or 100 % intensity; participants asked to judge gender of face; presentation for 2 s)	Fearful: left cingulate gyrus and insula Neutral: right superior temporal gyrus Disgusted: left anterior cingulate gyrus Happy: right superior frontal gyrus	Fearful: Left fusiform and middle temporal gyri Neutral: left superior temporal gyrus, right medial frontal gyrus Disgusted: left middle temporal gyrus, mid-cingulate gyrus, precuneus Happy: left postcentral gyrus	Increased activation of fusiform cortex, extrastriate cortex, superior temporal gyri, insula, cingulate gyri, medial frontal gyri during face processing vs. fixation cross	–	5-HT system affects limbic and face processing regions, but varies with emotion type
Passamonti et al. (2012)	Healthy males and females (mean age 25 ± 3 years)	Facial emotional processing (angry, sad, neutral expression alternating with fixation cross; presentation for 1 s)	–	Angry: amygdala and ventral anterior cingulate cortex and ventrolateral PFC	–	–	Supporting biological model of negative emotions related to aggression in amygdala-PFC-circuit
Grady et al. (2013)	Healthy males and females (mean age 31 ± 6 years)	Judging of gender of neutral, fearful and angry faces	Angry and fearful: bilateral amygdala, bilateral ventral striatum, fusiform gyri, posterior occipital cortex, medial PFC, right superior temporal sulcus, inferior frontal gyri, right rostral prefrontal cortex, temporal poles	–	Fearful: increased activity in bilateral amygdala, bilateral ventral striatum, fusiform gyri, posterior occipital cortex, medial PFC, right superior temporal sulcus, inferior frontal gyri, right rostral prefrontal cortex, temporal poles	–	ATD enhances the brains response to angry faces, making them indistinguishable from fearful faces

Table 1 continued

Study	Participants	Task	Increased activation after ATPD	Decreased activation after ATPD	Findings at baseline	Other findings	Conclusion
Robinson et al. (2013)	Healthy males and females (mean age 25 years)	Forced choice identification task with happy or fearful faces with word distracters ("HAPPY" or "FEAR"; presentation for 1 s)	Fearful rel. to happy: dorsal medial prefrontal cortex Generally: amygdala	–	–	Increase of dorsal medial prefrontal cortex-amygdala circuit connectivity during fearful faces	Serotonergic dysfunction may contribute to negative bias observed in, i.e., anxiety or mood disorder
Cools et al. (2005b)	Healthy males (mean age 23 ± 3 years)	Facial emotional processing (fearful, happy, neutral; participants asked to judge gender of face; presentation for 960 ms)	–	–	Fearful faces induced signal chance in bilateral amygdalae and fusiform gyrus	Modulation of amygdala and hippocampus activation after ATD in response to fearful vs. happy faces as a function of self-reported threat-sensitivity	"Individual variation in threat sensitivity interacts with manipulation of 5-HT function to bias the processing of amygdala-dependent threat-relevant stimuli"
van der Veen et al. (2007)	Healthy males and females vs. males and females with a family history of depression (mean age 21 ± 0.6 years)	Determining which of two faces showed the strongest emotion	Generally: increased activation in right amygdala	–	–	Intense vs. weak expressions: increased activation in right amygdala, bilateral fusiform gyrus, right medial frontal gyrus, right middle frontal gyrus, right cuneus, right insula	Effects of ATD on mood, performance and brain activation in facial emotion perception depend on family history of depression
Williams et al. (2007)	Healthy males (ages 18–30 years)	Front vs side-viewed faces; participants were asked to judge whether expression was neutral or emotional	Side viewed: right superior temporal sulcus, right amygdala, temporal pole. Front-viewed: BA 44, right amygdala	Generally: left superior temporal sulcus, anterior cingulate Side viewed: lateral orbitofrontal cortex Front-viewed: lateral orbitofrontal cortex, medial prefrontal cortex, temporal pole	–	"Effects of ATD were greater on side-viewing faces than front-viewing faces"	"Averted faces and reduced serotonin function facilitate attention to the external goal of gaze"

Table 1 continued

Study	Participants	Task	Increased activation after ATPD	Decreased activation after ATPD	Findings at baseline	Other findings	Conclusion
Daly et al. (2012)	Males with autism spectrum disorder (ASD) (mean age 31 ± 11 years) vs. healthy males (mean age 31 ± 13 years)	Facial emotional processing (happy, sad, disgust, fear, neutral; participants asked to judge gender of face)	Healthy: cingulate gyrus ASD: medial frontal and lingual gyrus/cuneus	Healthy: medial frontal gyrus and lingual gyrus/cuneus ASD: cingulate gyrus	Participants with ASD vs. control: increased activation in a cluster centered in the left lingual gyrus including left para-hippocampal gyrus, cuneus and middle occipital gyrus, right fusiform gyrus, inferior, middle, and superior temporal gyri; inferior and middle occipital gyri; and cuneus	Modulated activation intensity in bilateral cingulate cortex, left postventral gyrus, right cerebellum with respect to intensity of disgust	
Emotional processing of words							
Roiser et al. (2008)	Healthy males and females (ages 18–50 years)	Affective Go/No-Go with emotional words; participants were informed before each block which emotion to respond to	Emotional vs. neutral targets: left ventral putamen, left thalamus, left amygdala, right parahippocampal gyrus, bilateral parietal operculum and right anterior insula/putamen, right superior temporal gyrus, left posterior gyrus, bilateral caudate, right inferior temporal gyrus, right VLPFC and left posterior cingulate, right DLPFC Emotional vs. neutral distractors: right superior temporal gyrus, left posterior hippocampus, bilateral caudate, right inferior temporal gyrus, right VLPFC, left posterior caudate Negative vs. positive words: superior temporal gyrus, posterior cingulate cortex-	Emotional vs. neutral words: dorsolateral PFC and dorsal anterior cingulate cortex Emotional vs. neutral distracters: dorsal anterior cingulate cortex	Emotional vs. neutral: increased activation in posterior cingulate, ventromedial PFC, pregenual anterior cingulate, mid-cingulate cortex, superior temporal cortex Neutral vs. emotional: increased activation VLPFC	Self-reported anxiety state after ATD was connected to an increase in activation in the right caudate.	5-HT plays an important role in mediating automatic negative attentional biases in major depression, as well as resilience against negative distracting stimuli in never-depressed individuals

Table 1 continued

Study	Participants	Task	Increased activation after ATPD	Decreased activation after ATPD	Findings at baseline	Other findings	Conclusion
Roiser et al. (2009)	Healthy males and females vs. males and females with remitted major depressive disorder (rMDD)	Affective Go/No-Go with emotional words; participants were informed before each block which emotion to respond to	Controls: Neutral vs. emotional words: dorsal anterior cingulate cortex rMDD: left habenula	–	Controls: increased response to neutral words in thalamus, caudate and putamen rMDD: increased activation of dorsal anterior cingulate cortex	All subjects: Higher resting-state blood flow in amygdala associated with more negative emotional bias following ATD	"Alterations in neural processing of emotional stimuli following ATD in rMDD subjects, even in absence of overt mood-change"
Roiser et al. (2012)	Healthy males and females vs. males and females with remitted major depressive disorder (rMDD), genotyped for serotonin transporter polymorphisms (high risk vs. low risk)	Affective Go/No-Go with emotional words; participants were informed before each block which emotion to respond to	Controls (low risk): Negative words: hippocampus and subgenual cingulate cortex; medial temporal regions	Controls (high risk): Negative words: hippocampus and subgenual cingulate cortex rMDD(low risk): Negative words: hippocampus and subgenual cingulate cortex, SGC, medial temporal regions	–	–	"Increased neural responses to negative words following [ATD] may reflect an adaptive mechanism promoting resilience to mood change following perturbation of the serotonin system, which is reversed in sub-groups vulnerable to developing depressive symptoms"
Cognitive flexibility							
Rubia et al. (2005)	Healthy males and females (mean age 26 ± 4 years)	Go/No-Go task (arrows as stimuli)	No-Go: right middle temporal and left middle-superior and inferior temporal gyri	No-Go: Right inferior prefrontal cortex	–	–	"Serotonergic modulation of right inferior prefrontal during inhibitory motor control"
Evers et al. (2006a)	Healthy males (mean age 23 ± 2 years)	Go/No-Go task (No-Go: X preceded by X or Y preceded by Y)	–	Performance monitoring: dorsomedial PFC	Performance monitoring: increased activity in anterior cingulate	–	"More evidence for the suggested role of 5-HT in performance monitoring"

Table 1 continued

Study	Participants	Task	Increased activation after ATPD	Decreased activation after ATPD	Findings at baseline	Other findings	Conclusion
Helmbold et al. (2015)	Healthy females (mean age 24 ± 3 years)	Go/No-Go task	–	Medial orbitofrontal cortex and dorsal ACC	No-Go with punishment: Ventral and subgenual anterior cingulate	Neural activation in the medial orbitofrontal cortex (mOFC) and the dorsal ACC correlated positively with trait impulsivity	The results indicate a conjunction of reduced serotonergic functioning and trait impulsivity
Horacek et al. (2005)	Healthy males and females (mean age 23 ± 2 years)	Standard Stroop Color-word task	Generally: medial frontal gyrus, right middle frontal gyrus, superior frontal gyrus, right inferior frontal gyrus, right anterior cingulate, left inferior frontal gyrus Interference condition: bilateral medial, inferior, and superior PFC and anterior cingulate cortex	–	–	–	"[…] The serotonergic medial forebrain and cingulum bundle pathways play a role in the activity of cortical structures involved in Stroop test processing"
Evers et al. (2006b)	Healthy females (mean age 22 ± 1 years)	Standard Stroop color-word task	Congruent color words: left precuneus and cuneus; during the first Stroop-block: anterior cingulate cortex Generally: anterior cingulate cortex	–	Interference condition: increased activation in left inferior parietal, middle frontal and right superior temporal cortex	–	"[…] A temporary reduction of 5-HT improved Stroop performance and changed the underlying brain activation pattern […] Moreover, we replicated our previous finding that ATD modulated the BOLD response in the dorsomedial prefrontal cortex during tasks that require cognitive control"
Evers et al. (2005)	Healthy males (mean age 24 ± 3 years)	Probabilistic reversal learning task (learning in a two-choice pattern, which patterns trigger a positive feedback; adaptation to a switch halfway through the task is assessed)	Reversal switch errors vs. correct baseline responses: dorsomedial PFC	Dorsomedial PFC	–	–	"ATD affects reversal learning and the processing of aversive signals by modulation of the dorsomedial PFC"

Table 1 continued

Study	Participants	Task	Increased activation after ATPD	Decreased activation after ATPD	Findings at baseline	Other findings	Conclusion
Memory							
van der Veen et al. (2006)	Healthy males (mean age 23 years)	Episodic memory task with emotionally neutral words	–	Encoding: right hippocampus	Encoding: increased activation in inferior occipital gyrus, bilateral frontal gyrus, left superior frontal gyrus, left caudate nucleus, bilateral anterior cingulate gyrus, right globus pallidus, left posterior cingulate gyrus Recognition: bilateral occipital gyrus and cuneus, left middle frontal gyrus, bilateral inferior frontal gyrus	–	"5-HT is important in long-term memory processes, and that serotonin acts on the encoding phase and not on the retrieval phase"
Allen et al. (2006)	Healthy males and females (ages 23–35 years)	2-back verbal working memory and phonological verbal fluency task	Verbal memory: left middle and medial frontal gyri, left precentral gyrus, superior parietal lobule, right middle frontal gyrus, cingulate gyrus; posterior cingulate gyrus Verbal fluency: left insula, left superior, middle and inferior gyri, right cingulate gyrus	Verbal memory: right superior/medial frontal gyrus Verbal fluency: left medial frontal gyrus, precuneus	2 back-task vs. 0 back-task: increased activation in middle frontal gyrus and superior/inferior parietal lobule bilaterally, left superior temporal gyrus, right anterior cingulate gyrus Verbal fluency: increased activation in left superior, middle and inferior frontal gyri, left medial frontal and superior temporal gyri, right cingulate gyrus	–	"The engagement of prefrontal cortex during verbal working memory and verbal fluency tasks is significantly modulated by central serotonergic activity"
Wang et al. (2009)	Healthy males and females (mean age 24 ± 3 years)	Emotional oddball task (press one button in response to target, press other button in response to other objects)	All stimuli: left inferior frontal gyrus and anterior cingulate gyrus Negative stimuli: orbital IPG, dorsomedial PFC, bilateral angular gyrus, middle temporal cortex areas	Target stimuli: dorsomedial PFC, posterior cingulate and orbital inferior–frontal cortex	Target stimuli: deactivation of dorsomedial PFC, dorsolateral PFC, dorsal anterior cingulate cortex, insula, motor and sensory cortex, supplementary motor cortex, inferior parietal cortex, cerebellum Negative stimuli: increased activation dorsomedial PFC, ventromedial PFC, bilateral ventrolateral PFC, bilateral middle temporal cortex, hippocampus, bilateral fusiform gyrus, precuneus	–	"These findings highlight the importance of serotonin in negative memory with implications for mood disorders"

Table 1 continued

Study	Participants	Task	Increased activation after ATPD	Decreased activation after ATPD	Findings at baseline	Other findings	Conclusion
Epperson et al. (2012)	Postmenopausal, healthy females (ages 40–65 years)	N back-task (working memory) and emotion identification task	Emotion identification no estrogen: orbital frontal cortex and bilateral amygdala	2 back-task, no estrogen: right and left dorsal lateral prefrontal and middle frontal/ cingulate gyrus Emotion identification with estrogen: orbital frontal cortex and bilateral amygdala	–	–	"Preliminary evidence that serotonergic effects directly mediate the impact of estrogen on brain activation during working memory and affective processing"
Other studies							
Krämer et al. (2011)	Healthy males (mean age 25 ± 3 years)	Taylor Aggression Paradigm (competitive reaction time game; if lost, participants believed they would be punished by thermal stimulation by opponent; high vs. low provocation)	Low-trait aggressive patients: insula	Decision phase: right insula	Increased activation in the dorsal anterior cingulate cortex during decision phase	–	"[…] Aggression diminishing effect of ATD in low-trait aggressive participants […]"
Lamar et al. (2009)	Healthy females (mean age 63 ± 5 years)	Cognitive interference task (Simon task)	Neocerebellum and parietal lobe	Left inferior PFC, anterior cingulate cortex and basal ganglia	activation in left inferior PFC	–	"ATD modulates task-relevant brain activation for cognitive interference inhibition and is associated with an anterior-to-posterior activation shift"
Demoto et al. (2012)	Healthy males (ages 20–27 years)	Multi-step delayed reward choice task	–	–	–	Several temporal and frontal areas as well as cerebellum, insula, caudate, precuneus	"Individuals who have high neuroticism and low self-directedness as personality traits are particularly vulnerable to the effect of low serotonin on future reward evaluation accompanied by altered brain activation patterns"
Seymour et al. (2012a, b)	Healthy males and females	Probabilistic instrumental learning task in a four arm bandit decision-making task	–	Reward: ventromedial PFC Error: dorsolateral putamen	–	–	"Role for serotonin in reward processing, while illustrating its complex and multifarious effects"

Table 1 continued

Study	Participants	Task	Increased activation after ATPD	Decreased activation after ATPD	Findings at baseline	Other findings	Conclusion
Hindi Attar et al. (2012)	Healthy males (mean age 28 ± 5 years)	Fear learning [Pavlovian conditioning; stimuli consisted of phasic temperature increases (pain) or decreases (relief of pain)]	Orbitofrontal cortex	Amygdala	–	–	"First empirical evidence for a role of serotonin in representing formally derived learning signals for aversive events"
Salomon et al. (2011)	Patients remitted from Major Depressive Disorder (rMDD) (ages 18–50 years)	Resting state	Pontine region	Decreased functional connectivity between pontine raphé and anterior thalamus	–	–	"Supports using fMRI time-series features to assess pontine raphé function"
Labus et al. (2011)	Healthy females (ages 19–25) vs. female patients with constipation-predominant irritable bowl syndrome (ages 24–50 years)	Rectal distention up to 45 mmHg	Healthy baseline and distention: amygdala and emotional arousal network	–	–	In all subjects: ATD caused a loss of negative feedback inhibition in amygdala	"[…] Greater engagement of central serotonin system with more aversive visceral stimuli"
Macoveanu et al. (2013)	Healthy males and females (mean age 32 ± 6 years)	Go/No-Go task (with three trial types: Go, alternative Go, No-Go) under ATD, Citalopram	Response inhibition: right inferior frontal gyrus	(As compared to citalopram) No-Go: left IFG	–	ATD abolished the difference between No-Go and AltGo trials in right IFG "ATD produced a relatively larger No-Go response in the right IFG in subjects with low 5-HT2A [receptor binding in PET [but reduced the No-Go response in those with high 5-HT2A [receptor binding in PET]"	"Links between serotonergic function and response inhibition in healthy subjects may help to interpret serotonergic abnormalities underlying impulsivity in neuropsychiatric disorders"

Table 1 continued

Study	Participants	Task	Increased activation after ATPD	Decreased activation after ATPD	Findings at baseline	Other findings	Conclusion
PET and ATD							
Agren and Reibring (1994)	Healthy vs. unipolar depression	[^{11}C]5-HTP and [^{11}C]L-DOPA	–	–	Depressed vs. healthy: 30 % lower uptake of 5-HTP into the brain, higher 5-HTP utilization in medial prefrontal cortex	–	The reduced uptake of 5-HTP in depressed patients could suggest "a compensatory increase of serotonin synthesis"
Brain metabolism							
Bremner et al. (1997)	Patients with Major Depressive Disorder (MDD) (ages 18 to 65 years)	Fludeoxyglucose F^{18}	Relapse-prone patients: PFC and limbic regions	Dorsolateral PFC, thalamus, orbitofrontal cortex	–	Decrease in metabolism correlated with depressive symptoms	"[…] Middle frontal gyrus, thalamus and orbitofrontal cortex may mediate the symptoms of patients with major depression"
Morris et al. (1999)	Male patients with rMDD (mean age 39 years)	$H_2^{15}O$; during scan participants performed either a paced word repetition task or a paced orthographic verbal fluency task	–	Task-specific activation: left amygdala, left anterior cingulate cortex	–	Correlation between habenulae and raphé activity and depressed mood	"These data support a model of the serotonergic system in which the habenula projection to the raphé represents a convergent feedback pathway that controls the release of 5-HT throughout the brain"
Smith et al. (1999)	Male patients recovered from depression (mean age 39 years, ranging from 27 to 64 years)	$H_2^{15}O$; during scan participants performed either a paced word repetition task or a paced orthographic verbal fluency task	–	With increasing levels of depression: orbitofrontal cortex, subgenual anterior cingulate, left caudate nucleus, left extrastriate cortex, superior parietal cortex bilaterally	–	–	"[…] Changes in neural activity in distinct brain regions mediate the clinical phenomena of depression and depression-related cognitive impairment following [ATD]"

Table 1 continued

Study	Participants	Task	Increased activation after ATPD	Decreased activation after ATPD	Findings at baseline	Other findings	Conclusion
Neumeister et al. (2004)	Men and women with remitted MDD (rMDD) (mean age 39 ± 13 years) Healthy males and females (mean age 34 ± 11 years)	Fludeoxyglucose F^{18}	rMDD: orbitofrontal cortex, medial thalamus, anterior and posterior cingulate cortices, ventral striatum	–	–	–	"[A]TD unmasks a disease-specific, serotonin system–related trait dysfunction and identifies a circuit that probably plays a key role in the pathogenesis of MDD"
5-HT receptors							
Udo de Haes et al. (2002)	Healthy males (ages 19-62 years)	[^{18}F]MPPF and [^{15}O]H_2O	–	–	–	"[[^{18}F]MPPF distribution] was in agreement with previous results and with known 5-HT_{1A} receptor localization, with the highest uptake in the MTC and low uptake in the cerebellum […]"	"Mean binding potentials in the medial temporal cortex, cortical regions, and raphé nucleus did not significantly differ between the two conditions. [^{18}F]MPPF binding was not affected"
Praschak-Riedler et al. (2004)	Males patients remitted from major depressive disorder (rMDD) (mean age 42 ± 9 years)	[^{18}F]MPPF	–	–	–	"There was no change in 5-HT1ABP as found with [18F] MPPF PET in the hippocampus and other a priori selected brain regions"	"Large-magnitude changes in extracellular 5-HT are not crucial for the mood effects observed in SSRI-treated subjects after [ATD]. Therefore, greater consideration must be given to other mechanisms that involve vulnerability to small perturbations in extracellular 5-HT, such as impairment of signal transduction"
Yatham et al. (2001)	Healthy women (mean age 30 ± 8 years)	^{18}F-setoperone	–	"In an extensive cluster of voxels embracing frontal, temporal, parietal and occipital cortical regions"	–	–	"[…] These findings suggest that a decrease in 5-HT_2 binding following [ATD] might be an adaptive response that provides protection against depressive symptoms"

Table 1 continued

Study	Participants	Task	Increased activation after ATPD	Decreased activation after ATPD	Findings at baseline	Other findings	Conclusion
Yatham et al. (2012)	Male and female patients recently remitted from Major Depressive Disorder (rMDD) (mean age 41 ± 11 years)	^{18}F-setoperone	–	Right frontal, left medial frontal, right temporal and parietal, and right and left occipital regions	Decreased binding in: anterior cingulate, medial orbitofrontal cortex	–	"Reduction in brain serotonin 2 receptors might be a potential compensatory mechanism to prevent tryptophan depletion–induced depressive relapse"
Talbot et al. (2012)	Healthy males and females (ages 18-55 years)	^{11}C-MDL100907	–	Right prefrontal cortex	–	–	"[…] Support for 11C-MDL100907 as a PET tracer with very favorable properties for quantifying 5-HT2A receptors in the human brain"
5-HT transporters							
Talbot et al. (2005)	Healthy males and females (mean age 30 ± 5 years)	^{11}C-DASB	–	–	–	–	"These results suggest that ^{11}C-DASB in vivo binding is not affected by reductions in endogenous 5-HT"
Praschak-Rieder et al. (2005)	Healthy males and females (mean age 35 ± 8 years)	^{11}C-DASB	–	–	–	–	"Acute changes in 5-HTT density or affinity are unlikely to play a role in protecting healthy subjects against mood symptoms during [ATD]"
Neumeister et al. (2006)	Male and female patients remitted from Major Depressive Disorder (rMDD) vs. healthy controls	Fludeoxyglucose F^{18}	Orbitofrontal cortex, subgenual anterior cingulate, pregenual anterior cingulate rMDD with L/L genotype: left amygdala	rMDD with L/S genotype: left amygdala	–	–	"Variations in *5-HTTLPR* modulate the sensitivity of patients with rMDD and controls to the behavioral effects of TD"

Table 1 continued

Study	Participants	Task	Increased activation after ATPD	Decreased activation after ATPD	Findings at baseline	Other findings	Conclusion
Nugent et al. (2008)	Healthy males and females (mean age 34 ± 12 years)	Fludeoxyglucose F^{18}	–	–	–	"79 % of the cases were classified correctly by genotype, and 85 % […] by phenotype. In a leave-one-out cross-validation, 72 % of the subjects were classified correctly as carrying an s-allele, and 79 % of the subjects were classified correctly by primary diagnosis"	"[…] Much of the variance in metabolic response to ATD is accounted for by genotypic and phenotypic category"
Other studies							
Cox et al. (2011)	Male and female non-dependent cocaine users (mean age 25 ± 4 years)	^{11}C-raclopride; participants were administered cocaine or a placebo powder	Cocaine: ventral striatum, post-commissural putamen Without cocaine: right ventrolateral posterior putamen and bilateral anterior putamen	–	–	–	"[…] Low serotonin transmission can increase dopaminergic and appetitive responses to cocaine"
Sacher et al. (2012)	Healthy males and females (mean age 25 ± 8 years)	^{11}C-harmine (MAO-A substrate); ATD vs. levidopa as dopaminergic challenge	–	Prefrontal cortex	–	Increased binding in striatum after levodopa	"[…] Adaptive role [of] MAO-A in maintaining monoamine neurotransmitter homeostasis […]"
fMRI and ATPD							
Nagano-Saito et al. (2012)	Healthy males and females (mean age 24 ± 4 years)	Motion discrimination task and response time task	Primary and extrastriate visual areas, IPS	–	Random motion: fusiform gyrus, middle temporal area, anterior insula, ventrolateral prefrontal cortex, nucleus accumbens, ventral putamen, sublenticular extended amygdala, frontal corticobasal thalamocortical loop (dorsolateral prefrontal cortex, caudate nucleus, premotor cortex, bilateral thalamus), posterior cingulate gyrus, parahippocampal gyrus, cerebellum	–	"[…] Some reward-related functional MRI signals in the striatum are the result of dopamine neuron activity and […] mesolimbic dopamine transmission can influence perceptual and decision-making neural processes engaged to maximize reward harvest"

Table 1 continued

Study	Participants	Task	Increased activation after ATPD	Decreased activation after ATPD	Findings at baseline	Other findings	Conclusion
Coull et al. (2012)	Healthy males and females (mean age 26 years)	Temporal and color discrimination (working memory)	–	Sample stage: left putamen, SMA,	–	Decreased SMA activity under ATPD correlated with APTD-induced decrease in timing performance	"[…] In healthy humans […] DA manipulation perturbs timing by attenuating the activity in putamen and SMA that mediates initial storage of temporal information into WM"
Bjork et al. (2014)	Healthy males (mean age 29 ± 5 years)	Monetary incentive delay task	Loss notification: thalamus, left anterior insula, subgenual anterior cingulate cortex, posterior mesofrontal cortex	–	–	–	"(1) Dietary depletion of catecholamine precursors will blunt dopaminergic mesolimbic activity, and (2) in controls, synthetic pathways of this neurocircuitry maintain sufficient buffering capacity to resist an effect on motivated behavior"
PET and ATPD							
Ellis et al. (2007)	Healthy males (mean age 48 ± 11 years)	$H_2^{15}O$; participants performed a spatial working memory task during the scan	Left putamen, bilateral hippocampal region, left inferior and superior frontal gyri, putamen	Right inferior temporal gyrus, medial frontal gyrus bilaterally, anterior cerebellar lobe and pons	–	ATPD had no effect on task related regional blood flow	"These findings question the capacity of TPD to consistently modulate dopamine function and SWM neural networks in humans"

* [^{18}F]MPPF = [18F]4-(2 = -methoxyphenyl)-1-[2 = -[N-(2 ==-pyridinyl)-*p*-fluorobenzamido]ethyl]piperazine

emotion, is the fusiform gyrus, known as the fusiform face area (FFA) (Kanwisher et al. 1997). Many studies point toward dense serotonergic innervations of brain regions involved in facial processing (Bergström et al. 1997; Carli and Reader 1997; Varnäs et al. 2004). The 5-HT system is therefore quite possibly involved in the modulation of activity in and between regions in facial emotion-processing systems comprising the FFA and limbic regions. However, studies using ATD and fMRI have shown mixed results.

Fusar-Poli et al. (2007) examined the effect of ATD on emotional face processing in healthy male and female volunteers and described an attenuated activation of the right medial/inferior frontal gyrus, posterior cingulate cortex, bilateral occipital and parietal vortex, right hippocampus, claustrum and insula after ATD. However, the authors reported an increased activation in the left inferior frontal gyrus and, in response to emotional faces, in the right amygdala. After ATD intake, the authors observed an increased activation in the left superior temporal gyrus during the processing of sad faces; during the processing of happy faces activation was attenuated. Overall, ATD had differential effects on activation in the left superior temporal gyrus during the processing of happy and sad faces. The authors therefore concluded that there must be a serotonergic modulation of emotional facial processing.

Daly et al. (2010) used the same design in healthy males, but reported slightly different results. In this study, ATD differentially affected activity during the processing of happy faces in the left precentral gyrus (increased activation) and anterior cingulate gyrus (attenuated activation). Viewing fearful faces after ATD intake increased activation in the left cingulate gyrus and insula and decreased activation in the left fusiform and middle temporal gyri. Viewing neutral faces after ATD increased activation in the right superior temporal gyrus and caused attenuated activation in the left superior temporal gyrus and right medial frontal gyrus. Disgust caused increased activation in the left anterior cingulate gyrus and a decrease in activation in the left middle temporal gyrus, mid-cingulate gyrus and precuneus. Finally, viewing happy faces caused increased activation in the right superior frontal gyrus and decreased activation in the left postcentral gyrus. ATD modulated the activation intensity in the bilateral cingulated gyri, left postcentral gyrus and right cerebellum with respect to the expression intensity when viewing a disgusted face. These findings showed that 5-HT differentially modulates facial emotion processing and varies with emotion type.

Passamonti et al. (2012) used a similar design, studying ATD in healthy men and women, and reported that ATD significantly modulated the connectivity between the amygdala and the ventral anterior cingulated cortex as well as the ventrolateral prefrontal cortex when processing angry faces as compared to other expressions. Based on their results, they suggest a neurobiological model of 5-HT that suppresses negative emotions associated with aggression and other emotions that are generated in the amygdala–PFC circuit.

Grady et al. (2013) investigated the effects of ATD on emotional face processing in healthy male and female volunteers by comparing treatment with citalopram (to increase central nervous availability of 5-HT), ATD and a control condition, consisting of no pharmacological treatment. However, it should be noted that most studies compare ATD to a balanced amino acid mixture (Young et al. 1985), which consequently limits the comparability of the aforementioned study to other studies, and therefore is not the subject of this review. The authors described two separate networks: the first comprised areas involved in facial processing [the fusiform gyri, near the FFA (Kanwisher et al. 1997); the posterior occipital cortex, near the occipital face area (Rossion et al. 2003); the right superior temporal sulcus (Haxby et al. 2000)]; and also the left amygdala, bilateral ventral striatum, medial prefrontal cortex and brain stem, which showed a selective response to fearful faces during the control session. This particular network showed increased activation after ATD in response to angry and fearful faces, but not neutral faces. The second network included the bilateral amygdala, inferior frontal gyri, right rostral prefrontal cortex, temporal poles and regions consistent with the FFA, occipital face area and superior temporal sulcus. These regions showed selective activation when viewing fearful faces during the control condition. During ATD, this network showed more activity for angry and fearful faces, as compared to neutral faces, and increased activation for angry faces when compared to the control group. This finding suggests that ATD enhances the brain's response to angry faces, making them indistinguishable from fearful faces. Overall, these findings challenge conventional models of 5-HT repressing negative emotions.

Robinson et al. (2013) used a forced choice face identification task with distracters displaying words describing emotions (i.e., "HAPPY" or "FEAR") written across the faces that were presented to healthy male and female volunteers. They showed an increased response to fearful relative to happy faces under ATD in the dorsal medial prefrontal cortex, which was not observed in the control group. This negative bias was accompanied by a corresponding increase in positive dorsal medial prefrontal-amygdala circuit connectivity. This circuit, also known as the "aversive amplification circuit" (Robinson et al. 2013), might normally be inhibited by serotonergic projections. The authors suggested that serotonergic dysfunction subsequently may contribute to a negative affective bias.

A study by Cools et al. (2005b) added the aspect of individual traits, which they studied in healthy men. While no general modulation of activation of the amygdala and hippocampus after ATD in response to fearful relative to happy faces was detected, a co-variation with self-reported threat-sensitivity was found. An individual variation of the reaction to serotonergic malfunction with regard to threat-sensitivity was therefore suggested.

Following the train of thought of individual sensitivity toward serotonergic manipulation, van der Veen et al. (2007) investigated the effects of ATD on emotion perception in women with and without a family history of depression and found a generally increased activation of the right amygdala under ATD as compared with the control condition. In addition, a significant correlation of the activation of the right amygdala with mood effect after ATD was described. Whole brain analysis revealed a significant effect in the ventromedial prefrontal cortex and the rostral part of the anterior cingulate cortex (rACC) after ATD administration, which is to be expected due to strong projections from the amygdala to the rACC. The authors concluded that a family history of depression strongly influenced the effects of ATD on mood, performance and brain activation, thus generally supporting the findings of Cools et al. (2005b).

Williams et al. (2007) studied the effects of ATD on brain activation under a slightly different paradigm; they used healthy male subjects to investigate the differences in brain activation when participants were shown front- or side-viewed faces. Williams et al. found decreased activity in the left posterior superior temporal sulcus (STS) and the anterior cingulate cortex under the influence of ATD. After ATD intake, there was reduced activity in the medial prefrontal cortex, right temporal pole and orbitofrontal cortex for front-viewed faces and increased activity in the right STS for the side-viewed faces. The authors suggested that averted faces and reduced serotonin function might facilitate attention to the external goal of gaze and that these changes could be adaptive in a threatening context and markedly affect empathetic function in conditions associated with impaired central nervous serotonin function.

While all of the previously discussed studies investigated the effects of ATD on emotion processing in healthy individuals, Daly et al. (2012) studied this aspect in patients with autism spectrum disorder (ASD) as compared to control subjects. Both groups showed the established face processing networks under the control condition, including the fusiform and extrastriatal cortices, insula, superior temporal, cingulate and medial frontal gyri. Depending on the facial expression, Daly and colleagues reported differences between patients and controls. While processing disgust after ATD intake, the control group showed increased activity in the cingulate gyrus, but decreased activation in the medial frontal gyrus and lingual gyrus/cuneus was detected. Patients with ASD, however, showed decreased activation in the cingulate gyrus and increased activation in the medial frontal and lingual gyrus/cuneus. A contrasting effect of ATD on the subject groups was also observed when processing happy faces, where controls showed an increased response in the medial frontal gyrus, the subjects with autism spectrum disorder showed a decreased activation in the same region. Once again, a similar effect could be observed for sad faces; the control group showed increased activation in the middle/medial frontal ROI and decreased activation in the putamen under ATD, whereas the opposite was observed in patients with ASD. Lastly, during the fear experiment, under influence of ATD, the control subjects showed less activation in the left lingual gyrus than patients. The authors concluded that the serotonergic modulation of emotion processing is significantly altered in people with autism spectrum disorders.

In summary, the studies mentioned so far have shown a strong serotonergic modulation of facial emotion processing, the extent of which seems to depend on personal traits and risk factors. However, different brain regions were reported in each study and for each facial expression. The brain areas that seem to be involved in this 5-HT-dependent modulation of facial emotion processing are the cingulate area, amygdala, ventrolateral and medial PFC, but also other well-described parts of facial processing network are involved, namely the FFA, striatum, and insula.

Emotional processing of words

Another possible method to study emotion regulation consists of using words of emotional valence to elicit emotional responses. Three studies so far have used this paradigm to investigate the effects of ATD on emotion regulation in an fMRI study.

Roiser et al. (2008) investigated the effects of ATD on neural activation after viewing emotional relative to neutral words in a sample of healthy men and women. After intake of ATD, activation in response to emotional words was increased in the ventral striatum, hippocampal/parahippocampal cortex, anterior insula and VLPFC. The response to negative words was increased after intake of ATD in the superior temporal gyrus and posterior cingulate cortex. All of these brain regions have been implicated in the underlying pathophysiology of depression (Siegle et al. 2002; Drevets et al. 2002) and receive moderate-to-high densities of serotonergic projections from the dorsal and/or median raphé nuclei (Jacobs and Azmitia 1992; Varnäs et al. 2004). The response to emotional words after ATD intake was decreased in the right DLPFC as well as the right dACC, both of which are important in regulating

limbic structures. Neutral words caused increased activation in the STG and posterior cingulated cortex under ATD. This outcome may be caused by a loss of inhibitory tone provided by 5-HT receptor subtypes (Roiser et al. 2008). Participants who reported increased anxiety following tryptophan depletion showed an increased response in the caudate to negative relative to positive distractors. Also in the caudate, the BOLD signal response to emotional vs. neutral words was increased following tryptophan depletion. This region has been implicated in modulating anxiety responses to stress or threat (Charney and Drevets 2002).

Roiser et al. used the same task to investigate the effects of ATD in men and women remitted from major depressive disorder (rMDD) as compared with healthy controls (Roiser et al. 2009). ATD differentially affected the groups in terms of the BOLD response to emotional vs. neutral words, with a decreased activation in controls in the thalamus, caudate and putamen and the dACC, all of which have been implicated in the pathophysiology of MDD. There was little effect in subjects with rMDD after ATD intake, with a significant increase of activation only in the habenula. Roiser and colleagues interpreted these findings as evidence for elevated habenula blood flow and alterations in the neural processing of emotional stimuli following ATD in subjects with rMDD.

Recently, Roiser et al. (2012) published a study reporting the connections between the serotonin transporter (5-HTTLPR) genotype and neural responses to emotional words under ATD or a placebo. Patients remitted from MDD (rMDD) as well as healthy controls were studied. In controls with high expression of 5-HTTLPR, which were previously identified to have a low risk for mood changes after ATD administration, increased responses to negative words in both the hippocampus and subgenual cingulate cortex were observed for ATD conditions. In contrast, high-risk low-expression controls and subjects with high-expression rMDD showed a decrease in activation in these particular regions in response to negative words after ATD intake. The increased neural response to negative words after ATD was therefore interpreted as an adaptive mechanism, protecting the subject against mood changes following a challenge to the central nervous serotonin system. This mechanism is thought to be reversed in groups vulnerable to developing depressive symptoms, meaning that these particular subjects showed a decreased neural response to negative relative to positive words. In the subgenual cingulate cortex and medial temporal regions, neural responses following ATD were strongly influenced by genotype, which is consistent with previous reports (Neumeister et al. 2006). In low-expression controls and patients with high-expression and rMDD, negative words caused a decreased response in these regions. In contrast, in high-expression controls under ATD, an increased response was observed. Therefore, Roiser and colleagues concluded that the 5-HTTLPR genotype differentially modulated responses to emotional stimuli following tryptophan depletion in healthy and subjects with rMDD.

In summary, participants remitted from MDD seem to use different mechanisms of emotional processing during a serotonergic challenge such as an elevated habenula blood flow. The 5-HTTLPR genotype seems to play into this model, in particular, the subjects with a high risk for depression showed neural processing mechanisms that resemble those of subjects with MDD. 5-HTTLPR therefore seems to modulate the response to serotonergic challenges.

Cognitive flexibility

Several studies have also explored the influence of ATD on cognitive flexibility, which is the ability to adapt performance to changes in the task (Evers et al. 2006b). 5-HT's relevance in cognitive flexibility tasks has been demonstrated in animal and human research (Robbins 2005). Reduced serotonergic functioning after ATD has been shown to impair performance during reversal learning (Park et al. 1994; Rogers et al. 1999) and decision-making (Rogers et al. 1999, 2003) in healthy volunteers. Other studies, however, did not find an impact of 5-HT on cognitive flexibility (Anderson et al. 2003; Talbot et al. 2005). Cognitive flexibility is achieved through a number of different processes, such as response inhibition and performance monitoring (Evers et al. 2006b). For example, patients suffering from depression have been shown to fail to improve performance after perceived failure (Elliott et al. 1997), which suggests a lack of cognitive flexibility. Depressed patients were also reported to have an increased tendency to change their response strategy in the face of misleading negative feedback (Murphy et al. 2003). Cognitive flexibility has been studied using functional imaging techniques. Fallgatter et al. (2004) showed a significantly higher amplitude of error-related negativity, indicating an enhanced responsiveness of the anterior cingulate cortex (ACC) during error processing, in participants carrying the short allele of the 5-HT transporter. Some studies employing ATD to investigate serotonergic function in response inhibition did not yield any effects in healthy volunteers (Cools et al. 2005a; Clark et al. 2005). One study has shown an increase in response style upon receiving ATD (Walderhaug et al. 2002).

A cardinal facet of human cognition is the ability to suppress inappropriate behavioral responses, termed response inhibition (RI). RI can be studied using so-called Go/No-Go tasks, where the subject has to press a button for a certain stimulus and inhibit motor reactions for other stimuli.

Rubia et al. (2005) investigated the effects of ATD on a rapid Go/No-Go task in healthy volunteers and found an increased BOLD response after ATD in the superior and medial temporal cortices but a decreased BOLD response in the right inferior PFC during response inhibition in a Go/No-Go test. This observation led to the assumption that 5-HT is essential in the modulation of right inferior prefrontal engagement during inhibitory motor control, and increased activity in the temporal regions may reflect compensatory mechanisms for this.

Evers et al. (2006a) investigated the effects of ATD on response inhibition in a Go/No-Go task in healthy males. ATD decreased activation in the dorsomedial prefrontal cortex during performance monitoring, but did not affect neural activation during response inhibition. Helmbold et al. (2015) studied the effects of ATD in a punishment and reward implementing Go-/No-Go task in a healthy female sample controlled for menstrual cycle phase. Central 5-HT depletion magnitude in the ventral and subgenual anterior cingulate cortices (ACC) correlated positively with neural activation during No-Go trials in punishment conditions after a control AA mixture versus ATD administration. Differences in neural activation between ATD and a control AA mixture further correlated positively with trait-impulsivity in the medial orbitofrontal cortex (mOFC) and the dorsal ACC. The results indicate a reduced neural sensitivity to punishment after short-term depletion of 5-HT in brain areas related to emotion regulation, including the subgenual ACC, that increases with depletion magnitude, and in brain areas related to appraisal and the expression of emotions, including the mOFC and dorsal ACC, increases with trait-impulsivity. The results suggest a conjunction of reduced serotonergic functioning and trait-impulsivity in females.

Another subgroup of cognitive flexibility paradigms, Stroop tasks (Stroop 1935), comprise reaction time, selective attention and automation tasks. In the original Stroop task, subjects are asked to name color words printed in the same or a different color. Interference is caused when a word (i.e., "red") is printed in an incongruent color (i.e., in blue) and will lead to slower reaction time and more errors as compared to words printed in congruent colors (i.e., "red" printed in red) or neutral words not printed in color. This effect shows that it is impossible to ignore stimulus-aspects completely, even if they are irrelevant to the task.

Two studies using ATD and Stroop tasks in a neuroimaging setting investigated the impact of ATD administration in healthy volunteers.

Horacek et al. (2005) observed an increased activation of the bilateral medial, inferior, and superior prefrontal cortex (PFC) and the ACC during the Stroop task after an ATD challenge. It was concluded that a serotonergic medial forebrain and cingulum bundle pathway exists to mediate the activity of cortical structures involved in Stroop test processing.

Evers et al. (2006b) investigated the effect of ATD during a modified Stroop task combining cognitive with emotional stimuli in healthy females. The authors found an increased BOLD response in the left inferior parietal, middle frontal and right superior temporal cortex during the interference condition at baseline, which is in line with a meta analysis by Laird et al. (2005). ATD increased the response in the anterior cingulate cortex (ACC) during the interference condition of the first block, which is in line with the findings of Horacek et al. (2005). ATD also increased the response in the left precuneus and cuneus during congruent color words. ATD showed no effect on responses to emotional stimuli. In line with previous studies, a temporary reduction of 5-HT improved Stroop performance and changed the brain activation pattern. Both studies showed that ATD modulated the BOLD response in the dorsomedial prefrontal cortex during tasks requiring cognitive control.

Attempting to clarify more areas related to cognitive flexibility, Evers et al. (2005) examined probabilistic reversal learning during ATD administration in healthy male volunteers. Probabilistic reversal learning involves learning a rule to determine which of two abstract stimuli is selected. This rule changes halfway through the task, which requires the subject to adapt their reaction. Evers et al. demonstrated an increased BOLD response under the influence of ATD in the dorsomedial PFC (dmPFC). These results support the assumption that ATD modulates activity in the dorsomedial prefrontal cortex during actions requiring cognitive control.

In summary, the above-mentioned findings of various studies strengthen the assumptions that central nervous 5-HT plays a key role in cognitive flexibility in healthy subjects. It appears to modulate this particular process mainly in the prefrontal cortex and ACC.

Memory

Serotonin is suggested to be essential for memory function (Asberg et al. 1976). Animal studies have yielded mixed results, some report that decreasing central nervous system levels of 5-HT can increase memory function (Altman et al. 1990), some report that this will decrease memory function (Lieben et al. 2004), and some report no effect on memory (Blokland et al. 2002). With regard to neuroimaging, such studies can help to clarify the underlying connection between serotonin availability in the central nervous system and memory function.

Van der Veen et al. (2006) investigated the effects of ATD on a verbal episodic memory task in healthy males.

Encoding of information caused an increased activation of the occipital, middle, superior frontal, anterior and posterior cingulate and striatal areas. ATD caused decreased activation in the right hippocampus. This particular study demonstrated the importance of serotonin in long-term memory processes and in encoding information.

Allen et al. (2006) studied the effects of ATD on a verbal memory task in healthy volunteers. ATD caused an enhanced activation in the left middle and medial frontal gyri, left precentral gyrus, superior parietal lobule, right middle frontal gyrus, cingulate gyrus and posterior cingulate gyrus during the verbal working memory task and an increased activation of left insula, left superior, middle and inferior gyri and right cingulate gyrus in the verbal fluency task. ATD decreased activation in the right superior frontal gyrus during the verbal working memory task and in the medial frontal gyrus and precuneus during the verbal fluency task. This finding suggests a modulation of prefrontal engagement by serotonin activity during verbal working memory and verbal fluency tasks.

Wang and colleagues investigated the effects of ATD on emotional memory in healthy males using an emotional oddball task, which required the discrimination of infrequently presented targets from distracting negative and neutral pictures (Wang et al. 2009). ATD increased responses to negative distracters in the left orbital–inferior frontal, dorsomedial prefrontal and bilateral angular gyri. An increased frontal activation correlated positively with memory performance after ATD but not control mixtures. This outcome suggests a possible compensatory mechanism that improves coping with an increased task demand during serotonergic depletion. This study highlighted once more the importance of serotonin in negative memory, implicating its role in affect disorders.

Regarding 5-HT and memory, there are hints towards gender differences and the important role of sex hormones. In a review, Sambeth et al. (2007) concluded that female gender is the only factor that actually has the properties to serve as a serotonergic vulnerability factor for changes in declarative memory performance. In line with this assumption, Helmbold et al. (2015) showed that in young healthy women, verbal short-term memory function was more vulnerable to ATD than consolidation processes. These findings are in line with results of the mentioned fMRI study reporting reduced activation of the right hippocampus during encoding in an episodic memory task under the influence of ATD, whereas ATD did not affect brain activity in the retrieval phase (van der Veen et al. 2006). In accordance, applying the same ATD procedure as used in the current study, 5-HT was found to be reduced in the hippocampus in a rodent model (Biskup et al. 2012). Epperson et al. (2012) investigated the effects of ATD in healthy women with regard to affective processing after menopause. They examined the effects of estrogen on neural correlates of reduced serotonergic tone and found that ATD caused an attenuated activation in the right and left dorsal lateral prefrontal and middle frontal/cingulate gyrus, an effect which was attenuated by estrogen application. During the emotion identification task, the orbitofrontal cortex and bilateral amygdala showed increased activation, which was attenuated by estrogen application. The authors therefore concluded that these findings point towards an interaction of 5-HT and estrogen with respect to working memory and affective processing (Epperson et al. 2012).

In summary, all of these studies highlight once again the importance of 5-HT in memory function, mainly in the hippocampus, medial frontal gyrus and anterior cingulate cortex in healthy humans.

Other studies

The remainder of the investigations using fMRI and ATD had foci that do not fit any the categories discussed above and will therefore be reviewed separately in the following section.

Krämer et al. (2011) investigated the role of serotonin in reactive aggression in healthy male volunteers. The connection between serotonergic functioning and aggressive social interaction is a frequently studied subject. An inverse relationship between 5-HT and aggression has been shown in several animal species (Mehlman et al. 1994; Higley et al. 1996; de Boer et al. 1999; Fish et al. 1999). In humans, however, this particular connection is not quite as clear (Moss et al. 1990; Coccaro et al. 1997). A debate regarding the underlying mechanisms by which 5-HT influences reactive aggression is ongoing. Krämer et al. (2011) studied the role of 5-HT in reactive aggression using ATD and the Taylor Aggression Paradigm (TAP) (Taylor 1967). They observed an increased activity, which was not modulated by ATD, in the dorsal ACC during a decision phase in high compared to low provocation trials. ATD did however decrease the response in the right insula during the decision phase as compared to the balanced mixture, which contradicts previous reports of an inverse relationship between central nervous 5-HT levels and insula activation in emotion processing (Roiser et al. 2008; Arce et al. 2008). In low-trait aggressive participants, ATD increased activation in the insula, which more closely fits these previous reports. However, group main effects should to be interpreted with some caution as Krämer and colleagues used a between-group design, which must be considered to be a crucial limitation.

Lamar et al. (2009) investigated the effects of ATD on neural activation patterns during a cognitive interference task (Simon task) in elderly women. They found that ATD

caused an increased activation in the neocerebellum and parietal lobe, but a decreased activation in left inferior PFC, anterior cingulate and basal ganglia. This phenomenon can be summarized as a parietal shift of activation during cognitive interference caused by reduced serotonergic tone.

Demoto et al. (2012) investigated the effects of ATD and personality measures on neural correlates during a multi-step delayed reward task in healthy men. Though they could not find any changes in regional activity caused by ATD alone, subjects with high neuroticism and low self-directedness were particularly vulnerable to the effects of ATD on future reward evaluation and the accompanying neural activation patterns (Demoto et al. 2012).

Using a different approach to investigate the neural correlates of reward value and the effect of ATD, Seymour et al. (2012b) conducted a study in healthy male and female subjects during a probabilistic instrumental learning task. Decreased activation in the ventromedial PFC was caused by reward, and decreased activation in the dorsolateral putamen was caused by error. This outcome led the authors to conclude that 5-HT plays an important, though complex, role in reward processing (Seymour et al. 2012b).

Hindi Attar et al. (2012) investigated the role of serotonin in fear learning. Learning is essential to predict and respond to events and therefore adequately adapt to a rapidly changing environment. Models of reinforcement learning (RL) provide a method of probing how brain function is related to the ability to predict future events by using prediction error (PE) signal, meaning the difference between the predicted and actual outcomes (Sutton and Barto 1998). Animal studies have uncovered the role of midbrain DA neurons in the encoding of reward PEs (Schultz et al. 1997). Therefore, fMRI studies in humans have investigated the mesolimbic DA systems such as the ventral striatum and the prefrontal areas including the orbitofrontal cortex (OFC) (O'Doherty et al. 2003; McClure et al. 2003). However, recent studies have shown a specific effect of serotonin on reward processing (Seymour et al. 2012b), and evidence of a major role of 5-HT in aversive processing has accumulated (Evers et al. 2005; Crockett et al. 2009; Robinson et al. 2012). Studies on classical fear conditioning have shown that amygdala-related signal changes decay over the experiment, possibly reflecting a decrease in prediction error as the association is learned (Büchel et al. 1998; LaBar et al. 1998). This finding points toward theoretical accounts (Daw et al. 2002) suggesting that 5-HT serves as an opponent signal to the dopaminergic reward prediction error signal by encoding a prediction error rule for aversive events. Hindi Attar et al. observed the strongest effects of ATD in terms of an increased activation in the OFC, which is in line with results of animal studies demonstrating the importance of the OFC in reversal learning, conditioned reinforcement and extinction learning (Clarke et al. 2008; Walker et al. 2009). The amygdala, a core region in the neural circuit of Pavlovian fear conditioning (Büchel et al. 1998; LaBar et al. 1998; Davis and Whalen 2001), showed reduced activation after ATD intake, which once again underscores the role of the amygdala in aversive learning (Gläscher and Büchel 2005; Yacubian et al. 2006). However, group main effects have to be viewed with caution as Hindi Attar and colleagues used a between-group design, which must be considered as a crucial limitation.

Salomon et al. (2011) investigated the effects of ATD on resting-state signals in male and female patients remitted from depression. In this trial, increased signals in the pontine raphé during ATD where observed. Functional connectivity between the pontine raphé nucleus and the anterior thalamus was attenuated by ATD.

Another interesting approach using ATD in an fMRI setting was used by Labus et al. (2011), who studied the effective connectivity of emotional arousal circuitry during visceral stimuli, namely rectal distention, in female patients. They found that ATD caused an increased activation in the amygdala and emotional arousal networks (i.e., the amygdala and locus coeruleus complex), which was even greater during maximal distention. ATD also caused a loss of negative feedback inhibition of the amygdala in all subjects, suggesting "a greater engagement of central nervous serotonin system with more aversive visceral stimuli" (Labus et al. 2011).

Macoveanu et al. (2013) studied the effects of ATD in healthy adults on behavioral inhibition, which is a common feature of neurological and neuropsychiatric disorders. The central nervous serotonin system as well as the inferior frontal gyrus (IFG) both play important roles in inhibition-related processes. The group assessed 5-HT_{2A} receptors (which are related to impulsivity) using 18F-altanserin positron emission tomography. They subsequently investigated the effects of ATD as well as citalopram during an fMRI session. ATD caused an increased signal in the right IFG during response inhibition in subjects with low 5-HT_{2A} receptor binding. These results underscore the relationship between serotonergic function and response inhibition in healthy subjects and may help to interpret impulsivity and related behaviors in patients with neuropsychiatric disorders.

PET studies and ATD

In 1994, the first study combining ATD and PET was conducted by Agren et al. (1994). They investigated the effects of ATD on the transport of 5-HTP and L-DOPA, both of which were ^{11}C-labeled, across the BBB as well as the utilization of these monoamine precursors in eight

healthy subjects and eight volunteers suffering from unipolar depression. They found that depressed patients showed 30 % less 5-HTP uptake across the BBB under baseline conditions compared to healthy subjects. The study was also able to show that depressed patients showed higher 5-HTP utilization in the medial prefrontal cortex under baseline conditions compared to healthy volunteers. The study did not report an effect of ATD on uptake or utilization of 5-HTP or L-DOPA. The authors concluded that this result points toward "a compensatory increase of serotonin synthesis" (Agren and Reibring 1994).

Brain metabolism

Bremner et al. (1997) published a study on the effects of ATD on brain metabolism in patients with major depressive disorder in 1997 using a fludeoxyglucose F^{18} tracer in a between-subjects design. The F^{18}-tracer shows metabolic activity as the attached glucose molecule is metabolized. The authors reported that ATD resulted in a decrease in brain metabolism in the dorsolateral prefrontal cortex, thalamus and orbitofrontal cortex. This decrease in metabolism correlated with depressive symptoms. Metabolism was increased in the prefrontal and limbic regions in relapse-prone patients (Bremner et al. 1997). The authors concluded that the "middle frontal gyrus, thalamus and orbitofrontal cortex mediate the symptoms of patients with major depression" (Bremner et al. 1997).

The neural activation during different cognitive tasks after ATD intake in eight male rMDD patients was investigated by Morris et al. (1999) using $[^{15}O]H_2O$. Task-specific activation in the left amygdala and left anterior cingulate cortex was decreased by ATD. They also found a correlation between habenulae and raphé activity and depressed mood, which they concluded shows the "habenulae projections to raphé [represent] a convergent feedback pathway [controlling 5-HT release in the whole brain]" (Morris et al. 1999).

In another study by Smith et al. (1999), $[^{15}O]H_2O$ PET was used to identify brain regions involved in depressive relapses and determine their neural activity during a cognitive task in eight male patients with rMDD. They found that depressive symptoms after ATD were associated with diminished activity in the ventral anterior cingulate, orbitofrontal cortex and caudate nucleus regions and concluded that these changes mirror the clinical phenomena of clinical depression (Smith et al. 1999).

Neumeister et al. (2004) also investigated the effects of ATD on brain metabolism using fludeoxyglucose F^{18}, comparing healthy subjects to patients remitted from major depressive disorder (rMDD). They found that ATD caused increased regional glucose utilization in the orbitofrontal cortex, medial thalamus, anterior and posterior cingulated cortices and ventral striatum in subjects with rMDD, but not in healthy controls. The authors concluded that ATD "unmasks a disease-specific, serotonin system-related trait dysfunction and identifies a circuit that probably plays a key role in the pathogenesis of MDD" (Neumeister et al. 2004).

Overall, the mentioned studies have shown somewhat conflicting results, most of them showing decreases in neural activation patterns after ATD administration in patients with rMDD. Neumeister et al. (2004), however, showed increased activation in the very same regions in patients with rMDD. Other studies have demonstrated the amygdala, dorsal anterior cingulate cortex and insula as well as the striatum and dorsolateral PFC to be key regions in patients with depressive disorders (Hamilton et al. 2012). At this stage, further investigation of the influence of serotonin on neural activation in patients with depressive disorders is needed.

5-HT receptors

Several studies have investigated the effects of ATD on the binding of different ligands to serotonin receptors. Udo de Haes et al. (2002) studied the binding of [^{18}F] 2′-methoxyphenyl-(*N*-2′-pyridinyl)-*p*-fluoro-benzamidoethyipiperazine ([^{18}F]MPPF) to 5-HT_{1A} receptors, which are sensitive to central nervous 5-HT levels, in six healthy males using ATD in a within-subject repeated measures design. However, they were unable to find any effect of ATD on [^{18}F]MPPF binding.

Praschak-Rieder et al. (2004) replicated this experimental design in eight patients remitted from major depressive disorder and could not find any changes in [^{18}F]MPPF binding after an ATD challenge either. It can therefore be concluded at this stage that changes in 5-HT availability do not seem to have an effect on 5-HT_{1A} receptor binding. However, these results should be interpreted with some caution as the sample sizes were rather limited.

Other studies have examined the effects of ATD on 5-HT_2 receptor binding. Yatham et al. (2001) were the first to investigate this topic in healthy women using ^{18}F-labeled setoperone, a compound that is a ligand to the 5-HT_{2A} receptor, and found a decrease in 5-HT_2 receptor binding after ATD administration that was particularly prominent in the left fusiform gyrus, left insula, left superior temporal gyrus and left superior gyrus (Yatham et al. 2001). They concluded that, in light of findings showing a decrease in 5-HT_2 receptors during treatment with antidepressants (Peroutka and Snyder 1980; Goodnough and Baker 1994), these data show "an adaptive response against depressive symptoms" (Yatham et al. 2001).

In 2012, the same group published a study testing this hypothesis by administering ATD to patients remitted from

major depressive disorder and subsequently using ^{18}F-labeled setoperone to measure brain 5-HT_2 receptor density. ATD tendentially caused a reduction of brain 5-HT_2 receptor binding in all participants. However, a subgroup analysis showed that the reduction of brain 5-HT_2 receptor binding was only statistically significant in the non-depressed group in the right frontal, left medial frontal, right temporal, parietal and right and left occipital regions. This outcome led to the conclusion that the observed down regulation of brain 5-HT_2 receptor binding may be a compensatory mechanism to obviate a depressive relapse (Yatham et al. 2012).

Talbot et al. (2012) investigated 5-HT_{2A} receptors and their vulnerability to endogenous serotonergic tone in healthy subjects. They used the 5-HT_{2A} receptor-specific tracer ^{11}C-MDL100907. After intake of ATD, binding was only reduced in the right prefrontal cortex, which led the authors to conclude that binding of this tracer is not vulnerable to endogenous serotonergic tone.

In summary, it seems that 5-HT_{1A} receptors appear to be more or less unaffected by changes in serotonergic tone in healthy males. 5-HT_2 receptors seem to be more sensitive to changes in 5-HT levels in several brain regions in healthy subjects and might be involved in compensatory mechanisms in patients with rMDD. 5-HT_{2A} receptors are not affected by changes in serotonergic tone in healthy subjects.

5-HT transporters

Another topic that has been examined by several studies is the effect of ATD on 5-HT transporters (5-HTT). The first study to shed light on this topic was conducted by Talbot et al. (2005). They administered ATD to eight subjects in a within-subject repeated measures design and measured regional distribution volumes and binding potentials specific to the nonspecific equilibrium partition coefficient of 3-(11)C-amino-4-(2-dimethylaminomethylphenyl-sulfanyl)benzonitrile (^{11}C-DASB), a 5-HTT ligand. Finding small, significant reductions of regional distribution and binding potentials, they concluded that ^{11}C-DASB in vivo binding is not affected by endogenous 5-HT tone (Talbot et al. 2005).

A similar design was used by Praschak-Rieder et al. (2005), the regional binding potential of ^{11}C-DASB was investigated in 25 healthy subjects. Like Talbot et al. (2005), they did not find any changes in regional 5-HTT regional binding potentials under ATD compared with the control condition. They concluded that acute changes in 5-HTT density are not likely to be a compensatory mechanism against changes in 5-HT tone, and that other mechanisms should be explored (Praschak-Rieder et al. 2005).

Using a different approach to clarify the role of 5-HTT on the effects of ATD, Neumeister et al. (2006) used a PET study to investigate the differential effects of the functional length triallelic polymorphism in the promoter of the 5-HT (5-HTTLPR) genotypes in patients remitted from major depression (rMDD) and healthy subjects. They then measured regional changes in metabolism using a fludeoxyglucose F^{18} tracer. All subjects' behavioral responses to ATD were affected by their genotype. The genotype of subjects with rMDD, but not healthy participants, influenced regional cerebral metabolic rates. They showed increased metabolic rates in the orbitofrontal cortex and subgenual and pregenual anterior cingulate cortex. Subjects with rMDD and with an L/L genotype showed an increased metabolism in the left amygdala. Contrastingly, subjects with rMDD with an S/L genotype showed a decreased activation in the left amygdala. Neumeister et al. (2006) concluded that these findings underscore the importance of 5-HTTLPR in the sensitivity of patients with rMDD on a metabolic basis and in all subjects on a behavioral basis.

Following this approach, Nugent et al. (2008) conducted a phenotype and genotype determination by discriminant analysis of brain glucose metabolism using the fludeoxyglucose F^{18} tracer after intake of ATD in healthy and subjects with rMDD. Nugent et al. (2008) correctly classified 79 % of subjects by genotype and 85 % of subjects by phenotype after analyzing the ^{18}FDG-PET scans after ATD intake. They concluded that much variance in the central nervous metabolic response to ATD is accounted for by genotypic and phenotypic categories (Nugent et al. 2008).

These studies have shown that 5-HTT density does not seem to be affected by acute changes (in terms of ATD) in serotonergic tone. The genotype of the 5-HTT promoter, however, does seem to influence neural correlates of reduced serotonergic tone.

Other studies

There have been other approaches using ATD and PET that could be seen as overlaps of serotonin research with other areas. One example of such a study by Cox et al. examined the effects of ATD on cocaine-induced striatal DA responses using ^{11}C-raclopride, a D2 receptor antagonist, as a tracer. Subjects were non-dependent cocaine users. They found that cocaine-craving and striatal DA responses to cocaine were increased by ATD, which suggests a possible mechanism by which individuals with lower serotonergic function might have an elevated risk for substance abuse (Cox et al. 2011).

Sacher et al. (2012) investigated the effects of ATD on the binding of ^{11}C-harmine, a monooxidase A (MAO-A) substrate. They found that ATD caused a decreased binding

in the prefrontal cortex. This study also investigated the dopaminergic system by applying carbidopa–levodopa, increasing dopaminergic function, after which the binding of ^{11}C-harmine was increased in the striatum. These findings suggest an adaptive function of MAO-A as a fast compensatory mechanism for fluctuating monoamine levels (Sacher et al. 2012).

Dopaminergic system

The neurotransmitter DA plays an important role in human incentive-motivational behavior and has high clinical relevance. Impaired central nervous DA function is thought to lead to anhedonia and motor symptoms of depression (Stein 2008). Reward-related signaling is in part mediated by the phasic activity of dopaminergic neurons that project to the ventral striatum (Hernandez et al. 2007; Stuber et al. 2008).

fMRI and APTD

The dopaminergic system can be modulated in the short term by using ATPD, which reduces striatal DA release (Montgomery et al. 2003; Leyton et al. 2004) and modulates frontostriatal connectivity (Nagano-Saito et al. 2008).

The first study using ATPD in an fMRI setting was published by Nagano-Saito et al. (2012). They investigated the effects of ATPD on the neural correlates of decision-making. They found that corticostriatal activation and the correlation between striatal activation and decision threshold were abolished with reduced dopaminergic transmission. This result suggests that an increased reward-related activation of the striatum is the result of dopaminergic activity. These data also show that perceptual and decision-making neural processes are influenced by mesolimbic DA transmission (Nagano-Saito et al. 2012).

Coull et al. (2012) used ATPD to investigate the role of DA in a perceptual timing task. They showed that timing-specific activity in the putamen and single-cell motor area (SMA) was decreased by ATPD, and these changes correlated with impairments in timing performance. They concluded that these data show that a depletion of DA disrupts timing by decreasing the activity in the putamen and SMA (Coull et al. 2012).

A recent study by Bjork et al. (2014) investigated the effects of ATPD on motivational processes and their neural correlates in healthy subjects. While ATPD was not associated with main effects on mood or task behavior, it did attenuate activation in the right nucleus accumbens when large rewards were anticipated. In subjects whose mood worsened following ATPD, the left nucleus accumbens showed a decreased activation compared with the control condition. The anterior insula was activated under ATPD as a response to loss outcomes. The authors concluded that APTD blunts dopaminergic mesolimbic activity, and that the effect on the neurocircuitry is small enough to be buffered and not show any behavioral effects (Bjork et al. 2014).

In summary, these studies demonstrate that ATPD can cause changes in neural activation patterns during different tasks. The corticostriatal network mirrors the effects of decreased dopaminergic function during decision-making, the SMA and putamen are affected during timing tasks, and the nucleus accumbens and anterior insula are regions of effect after ATPD intake during motivational tasks.

PET studies and ATPD

Ellis et al. (2007) conducted an ATPD study using PET with $H_2^{15}O$ to measure changes in regional cerebral blood flow after ATPD administration in healthy males. They found that blood flow was increased in the parahippocampal gyrus bilaterally and in the left inferior frontal gyrus and the putamen after an ATPD challenge. ATPD caused a decrease in blood flow in the medial frontal gyrus bilaterally and in the right inferior temporal gyrus and the pons (Ellis et al. 2007). No effect on spatial working memory was observed. The authors concluded that these results question the "capacity of (ATPD) to consistently modulate DA function and (spatial working memory) neural networks in humans" (Ellis et al. 2007).

Discussion

Many studies have used amino acid challenges or depletion concepts in combination with different neuroimaging techniques. Manipulating central nervous system serotonin synthesis in an fMRI setting has allowed the investigation of the effects of a reduced serotonergic synthesis on different emotions, tasks and disorders. 5-HT seems to play a major role in the neural processes underlying facial emotion processing, mainly in the cingulate area, amygdala, ventrolateral and medial PFC, but also in other well-described areas of the facial processing network, namely the FFA, striatum, and insula. The visual presentation of emotional words in healthy subjects was not changed by reduced central nervous serotonergic tone, but seems to cause a differential habenula activation in healthy and subjects with rMDD. The 5-HTTLPR genotype seems to modulate the serotonergic response to such stimuli. Regarding cognitive flexibility, changes in serotonergic function seem to be focused in the prefrontal cortex and the ACC; while in memory function, 5-HT modulation affects the hippocampus, medial frontal gyrus and anterior

cingulate cortex. However, the influence of sex hormones on serotonergic neural transmission still remains unclear. Some studies have used ATD as an alternative to the administration of psychotropic agents to investigate numerous aspects of the brain that are related to 5-HT. It has been shown that aggression and activation of the insula show an inverse correlation (Krämer et al. 2011). ATD seems to cause a parietal shift in activation patterns in elderly women during cognitive interference (Lamar et al. 2009) and has more pronounced effects in individuals with neurotic personality traits during reward evaluation (Demoto et al. 2012). 5-HT's role in reward processing was confirmed by Seymour et al. (2012a). 5-HT is important in fear learning (Hindi Attar et al. 2012) and causes a greater engagement of the serotonergic system during aversive visceral stimuli (Labus et al. 2011).

In summary, all studies using ATD and PET have shown that 5-HT_2 receptors, but not 5-HT_{1A} or 5-HT_{2A} receptors, generally seem to be sensitive to acute changes in central nervous serotonergic tone. 5-HTT density was not influenced by ATD administration; however, the 5-HTTLPR genotype influenced the pattern of neural activation after an ATD challenge. It should be noted that many questions that are yet to be answered could well be investigated using ATD in a PET-setting.

ATPD has been used to investigate the effects of reduced dopaminergic tone on neural activation patterns in fMRI studies. The corticostriatal network mirrored the effects of decreased dopaminergic function during decision-making, the SMA and putamen are affected during timing tasks, and the nucleus accumbens and anterior insula are regions of effect after ATPD intake during motivational tasks.

However, some limitations to the studies discussed in this review should be noted. Several of the studies discussed used a subject group of mixed genders. This can be problematic, especially because women have been shown to be somewhat more vulnerable to the effects of ATD (Nishizawa et al. 1997) and because sex hormones, which vary during the menstrual cycle and were not always controlled, influence the serotonergic system (Rubinow et al. 1998; Carretti et al. 2005). Some studies, in particular studies using PET, did not report the handedness of their subjects. This information, however, is crucial for the comparison of neuroimaging studies because there are significant functional differences between left- and right-handed humans (Gurd et al. 2013). Some studies had rather small sample sizes ($n < 12$), which also makes an interpretation of the results difficult. Another critical point is that most studies did not control for a homogenous distribution of intelligence within their sample, which is supposed to ensure a homogenous understanding of the administered tasks and may exclude certain genetic conditions. Additionally, most studies did not control for body weight. This factor is important because a correlation between body weight and plasma TRP has been shown (Demisch et al. 2002; Kewitz 2002). Only very few studies controlled for the smoking habits of their subjects, which is relevant because nicotine use has been shown to have an influence on the BOLD response in fMRI and on ATD effects (Mobascher et al. 2012; Knott et al. 2013). While most studies tested for the success of their depletion paradigm using blood samples, some studies did not report measuring the respective AAs in the blood. Therefore, any effects that were observed cannot safely be attributed to the administered depletion paradigms. Other limitations that should be mentioned are that one study (Grady et al. 2013) conducted fMRI scans at different time points after the ingestion of ATD and compared ATD to no pharmacologic treatment at all, whereas most ATD studies compare the observed effects to a tryptophan-balanced mixture. Another study (Wang et al. 2009) used an open label design, which is also rather unusual for ATD studies. Yet another group (Sacher et al. 2012) used a between-group design, which makes the data more difficult to compare with other studies, usually conducted by a within-subject repeated measures design. A detailed overview of the limitations of the studies is shown in Table 1.

There are also some general methodological limitations that should be noted. To date, there is a lack of standardization in depletion/challenge paradigms, resulting in a somewhat limited comparability of study results. To our knowledge, there have not been any translational studies investigating the effects of one AA depletion method on neuroimaging parameters across several species. This would provide more insight as regards the ability to translate behavioral findings in particular across species (i.e., rodents and humans), as it would allow for a more dynamic assessment of animal brain function as compared to studying human brain function, in particular because animal-related research allows far more invasive techniques to assess neurotransmitter function in the brain. This approach, however, is certainly also limited by critical methodological aspects (i.e., such as a required anesthesiology of animals during the scanning procedure, etc.). Developmental aspects of this topic and approach also remain to be uncovered because studies in children and adolescents are scarce. The long-term effects of AA manipulations on neural activation are also yet to be described. Another aspect that could well be investigated is the direct comparison of AA manipulations to pharmaceutical agents with similar, somewhat opposite or even antagonistic effects, i.e., the comparison of SSRIs to ATD or ATPD to neuroleptic medications. Future studies are also needed to further clarify the complex interplay of sex hormones with AA manipulations and their effects on

Table 2 Methodology of reviewed studies

Study	Subjects	*n*	Handedness	IQ	Weight	Smoking	TRP in blood	Other
Fusar-Poli et al. (2007)	m f	10	Right	+	−	−	+	
Daly et al. (2010)	m	14	Right	+	−	−	+	
Passamonti et al. (2012)	m f	30	n.r.	−	−	−	+	
Grady et al. (2013)	m f	30	n.r.	−	−	−	+	Scans at different time points after challenge ingestion; ATD not compared to BAL but no treatment at all
Robinson et al. (2013)	m f	19	n.r.	−	−	−	+	
Cools et al. (2005b)	m	12	Right	−	−	−	+	
Van der Veen (2007)	f	14	n.r.	−	−	−	+	
Williams et al. (2007)	m	10	Right	−	−	−	+	
Daly et al. (2012)	m	14 vs. 14	Right	+	−	−	+	
Roiser et al. (2008)	m f	20	Right	−	−	+	+	
Roiser et al. (2009)	m f	20	Right	−	−	+	+	
Roiser et al. (2012)	m f	20	Right	−	−	+	+	
Rubia et al. (2005)	m f	9	Right	+	−	−	+	
Evers et al. (2006b)	m	17	Right	−	−	−	+	
Helmbold et al. (2015)	f	18	Right	+	+	+	+	
Horacek et al. (2005)	m f	20	Right	−	−	−	+	
Evers et al. (2006a)	f	19	Right	−	−	−	+	
Evers et al. (2005)	m	11	Right	−	−	−	+	
Van der Veen et al. (2006)	m	17	Right	−	−	−	+	
Allen et al. (2006)	m f	10	Right	+	−	−	+	
Wang et al. (2009)	m	12	n.r.	−	−	−	+	Open label design
Epperson et al. (2012)	f	8	n.r.	−	−	−	+	
Krämer et al. (2011)	m	30	Right	−	−	−	−	
Lamar et al. (2009)	f	9	Right			+	+	
Demoto et al. (2012)	m	18	Right	+	−	−	+	
Seymour et al. (2012a)	m f	30	n.r.	−	−	−	+	
Hindi Attar et al. (2012)	m	39	Right	−	−	−	+	
Salomon et al. (2011)	m f	11	n.r.	−	−	−	+	
Labus et al. (2011)	f	12	Right	−	−	−	−	
Macoveau et al. (2013)	m f	22	Right	−	−	−	+	
Agren et al. (1994)	m	8 vs. 6	n.r.	−	−	−	−	
Bremner et al. (1997)	m f	21	n.r.	−	−	−	+	

Table 2 continued

Study	Subjects	*n*	Handedness	IQ	Weight	Smoking	TRP in blood	Other
Morris et al. (1999)	m	8	n.r.	–	–	–	+	
Smith et al. (1999)	m	8	n.r.	–	–	–	+	
Neumeister et al. (2006)	m f	27 vs. 19	n.r.	–	–	–	+	
Udo de Haes et al. (2002)	m	6	n.r.	–	–	–	+	
Praschak-Rieder et al. (2004)	m	8	n.r.	–	–	–	+	
Yatham et al. (2001)	f	10	n.r.	–	–	–	+	
Yatham et al. (2012)	m f	17	n.r.	–	–	–	+	
Talbot et al. (2012)	m f	39	n.r.	–	–	–	+	
Talbot et al. (2005)	m f	8	n.r.	–	–	–	+	
Praschak-Rieder et al. (2004)	m f	25	n.r.	–	–	+	+	
Neumeister et al. (1997)	m f	27 vs. 26	n.r.	–	–	–	+	
Nugent et al. (2008)	m f	27 vs. 26	n.r.	–	–	–	+	
Cox et al. (2011)	m f	17	n.r.	–	–	+	+	
Sacher et al. (2012)	m f	19	n.r.	–	–	–	+	Between-group design
Nagano-Saito et al. (2008)	m f	17	Right	–	–	+	+	
Coull et al. (2012)	m f	16	Right	–	–	–	+	
Bjork et al. (2014)	m f	16	n.r.	–	–	–	+	
Ellis et al. (2007)	m	11	Right	+	–	+	+	

n.r. not reported; *m* male, *f* female

neural activation. Future studies also need to address a general issue that has been brought up about dietary amino acid manipulations, which is their specificity to their respective neurotransmitter pathways and effects on other neurotransmitter pathways and related behavioral effects. The suggested iterative translational approach studying such pathways across species as outlined above could be a first future avenue for such research because it would involve identical depletion strategies and would combine the benefits of human and animal related research (Table 2).

Overall, these studies have shown that AA challenge techniques are valid and effective translational research tools that allow the investigation of serotonergic and dopaminergic systems without causing serious side effects or long-term damage to the subjects.

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