Chronic Stress Disturbs Regulation of Serotonin Transporter in Major Depression: Intracellular Mechanism

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Abstract
Major depression is a complex neuropsychiatric disorder which is becoming a world wide health problem. Several studies indicate that chronic inflammation or chronic psychosocial stress can induce, or at least contribute to, major depression. In this review the precise molecular pathway of p38 MAPK in which chronic stress can eventually cause depression is discussed, with a synergistic role for proinflammatory cytokines. We propose a mechanism in which chronic stress acts on the adrenergic pathway, activating the p38 MAPK and eventually increase the activity and affinity of the serotonin transporter leading to a decrease of serotonin in the synapse. In addition, proinflammatory cytokines present due to systemic inflammation or peripheral inflammation, provide a positive feedback loop resulting in a chronic state of inflammation. Proinflammatory cytokines can also activate the adrenergic system and thereby creating a synergistic effect combined with chronic psychosocial stress resulting again in serotonin transporter disfunctioning. Alterations in the serotonergic system are known to have wide spread behavioral effects, which could lead to depression-like features. Furthermore, future research prospectives, new treatments possibilities and additional monitoring options are discussed. The serotonergic system is implicated in many psychiatric diseases, other than depression, such as schizophrenia, obsessive compulsive disorder and autism. Therefore, the clear overview of the p38 pathway described in this review can contribute to the overall knowledge of many more psychiatric disorders. Furthermore, the new research, therapeutic and monitor options could also be of relevance to, in addition to depression, these psychiatric disorders.

Keywords
Depression, inflammation, proinflammatory cytokines, stress, serotonin, serotonin transporter (SERT)

Introduction – Major depression and inflammation

Major depression (also known as major depression disorder) is a complex neuropsychiatric disorder with a broad range of recurrent symptoms such as mood alterations and cognitive deficits. The prevalence for major depression has been increasing over the last generations (Anonymous, 1992; Fiest et al., 2011; Kessler et al., 2003) resulting in a world wide health problem. Depression significantly increases susceptibility for the development of several medical conditions such as cardiovascular disease, diabetes, and cancer (Brintzenhofe-Szoc et al., 2009; Chan et al., 2011; Ko et al., 2010). In addition, patients suffering from cancer, diabetes and cardiovascular diseases show a higher susceptibility for major depression (Brintzenhofe-Szoc et al., 2009; Chan et al., 2011; Ko et al., 2010). Major depression is seen as a multifactorial disease with a correlation of environmental and genetic factors, although the precise aetiology is still unknown (Bufalino et al., 2012; Homberg et al., 2012). In the last decade the focus has shifted towards the involvement of the immune system in the pathophysiology of major depression. Supporting the role of proinflammatory cytokines in depression, first line of evidence came from treatment studies. 40% of patients treated with interferon (IFN) α significantly displayed depressive symptoms, providing a link between proinflammatory cytokines and depression (Hauser et al., 2002; McNutt et al., 2012; Raison et al., 2007). Administration of IFNα is associated with increased levels of proinflammatory cytokines such as, intercellular adhesion molecule 1, interleukin (IL) 1, IL6, IL8 and tumor necrosis factor (TNF)-α in the cerebrospinal fluid (CSF) (Capuron et al., 2006; Raison et al., 2007; Wichers et al., 2007). In addition, depression and depressive symptoms can be observed in patients associated with inflammatory markers in several diseases such as cardiovascular disease, cancer, arthritis, psoriasis, inflammatory bowel disease and postviral infections (Lesperance et al., 2004; Musselman et al., 2001; Owen et al., 2001). More recently, multiple studies have reported increased levels of proinflammatory cytokines in patients with major depression (Maes et al., 2012; Rethorst et al., 2012; Simen et al., 2006; Vogelzangs et al., 2012). In most studies an increase of IL6 concentration is found and more rarely elevations of IL1 and TNFα levels in plasma and periphery fluid. (Maes et al., 2012; Rethorst et al., 2012; Vogelzangs et al., 2012).

In general, proinflammatory cytokines can influence neurotransmitter metabolism, neuroendocrine function, synaptic plasticity and regional brain activity which are all relevant for depression. Cytokines can enter the brain through leaky regions in the blood brain barrier or through active transport. In the brain they can activate several pathways which will induce the release of even more inflammatory cytokines by influencing astrocytes and microglia cells (Dantzer et al., 2008; Vitkovic et al., 2000). These chronic released proinflammatory cytokines may influence certain molecules in different pathways (i.e corticotrophin-releasing hormone, glucocorticoid receptor, serotonin (5-HT), norepinephrine (NE) and dopamine (DA)), which are associated with major depression, eventually leading to behavioral changes (figure 1). In short, the hypothalamic-pituitary-adrenal (HPA) axis and corticotrophin-releasing hormone (CRH) can be activated and released, while the glucocorticoid receptor can be reduced through cytokine involvement. Disturbed HPA axis is a hallmark of depression which can be caused by chronic inflammation and may in part contribute to the pathophysiology of depression (Sapolsky et al., 1987; Webster et al., 2001). Furthermore, proinflammatory cytokines can induce behavioral changes by increasing regional brain activity (e.g. dorsal anterior cingulated cortex, frontal cortex and basal ganglia) as shown by functional magnetic resonance imaging (fMRI) studies in patients receiving IFNα treatment (Capuron et al., 2006, 2007; Juengling et al., 2000). Another pathway involves the role of synaptic plasticity in major depression. Physical and psychological stressors can activate the innate immune system hereby inducing proinflammatory cytokines. These cytokines can influence growth factors (e.g. brain-derived neurotrophic factor) and thereby disturb synaptic plasticity. For the development of, and also vulnerability to, major depression growth factors and synaptic plasticity have been indicated to be important (Barrientos et al., 2003; Schmidt et al., 2007). Another studied pathway involves monoamine metabolism. Here the monoamine transporters are influenced by either the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) in the Indoleamine 2,3-Dioxygenase (IDO) pathway or mitogen activated protein kinase (MAPK) pathway. Both of these pathways will influence monoamine transporters and thereby regulate monoamine availability in the synapse. Changes in monoamine availability in the synapse has been implicated in major depression (Dunn et al., 2005; Felger et al., 2007; Shuto et al., 1997).

The role of MAPK in the regulation of monoamine transporters has become of interest in the last decade. Multiple MAPKs have been described, although most research have been performed on p38 MAPK. Several acute stress exposures or chronic stress will activate the p38 MAPK pathway (therefore also called the stress activated MAPK), however, activation induced by cytokines has also been proposed (Chang et al., 2012; Miller et al., 2006; Raison et al., 2006). Activation of p38 MAPK will result in direct or indirect phosphorylation of the monoamine transporter, in this case specifically the serotonin transporter (SERT) and possibly also the dopamine transporter (DAT) (Chang et al., 2012; Morón et al., 2003). Phosphorylation of the SERT or DAT might alter their surface density, affinity and activity. Consequently, alterations in the performance of the SERT and the DAT will influence 5-HT and DA availability in the synapse and will eventually influence the behavior of the organism. Interestingly, several post-mortem and fMRI studies have suggested a decrease of the SERT density in depressed patients compared to healthy subjects (Arango et al., 1995; Eggers et al., 2003; Joensuu et al., 2007, 2010; Leake et al., 1991; Malison et al., 1998; Newberg et al., 2012). While, other studies showed an increase of the SERT density in depressed patients (Conway et al., 1985; Lawrence et al., 1997; Reivich et al., 2004; Staley et al., 2006). Consistent with the latter, studies involving depressed patients mainly showed decreased 5-HT levels in the synapse (Yatham et al., 2012). The discrepancy between these studies can be caused by age, gender, symptom severity, type of depression and methodology differences (Newberg et al., 2012; Raison et al., 2006). Looking at in vitro and in vivo studies the evidence is more consistent, stress exposure or cytokine induced activation of p38 MAPK resulted in increased density of the SERT hereby causing behavioral changes which can be linked to depression (Bruchas et al., 2011; Lai et al., 2009).

Recent research have elucidated parts of the p38 MAPK pathway involved in depression, however, certain steps are still unknown. For instance, the precise role of proinflammatory cytokines in major depression is still under debate and if p38 MAPK is involved is not sure. In addition, how the p38 MAPK pathway affects the SERT is not completely known. Furthermore, how alterations in monoamine transporters will result in behavioral changes also needs to be elucidated (Miller et al., 2008). This review will examine the latest literature to describe a more precise molecular intracellular mechanism of p38 MAPK which is induced by stress and can eventually contribute to the pathophysiology of major depression. In addition, the role of proinflammatory cytokines in major depression, and more specifically in the p38 MAPK mechanism, is described. Furthermore, new therapeutic insights for major depression will be discussed as well as techniques to monitor their effectiveness.

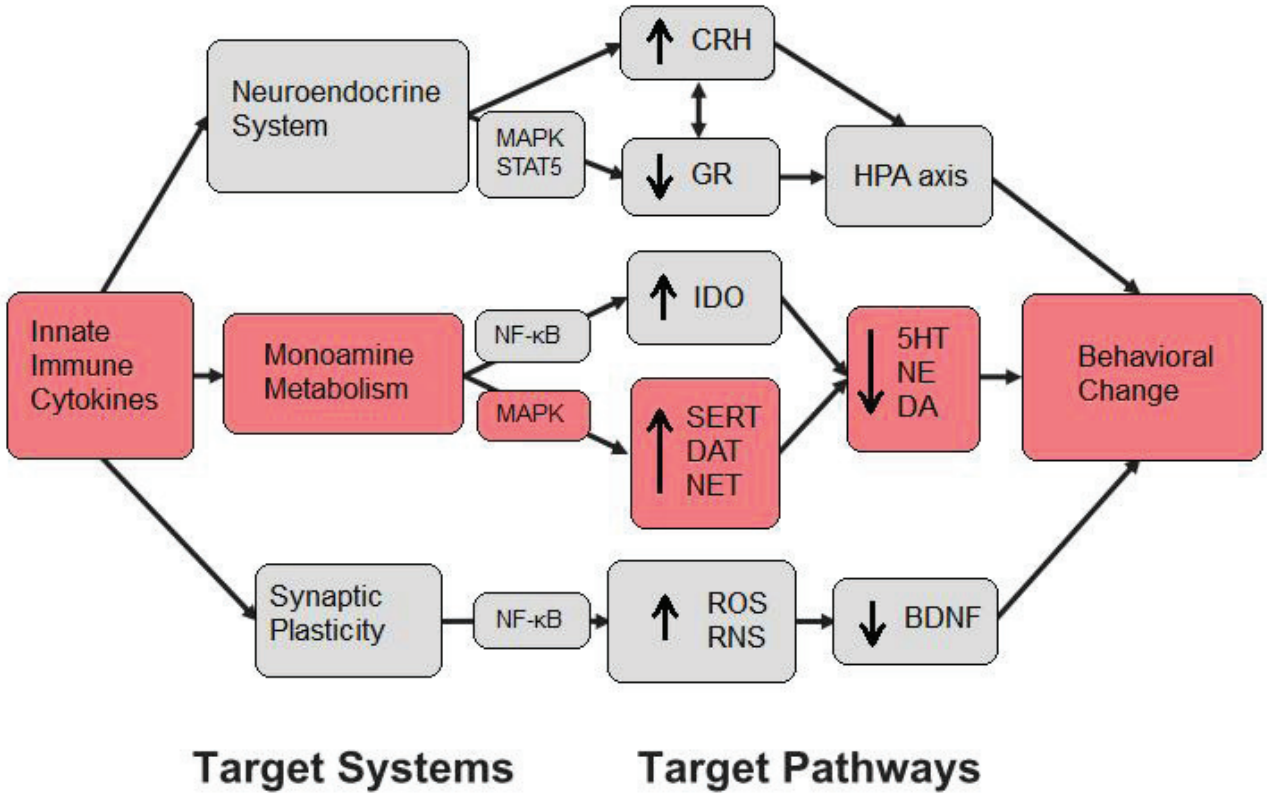

Figure 1. Pathways by which innate immune cytokines can influence the CNS and eventually cause behavioral changes and may manifest in psychological diseases such as depression. Proinflammatory cytokines can exert their effect on the CNS through several pathways. They may activated the HPA axis through the neuroendocrine system, or alter the synaptic plasticity through NF-κB. This review, however, will focus on alteration of the monoamine transporter following MAPK activation. Especially the alterations of the SERT by induction of p38 MAPK eventually leading to decreased 5HT in the synapse accompanied by behavioral changes. (Miller et al., 2008)

Chronic inflammation may cause major depression

Inflammation has been implicated with depression over the last years with support of several studies indicating increased proinflammatory levels in depressed patients. However, these findings could not always been reproduced. Therefore, the possible role for inflammation in major depression is discussed below.

Proinflammatory cytokines may contribute to the pathophysiology of major depression

High levels of proinflammatory cytokines are thought to be a hallmark of major depression (Maes et al., 2012; Rethorst et al., 2012; Simen et al., 2006; Vogelzangs et al., 2012). As mentioned before, increases in proinflammatory cytokines can have profound effects on neurotransmitter metabolism, neuroendocrine function, synaptic plasticity and regional brain activity and therefore may contribute to the pathophysiology of major depression (Dantzer et al., 2008; Vitkovic et al., 2000). Immune responses are tightly regulated under normal circumstances, however in major depression dysregulation of the immune response seems to be apparent. Multiple external but also internal stimuli (e.g. lipopolysacharide, viral infections, prostaglandins, physiological stress and psychological stress) can activate the immune system direct or indirect resulting in increased production of proinflammatory cytokines such as IL1, IL6 and TNFα (Szelényi et al., 2007).

However, valid evidence for immune activation in depressed patients was not always found (Natelson et al., 1999). The same observations were made in animal models. Several studies report increased proinflammatory cytokines (Felger et al., 2010; Golovatscka et al., 2012; Sen et al., 2008), while others contradict these results (Einvik et al., 2012). Noteworthy, most of these studies solely investigate the acute response of cytokines, the chronic effects have not been investigated thoroughly and could have a different effect in this regard. Overall, evidence for association of increased proinflammatory cytokines with depression is not very consistent. However, several subgroup of depression are reported given another explanation. It may be possible that solely in a subgroup of depression elevated proinflammatory cytokines play a role in the causation of the disorder. Therefore, it is postulated that proinflammatory cytokines may contribute to the symptoms and prolong periods of depression

and provide a synergistic effect together with stress but were not, in most cases, the cause of the illness first hand.

High proinflammatory cytokine levels are caused by repeated stress

Several studies indicate that stress can alter migration, proliferation and function of immune cells by influencing the production of cytokines (Reis et al., 2012). Exposure to chronic or repeated stress can contribute to the onset of depression by activating several physiological processes resulting in e.g. hyperactivity of the HPA axis, decreases of neurogenesis, elevation of cytokines and alterations in CNS monoamines (Carpenter et al., 2010; Choi et al., 2008; Pace et al., 2006; Raison et al., 2010; Schmidt et al., 2007). Indirect stressors such as acute and chronic psychological stress are considered a risk factor for the development of major depression. Laboratory animals encountering an acute psychological stressor (e.g. social isolation, social defeat, restraint, water escape or tilted cage) display increased concentrations of IL1, IL6 and TNFα in plasma and periphery fluid (Felger et al., 2010; Golovatscka et al., 2012; Sen et al., 2008). The same results are obtained for chronic mild stress, laboratory animals display increased levels of IL-1 after a chronic stress paradigm compared to wildtype animals (Goshen et al., 2008). Moreover, treatment of soluble IL1 receptor antagonist attenuated, but did not resolve, the behavioral changes induced by social isolation (Capuron et al., 2003). Similar results were obtained in humans, acute stress and chronic stress were associated with increased production of proinflammatory cytokines and decreased production of anti-inflammatory cytokines (Wichers et al., 2007). Humans with a history of early life stress displayed enhanced levels of IL6 in response to an acute stressor (Pace et al., 2006). In addition, people with moderate to severe childhood maltreatment showed not only greater IL6 release in plasma but also overall higher IL6 plasma concentrations (Carpenter et al., 2010).

Recently, most of the mechanism by which an acute stressors influence cytokines levels has been revealed (chronic stress has been postulated to follow the same pathway). Stress will act on the adrenergic pathway to induce higher cytokine levels through the activation of MAPK pathways. Acute stress in laboratory animals (e.g. repeated foot- or tailshock) revealed elevated levels of IL1β, TNFα and IL6 (Blandino et al., 2006; Johnson et al., 2005; Lv et al., 2012). Pretreatment with α-adrenergic antagonist attenuated this increase in proinflammatory cytokines (Johnson et al., 2005), while treatment with a β-adrenergic receptor (βAR) antagonist completely blocked the production of IL1β and IL6 (Blandino et al., 2006; Johnson et al., 2005; Johnson et al., 2008). Consistent with these findings, β-adrenergic receptor agonists elevated concentration levels of IL1β. βAR is a G protein-coupled receptor and can be activated by catecholamines (including norepinephrine and epinephrine). Indeed, treatment with a norepinephrine reuptake inhibitor showed elevated proinflammatory cytokines, suggesting that the main activator in this pathway is NE (Blandino et al., 2006). Consistent with this finding, depletion of NE attenuated the exaggerated IL1β production (Johnson et al., 2005). Recently, a subchronic stress paradigm of four days deciphers the effect of stress the adrenergic system even further. Porterfield et al., (2012) showed that βAR expression following subchronic stress was decreased in the limbic area's (including hypothalamus, amygdala and hippocampus) but increased in the brainstem. However, the NE turnover in the limbic area's was increased. Interestingly, no increased levels of IL1 were found, but treatment with βAR agonist indeed gave an exaggerated IL1 production response in stressed animals compared to control (Porterfield et al., 2012). The decrease in βAR expression might be due to compensation behavior, it is widely accepted that chronic activation of a receptor results in downregulation of that receptor. Indeed, increased βAR due to stress will increase cAMP (cyclic adenosine monophosphate), which will downregulate βAR expression through CRE-dependent (cAMP response element) gene transcription at the βAR promoter (Collins et al., 1992). During acute stress this negative feedback loop may not be activated yet, but during (sub) chronic paradigms it is activated. This decrease in βAR and increase in NE is also seen in other chronic stress paradigms (Asanuma et al., 1999; Woo et al., 1996). Overall, the involvement of the adrenergic system and NE in stress-mediated proinflammatory cytokine release is now well established.

Mechanism by which chronic stress induces proinflammatory cytokines

Adrenergic receptors are common receptors found throughout the whole body, but microglia and macrophages are the main cell types who produce IL1β. Blocking of microglia resulted indeed in decreased IL1 production following stress exposure (Blandino et al., 2006). This suggest that stress promotes βAR activation on microglia cells by either enhancing the receptor's expression or by increasing NE levels resulting in increased proinflammatory cytokine release. The activated βAR will initiate new pathways which will lead to increased proinflammatory cytokines (figure 2). Most research have been performed on protein kinase A (PKA) and NFκB, however, inhibitors of PKA and NFκB failed to inhibit the production of IL1 and IL6, suggest-

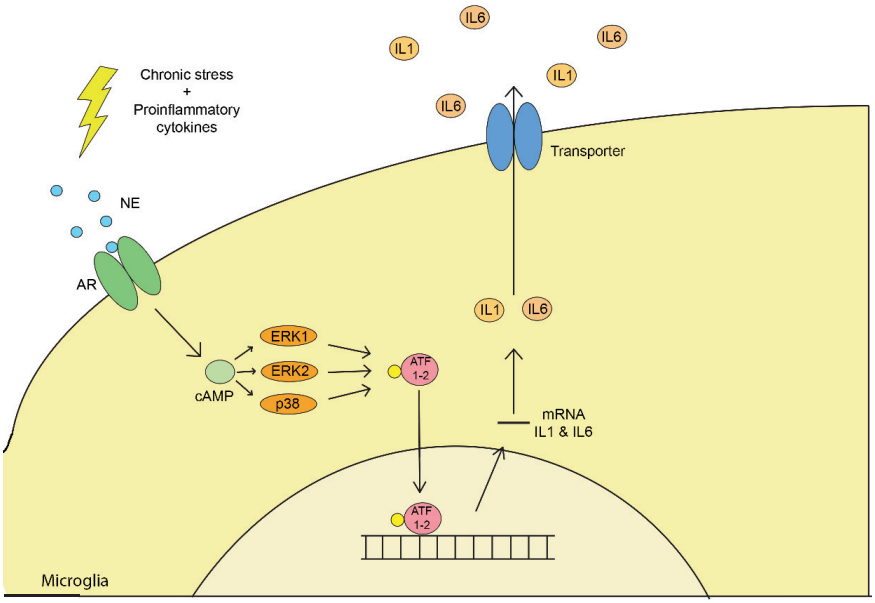

Figure 2. schematic overview of the mechanism in which chronic stress can induce proinflammatory cytokines in microglia cells. Chronic stress is known to induce higher levels of norepinephrine (NE) concentration leading to exaggerate adrenergic receptor (AR) activation. Activated AR acts on ERK1, ERK2 and p38 MAPKs through cAMP signaling. In turn, ERK1, ERK2 and p38 MAPK phosphorylates Activating Transcription Factor 1 and 2 (ATF1&2). ATF will increase the transcription and translation of Interleukin(IL)1 and IL6 resulting in higher levels of IL1 and IL6 in the brain. (Lemstra, unpublished)

ing the involvement of an other pathway. Indeed, it appears that βAR activation leads to ERK1, ERK2 (extracellular signal-regulated kinases 1 and 2) and P38 MAPK activation through cAMP in a PKA and NFκB-independent manner. cAMP agonist solely increases IL1 and IL6 production, proposing a role for cAMP in this process. Consequently, activated ERK1, ERK2 and P38 will phosphorylate transcription factor ATF (cyclic AMP-dependent transcription factor) 1 and 2. These transcription factors will promote the transcription of proinflammatory cytokines such as IL1 and IL6 (Tan et al., 2007). Chronic stress or repeated stress exposure could therefore lead to a chronic state of proinflammatory cytokines i.e. chronic state of inflammation by the positive feedback loop described above. However, the mechanism in which stress alters behavior and can cause a depressive-like phenotype involves yet a different pathway.

Chronic psychological stress can cause SERT alterations through the p38 MAPK pathway

Stress can, next to inducing proinflammatory cytokines, also induce changes in the SERT activity, affinity and surface expression in serotonergic neurons by activating the adrenergic system, eventually resulting in decreased 5-HT concentration levels in the synapse. Changes in concentration levels of monoamines have been implicated with major depression, but also with other psychiatric disorders (Millan et al., 2008; Prasad et al., 2005; Sen et al., 2008; Sutcliffe et al., 2005; Wendland et al., 2008; Zhang, et al., 2007). The SERT is a major determinant of 5-HT signaling due to its reuptake ability of 5-HT and localization on the presynaptic neuron. It shows close resemblance to the transporters of NE and DA (respectively NET and DAT). The SERT is composed of 12 transmembrane domains with an intracellular NH2 and COOH terminal. The SERT is regulated by various G protein-coupled receptors and protein kinase-linked pathways, making the SERT a sensitive modulatory transporter of 5-HT (Blakely et al., 1998; Miller et al., 1994; Reith et al., 1997; Samuvel et al., 2005; Zhu et al., 2004, 2005.

Zhu et al. (2004) was the first to provide solid evidence regarding the pathway in which stress could alter the SERT (figure 3 and 4). Activation of adrenergic receptors (AR) resulted in active Ga-linked PLC which can increase intracellular Ca2+ levels by releasing internal calcium and by increasing calcium influx. Furthermore, after activation of AR a rise in NOS was found. NOS is a known Ca2+/calmodulin–dependent enzyme and indeed calmodulin antagonist decreased the SERT activity (Miller et al., 1994). NOS stimulated by calcium will promote the production of NO, which in turn will activate soluble guanyl cyclase. The latter can activate protein kinase G (PKG) by phosphorylation when it is stimulated by NO (Zhu et al., 2005, 2004). It is proposed that PKG can influence the SERT directly by phosphorylation what will lead to increased affinity, activity and surface expression of the SERT (Ramamoorthy et al., 2007; Zhu et al., 2005, 2004). Furthermore, PKG-induced activation, but not surface expression, of the SERT can be blocked by p38 MAPK inhibitors, suggesting a role for p38 MAPK in this mechanism (Zhu et al., 2005, 2004). It is proposed that activated PKG may lead to phosphorylation of p38 MAPK on Thr180 and Tyr182 (Zarubin et al., 2005). Active p38 MAPK will, in turn, also influence the SERT. However, the manner in which p38 MAPK will exert its function and the effect it will induce are under debate and are discussed below.

p38 MAPK may affect the SERT through direct and indirect pathways

In this review we attempt to elucidate the precise mechanism of action by which active p38 MAPK influence the SERT (figure 4). At the moment it is still unclear whether p38 MAPK alters the SERT functioning by directly phosphorylating the SERT or by indirect pathways. Several points of evidence lead to the latter. Stress activated p38 MAPK is known to have several diverse responses carried out by various downstream targets (including MAPK-activated protein kinases, MAPK interacting kinases, p38-activated protein kinases and several transcription factors) (Gong et al., 2012; Johnson et al., 2008). Furthermore, it is proposed that stress activated p38 MAPK is transported to the nucleus along the microtubule with the help of a chaperone, i.e. HSP70. In the nucleus, p38 MAPK has access to nuclear substrates to exert its function in activating transcription factors (Gong et al., 2012). However, since p38 MAPK is a kinase it can also exert its function outside the nucleus, although direct phosphorylation of the SERT by p38 MAPK has not been shown yet. Indeed, a recent study shows that a cascade induced by PKG is most likely responsible for the phosphorylation of the SERT rather than p38 MAPK (Wong et al., 2012). Due to this fact, several interactors of p38 MAPK might alter the SERT basal phosphorylation, activity and affinity. Syntaxin1A and protein phosphatase 2A are examples of p38 MAPK interactors who associate with the SERT. In line with this view, p38 MAPK antagonist shows downregulation of syntaxin1A and PP2A in synaptosomes compared to control (Samuvel et al., 2005).

Syntaxin1A alters the conductance state and surface expression of the SERT

Syntaxin1A is a protein which is known mostly for it's interaction with the SNARE complex and is therefore associated with vesicle trafficking (Jahn et al., 2006; Rizo et al., 2002; Sollner et al., 1993). There are two main processes in which syntaxin1A may influence the SERT. First, as syntaxin1A is involved in vesicle trafficking with the SNARE complex, it is possible that syntaxin1A can influence the SERT surface expression. Studies involving 5-HT uptake, biotinylation and immunohistochemistry showed that cleavage of syntaxin1A from the SERT resulted in decreased SERT functioning and a decrease in surface expression (Quick et al., 2002, 2003; Samuvel et al., 2005). Therefore it is speculated that activation of syntaxin1A by p38 MAPK may increase the insertion of the SERT in a syntaxin1A-SNARE-dependent manner. Secondly, syntaxin1A has been shown to interact directly with the SERT and this interaction may determine the conducting state of the SERT (Ciccone et al., 2009; Quick et al., 2003). The interaction of the SERT with syntaxin1A will convert the SERT from an electrogenic 5-HT carrier to one that is electroneutral. Thus, when syntaxin1A is not bound to the SERT, a voltage-dependent flux of excess sodium will occur resulting in an electrogenic state where 12 additional sodium ions are necessary to transport one 5-HT at 80 mV. During this state 5-HT-induced ionic currents or 5-HT-independent sodium leak currents are present. Consequently, when the SERT and syntaxin1A do form a complex, 5-HT uptake will proceed in an electroneutral manner meaning that along with 5-HT uptake also one sodium and one chloride ion is transferred inward while one potassium ion being

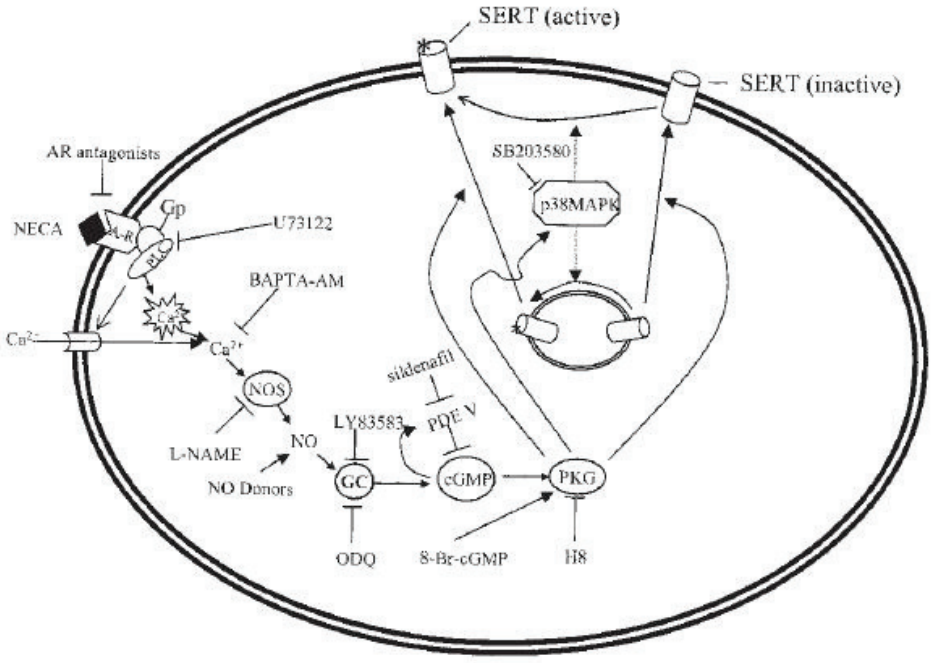

Figure 3. schematic illustration of signaling pathways implicated in adrenergic receptor (AR) upregulation of the SERT. AR activation will result in Gq-linked PKC stimulation to increase intracellular Ca2+. Increases in Ca2+ will stimulate NOS to produce NO which will activate guanyl cyclase (GC), producing elevated levels of cGMP. cGMP will activate PKG which, in turn, might activate p38 MAPK. PKG and p38 MAPK togethery will increase the activity, affinity and surface expression of the SERT (Zhu et al., 2004).

transferred outward. Hereby, 5-HT-induced ionic currents or 5-HT-independent sodium leak currents will not be present. When 5-HT-independent leak currents and 5-HT-induced transport-associated currents are absent, the neuron will not be able to depolarize. Thus, when syntaxin1A is bound to the SERT it will block depolizarization (Ciccone et al., 2009; Quick et al., 2002, 2003). To speculate further, when a neuron is not able to depolarize, a decrease in 5-HT release could be observed resulting in decreased 5-HT levels in the synapse which can eventually affect behavior. In addition, it is possible that inducing the electroneutral state by syntaxin1A binding to the SERT, might resulted in changed activity and/or affinity of the SERT (Ciccone et al., 2009; Goldshleger et al., 1990; Quick et al., 2002, 2003).

PP2A inhibits internalization of the SERT

Another protein which associates with the SERT is PP2A. The catalytic subunit of PP2A has been shown to bind directly with the SERT (Bauman et al., 2000). It has been proposed that phosphorylation of the SERT has a great influence on the expression, activity and affinity of the SERT (Jayanthi et al., 2005). Interestingly, it seems that PP2A might play a role in this mechanism as inhibition of PP2A results in increased phosphorylation, reduced surface expression and loss of activity of the SERT (Bauman et al., 2000; Prasad et al., 2009). It is thought that PKC might phosphorylate on serine residues – triggering internalization of the SERT while PKG (and possibly also a role for p38 MAPK) phosphorylates the SERT on threonine residue to alter affinity and activity (Ramamoorthy et al., 2011; Ramamoorthy et al., 2007; Zhang et al., 2007). Recently a part of this mechanism has been unravelled. Ramamoorthy et al. (2007) showed that PKG can phosphorylate the SERT on threonine residue 276, controlling SERT trafficking but also increasing the affinity of the receptor. In addition, PKG will activate p38 MAPK which in turn will activate PP2A that will keep the inhibitory serine residue dephosphorylated hereby blocking PKC-induced internalization of the SERT so that SERT activation is not opposed. Consistent with this view, PKC activation triggers PP2A dissociation of the SERT before internalization can occur (Bauman et al., 2000; Ramamoorthy et al., 2007; Samuvel et al., 2005). The precise process in which p38 MAPK might activate PP2A is not completely elucidated yet. As p38 MAPK might exert its function inside or outside the nucleus it is possible that PP2A activation occurs by direct phosphorylation of p38 MAPK or by slow upregulation via gene transcription of the regulatory subunit of PP2A (Fey et al., 2012). All together, activation of PP2A by p38 MAPK will lead to decreased internalization of the SERT and might enhance affinity and activity of the SERT by physical changes upon binding.

P38 MAPK increases the activity and affinity of the SERT

Although some suggestion have been made in what manner p38 MAPK affects the SERT, the influence of p38 MAPK on the performance of the SERT is another point of debate. Studies suggest that p38 MAPK upregulates surface expression, increases affinity and activity of the SERT (Lau et al., 2009; Samuvel et al., 2005). Blocking of p38 MAPK resulted in lower 5-HT uptake and a decrease in surface expression measured with surface biotinylation. However, there is some discrepancy about this finding. Other studies only showed an increase in 5-HT uptake after p38 MAPK activation, which could be explained by the observed decrease in Km (Zhu et al., 2005, 2004). A decrease in Km indicates that the affinity of the receptor has increased. Therefore, a higher uptake of 5-HT can be a consequence of higher affinity rather than higher surface expression of the SERT. However, multiple studies implicate a raise in surface expression of the SERT while only 5-HT uptake is measured (implicated by Lau et al., 2009; Samuvel et al., 2005). More evidence showing solely a decrease in Km and an increase in activity of the SERT was supplied by Zhu et al., (2005). Their study showed that p38 MAPK inhibitors could not attenuate the increase in surface expression of the SERT. The discrepancy between these studies can be due to various aspects. As the studies used different kinds of cells, inhibitors and time periods, inconsistencies should be expected. To solve the contradicting results, more research needs to be performed. However, based on the more valid studies of Zhu and coworkers it is suggested that PKG might be involved in the upregulation of the SERT surface expression while p38 MAPK might be more involved in the activity and affinity of the SERT.

P38 MAPK is not involved in DAT regulation

In addition to the SERT, also other monoamine transporters are influenced by the p38 MAPK pathway. The influence of p38 MAPK on the function of the norepinephrine transporter has been well characterized and have been the focus of several studies and reviews (Mannangatti et al., 2011; Ramamoorthy et al., 2011), for that reason this review will not focus on the norepinephrine transporter. The last decade a lot of interest went into elucidating the regulation of the dopamine transporter (DAT). Reduction of dopamine concentration levels is also of interest in major depression and other psychological diseases. Although the precise mechanism is still

unknown, a clear role for p38 MAPK is uncertain. Zhu et al. (2005) and Morón et al. (2003) both found that the DAT is regulated by mitogen-activated protein kinases. However, there are multiple MAPK of which ERK1 and ERK2 seem to be responsible for DAT regulation rather than p38 MAPK (Blakely et al., 2005; Jayanthi et al., 2005; Nakajima et al., 2004; Ramamoorthy et al., 2010; Ramamoorthy et al., 2011; Zhu et al., 2004). Inhibition of p38 MAPK did not influence the activity or transport of DAT in striatal neurons, suggesting a pathway different from p38 MAPK (Morón et al., 2003). Consistent with these findings, activation of p38 MAPK did not influence the activity of the DAT in chinese hamster ovary cells (Zhu et al., 2005). Thus, the regulation of the DAT probably does not involve direct p38 MAPK influence but is likely to be monitored by another mechanism.

However, second messengers from p38 MAPK activation might have an influence on the regulation of the DAT. As mentioned before, PP2A is activated by p38 MAPK, and has also been shown to form a complex with the DAT (Jayanthi et al., 2005; Ramamoorthy et al., 2010). DATs are phosphorylated to influence their activity, similar to SERTs. Therefore, the active PP2A by p38 MAPK might, in addition to the SERT, also influence the DAT. Interestingly, PP2A inhibitor downregulates DAT activity, which might suggest that activation of PP2A might stabilize the DAT (Bauman et al., 2000; Jayanthi et al., 2005). However, as activation of p38 MAPK resulted in, if anything, decreased activity of DAT, it is more likely that PP2A activation does not have a major influence on the DAT. It is possible that PP2A has a small role in DAT activity, but that the DAT regulation is governed by other systems which will compensate the small effect of PP2A on the DAT.

SERT dysregulation can increase the susceptibility to depression

Several indications are now given that activation of the p38 MAPK can lead to alterations in the SERT. However, as p38 MAPK is involved in many other processes such as proliferation, differentiation, and apoptosis it is not surprising that activation of this pathway can have wide spread effects (Deneris et al., 2012; Jayanthi et al., 2005; Lau et al., 2012; Ramamoorthy et al., 2011). Furthermore, it is important to note that activation of the adrenergic system will not solely activate PKG and p38 MAPK but it will also influence the HPA axis and NF-κB pathway and probably even more. All these different pathways can have an effect on the SERT and change it's expression, activity and affinity (for more information see review: Miller et al., 2008). Probably all these pathways, including p38 MAPK orchestrate together to regulate the basal SERT function. For that reason it is very difficult to solely elucidate the effect of the p38 MAPK pathway on behavior. Therefore, the focus will remain on SERT dysregulation rather than the effects of activated p38 MAPK on behavior, to elucidated the cause of depression further.

Active p38 MAPK through the adrenergic system will increase activity, affinity but also surface expression (most likely through PKG) of the SERT. As changes in the kinetics and number of the SERT will strongly influence 5-HT availability in the synapse, it is suggested that with p38 MAPK activation a decrease of 5-HT in the synapse is observed. Elevated functioning of the SERT will take up more 5-HT resulting in less 5-HT in the synapse. The serotonergic system innervates seemingly all regions of the brain and spinal cord (Deneris et al., 2012). Its neurotransmitter serotonin is implicated in various processes such as vasoconstriction, gastointerstinal motility and secretion, respiration, sleep, appetite, aggression and mood (Deneris et al., 2012; Gaspar et al., 2003; Homberg et al., 2012; Muller et al., 2010; Pae et al., 2012). Therefore it is not surprising that alterations in 5-HT concentrations in the synapse can lead to serious behavior disabilities. Indeed, a wide spectrum of disorders are implicated with serotonin dysregulation such as irritable bowl syndrome, sudden infant death syndrome, anorexia, obsessive-compulsive disorder, autism, suicide and major depression (Bale et al., 2010; Deneris et al., 2012; Eggers et al., 2003; Lira et al., 2003; Prasad et al., 2009; Sutcliffe et al., 2005; Wendland et al., 2008). Disrupted 5-HT signaling has been proposed as a cause for major depression. The classic model for the causation of major depression describes a decrease of 5-HT in the synaps. Although, human studies have never confirmed this theory due to technical dificulties. Post-mortem studies are not conclusive due to reduced levels of 5-HT after death and *in vivo* research in humans with microdialysis is for obvious reason not possible. Current antidepressant medications mainly target the monoamine transporters, this treatment is only effective in a third to a half of patients suffering from major depression (Pigott et al., 2010). This indicates that alterations in monoamine levels can not be solely the cause of depression. Therefore, it is plausible that serotonergic alterations might contribute to the development or symptoms of major depression, as seen for immune dysregulation. To speculate how p38 MAPK-induced changes in the function of the SERT can alter behavior, several studies of SERT-KO, but also, SERT-gain-of-function are discussed. Furthermore, the effect of chronic stress and chronic inflammation on behavior are examined.

SERT-KO models

Several studies are performed examining the role of serotonin on behavior in laboratory animals by creating knockouts of the SERT or by administrating selective serotonin inhibitors (SSRIs). The effect of SERT depletion or inhibition will logically result in an increase of 5-HT in the synapse, CSF or plasma, due to a lack of uptake by the SERT. However, there is discrepancy between studies about the alterations in 5-HT levels following depletion or inhibition of the SERT. Several studies indeed report an increase in 5-HT while other studies describe a decrease of 5-HT (Haenisch et al., 2011; Homberg et al., 2010; Homberg et al., 2012; Jayanthi et al., 2005; Lira et al., 2003; Zhong et al., 2012). There are multiple explanations for this discrepancy. For example, in addition to the SERT, 5-HT can be taken up by other transporters during acute stages. 5-HT uptake by the DAT has been observed as compensating behavior. The same phenomena could be apparent in this case (Zhou et al., 2002). Another possibility is the differences in animal models that were used. In some studies a KO of the SERT was created. As the serotonergic system is involved in

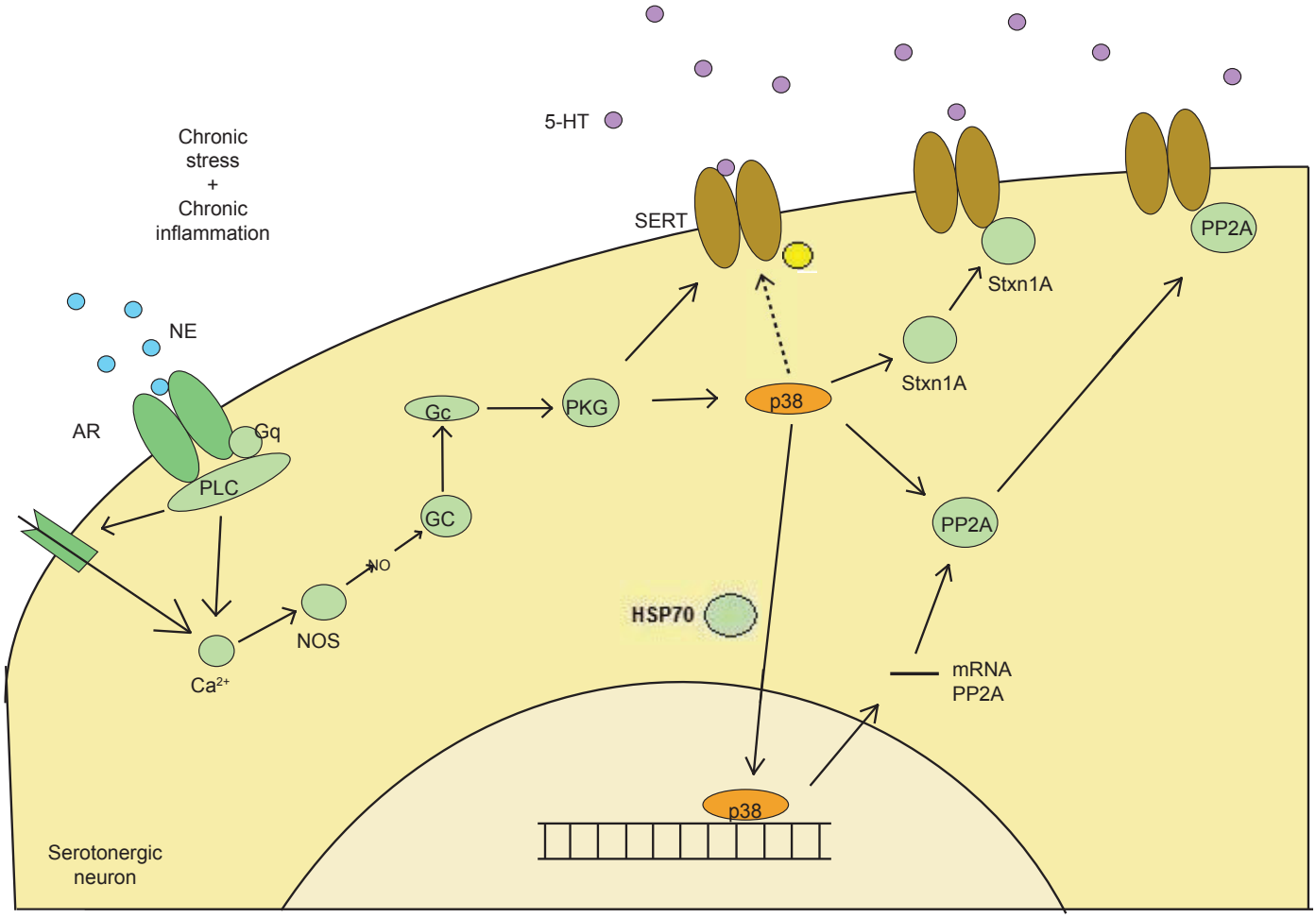

Figure 4. Hypothetical schematic overview of the mechanism in which stress can alter the monoamine serotonin transporter (SERT) and induce behavioral changes. Chronic stress will increase norepinephrine (NE) and activate the adrenergic receptor (AR). AR activation will lead to Gq-linked PKC stimulation to increase intracellular Ca2+. NOS will be stimulated by the elevated levels of Ca2+ and produce NO which in turn will activate guanyl cyclase (GC). GC will produce cGMP which will activate PKG. Next, we hypothesize that PKG will increase the surface density of the SERT and will activate p38 MAPK. P38 MAPK may affect the SERT in three ways. It may act directly on the SERT increase its activity and affinity or it may affect the SERT through several second messengers such as syntaxin1A (stxn1A) and protein phosphatase 2A (PP2A). Syntaxin1A will get activated by p38 MAPK and changes the conductance state of the SERT to electroneutral. An electroneutal state may inhibit depolarization of the protein resulting in a decrease amount of serotonin (5HT) release. Furthermore, the physical interaction of syntaxin1A with SERT might increase the activity and affinity of the SERT. PP2A will get activated by p38 MAPK directly or by slow upregulation via gene transcription. In the latter case, p38 MAPK has been shown to travel to the nucleus accompanied by HSP70. Activated PP2A is associated with the SERT and will inhibit PKC internalization resulting in a prolonged surface expression of the SERT. Eventually chronic stress will increase the surface expression (PKG mediated), affinity and activity (p38 mediated) of the SERT. These increases of SERT function will result in decreased concentrations levels of 5HT in the synapse leading to multiple behavioral deficits but can also lead to developmental and physiological deficits as the serotonergic system is implicated in many of these processes. (Lemstra, unpublished)

many processes, it is also involved in neurodevelopmental processes including cell division and differentiation, neuronal migration and maturation and synaptogenesis (Gaspar et al., 2003). Consequently, when genetic changes already occur during the start of development it will impact the whole organism on a greater scale than by only blocking the SERT temporarily. Therefore, studies using blocking agents for the SERT might be a more valid model. Indeed, administration of SSRIs will block the SERT resulting in consistent higher levels of 5-HT in the synapse. Interestingly, the acute increase in 5-HT does not immediately treat the depressive symptoms. Traditional antidepressants require 2 weeks or more to become therapeutic effective. This suggest that the changes necessary for the effectiveness of antidepressant are involved in synaptic plasticity and neuronal changes in addition to normalized 5-HT levels (Zhong et al., 2012). Also, it suggests that, for depression, research should focus on the chronic effects of decreased 5-HT in synapses rather than acute effects. Importantly, the classic theory of depression represents a decrease of 5-HT in the synapse due to increased activity of the SERT. This model is dificult to validate in humans as in post-mortem brains the amount of proteins often is reduced and in vivo microdialysis in humans is out of the question. However, assuming the classic theory about depression is correct, models where the SERT is inhibited seem less sufficient for depression, as inhibition of the SERT will result in an increase of 5-HT in the synapse, while in depressed patients a decrease of 5-HT may occur. For this reason the field now starts to focus on animal models with a SERT that contains a gain-of-function polymorphism.

SERT gain-of-function models

Gain-of-function polymorphisms of the SERT promoter were recently indentified for several psychiatric disorders such as depression, autism and rigid-compulsive disorder (Deneris et al., 2012). One gain-of-function polymorphism was generated in the mouse. In this mouse model it was shown that the levels of serotonergic markers were associated with markers in patients with depression. This confirms the idea that major depression indeed reflects decreased 5-HT levels in the brain. Kinetic studies revealed increased 5-HT uptake with the altered promoter. Furthermore, Vmax was increased and Km was decreased for the SERT indicating an increase in activity and affinity. In addition, the SERT surface density was elevated (Deneris et al., 2012; Prasad et al., 2005). Animal studies with mice exerting increased 5-HT clearance in the brain showed normal growth and fertility. The animals were tested on behavioral tasks to assess any changes which might be associated with depression. The mice displayed a twofold decrease in vocalization, which might represent the withdraw from social interaction which can be observed in depressed patients. Indeed, when tested on social preference the mutant mice did not show any preference for social interactions and upon encountering another mice it withdrew itself from the scene. Interestingly, these mutant mice did not show any differences in anxiety-like behavior, in contrast with many loss-of-function SERT animals (Veenstra-VanderWeele et al., 2012). Although this study suggest that characteristics of major depression are caused by a decrease of 5-HT in the CNS through elevated SERT functioning, more behavioral research should be performed for gain-of-function SERTs to substantiate these results. Furthermore, as mentioned earlier, many psychiatric disorders show decreased 5-HT levels in the brain, suggesting that the behavior observed may not solely display depressive behavior. However, more research investigating the gain-of-function, rather than the loss-of-function, phenotype should elucidate the contribution of the serotonergic pathway in depression, and other psychological disease. Furthermore, animal models should be generated with chronic, but not permanent, changes in the SERT – as the serotonergic pathway is also involved in many other physiological processes. Unfortunately, such a model is not available yet, and the field just started to focus on the gain-of-function SERT phenotypes by genetic alterations.

Induced inflammation as animal model for depression

For other models of depression, proinflammatory cytokines are used. As cytokines have been associated with depression, many animal models for depression were created with enhanced proinflammatory cytokine production or by administrating proinflammatory cytokines. Animals with enhanced proinflammatory cytokines (including IL1, IL6 and TNFα) exhibit behavioral symptoms referred to as sickness behavior (Capuron et al., 2006; Dunn et al., 2005; Simen et al., 2006; Wohleb et al., 2011). Sickness behavior is associated with behavioral changes seen in laboratory animals, but also humans, when suffering from microbial infections. These behaviors include fatigue, anorexia, anhedonia, sleep alterations, cognitive dysfunction and increased sensitivity to pain (Kent et al., 1992). Although certain features of sickness behavior have been linked to major depression (anhedonia, sleep alterations and cognitive dysfunction), it is not clear whether the two phenomena are related. The symptoms of sickness behavior are rather nonspecific and can be associated with many diseases (Deneris et al., 2012; Simen et al., 2006). Therefore, these animal models do not seem to be a valid model for major depression.

Chronic stress animal models for depression

Overall, decreased 5-HT levels in the synapse due to increased SERT activity, affinity and density might create a wide spectrum of behavioral changes. To further investigate the influence of serotonergic dysregulation in major depression, it is important to acknowledge the affected brain area's. As the serotonergic system can influence practically all brain area's the field should focus on the brain area's which are affected by chronic stress exposure – as this seems to be a key factor for major depression. Previously mentioned, subchronic stress followed by adrenergic receptor expression was decreased in the limbic area's including the hypothalamus, amygdala, nucleus accumbens and hippocampus. Also, an increase in the AR was observed in the brainstem (Dunn et al., 2005; Porterfield et al., 2012; Szelényi et al., 2007). As the limbic system is mostly involved in mood, motivation and memory it is not surprising that changes in this area can lead to behavioral dysfunction. Exactly how extensive the behavioral changes are could depend on the duration (acute versus chronic) and/or the cause (stress or cytokine induced SERT changes) of the stressor. Both chronic stress or chronic inflammation might manifest disturbances in attention and memory (Capuron et al., 2006). Animals subjected to the chronic mild stress (CMS) paradigm displayed increased immobility during the forced swim test compared to vehicle (Pao et al., 2012). However, while CMS reproduces specific core depressive symptoms such as anhedonia and helplessness, chronic social defeat stress model is proposed to be more relevant to stress-induced human psychiatric disorders such as major depression. Primarily focussing on depression, it seems that especially loss or fear of loss of a social status is thought to play a major role in inducing depression in humans. Therefore, it seems that animal models experiencing chronic social stress might be a very naturalistic model for depression. Indeed, chronic social defeat stress induced anhedonia, hyperactivity, anxiety and social avoidance, signs common to major depression, anxiety disorders and posttraumatic stress disorders (Goshen et al., 2008; Porterfield et al., 2012; Venzala et al., 2012; Ye et al., 2012). Both animal stress models (CMS and chronic social defeat) might overlap at some points but also cover other symptoms. Therefore, both models should be used to unravel the underlying mechanism further to investigate the role of serotonin dysregulation.

However, again, it is important to note that altered 5-HT signaling is apparent in the aetiology of many psychiatric and neurodevelopment disorders such as depression, anxiety, obsessive compulsive disorder, disorders of energy balance, autism and schizophrenia (Muller et al., 2010). The ongoing hypothesis states that interactions of genetic determinants with environmental factors eventually program too much or too little 5-HT in serotonin neurons (Capuron et al., 2006; Deneris et al., 2012; Reith et al., 1997). As the serotonergic system is innervated in many brain regions and implicated in the modulation of nearly al human physiological and psychological processes orchestrated by the CNS, it is not surprising that alterations in this system can cause such wide spread changes in physiological and psychological processes. Moreover, changes in this system during developmental stages or throughout life will consequently disrupt neuronal trajectories in the CNS and thereby develop a higher susceptibility to mental illness, including major depression (Gaspar et al., 2003).

From stress to depression – the intracellular mechanism

Based on the literature available at this moment, we would like to propose the mechanism of action from stress to depression involving p38 MAPK (Figure 4). Certain steps of the proposed pathway are still uncertain, in box 1 several research possibilities to clarify some steps are mentioned per number which can be find in the text. Chronic physiological stress will act on the adrenergic system by activating adrenergic receptors (1). The activated AR will in turn activate Gq-linked PLC which will increase intracellular calcium levels. Due to high calcium availability, NOS will interact with calcium and subsequently promote NO production. Afterwards, soluble guanyl cyclase will be activated by NO and will in turn phosphorylate PKG. Next, p38 MAPK is activated by PKG, however in which manner is still unknown. It is possible that PKG phosphorylates p38 MAPK into an active form (2). Next, the affect that active p38 MAPK exerts on the serotonergic system is under debate and will be discussed below.

P38 MAPK activation will cause alterations in the SERT activity by changing Km and Vmax. An increase in Vmax and a decrease in Km is observed in the SERT suggesting a higher uptake rate with increased affinity for 5-HT. Also the surface density of the SERT is increased which is, in my believe, due to PKG activation (although a complete role of p38 MAPK can not be excluded) (3). P38 MAPK might act directly on the SERT by phosphorylating parts of the protein, it might phosphorylate second messengers which in turn alter SERT activity or it may travel to the nucleus and effect the SERT through several transcription factors (4). A combination of the above listed possibilities is also not excluded. A few interactors of p38 MAPK are known to associate with the SERT. For instance, syntaxin1A is

directly or indirectly activated by p38 MAPK and interacts with the SERT. It might influence surface expression of the SERT through SNARE-dependent transporter, resulting in increased surface density of the SERT. In addition, syntaxin1A can also change the conducting state of the SERT. When bound to the SERT it will favour a electroneutral state in which the neuron will not be able to depolarize resulting in a decreased release of 5-HT into the synapse. Whether syntaxin1A indeed influence the depolarization of serotonergic neurons needs to be further investigated (5). Another interactor of p38 MAPK is PP2A. It is not completely known how PP2A is activated by p38 MAPK. P38 MAPK might activate PP2A through direct phosphorylation or by slow upregulation via gene transcription (4). PP2A will, when activated, keep the SERT dephosphorylated on the serine residue to inhibit internalization by PKC. Decreased phosphorylation of the serine residue will keep the SERT prolonged in an active state. Overall, a decrease of 5-HT will be observed in the synapse due to increased SERT density, activity and affinity after stress exposure.

Decreased 5-HT levels should observed in mostly all depressed patients according to the classical theory of the causation of depression (6). As mentioned before, it is dificult to measure the 5-HT concentration in living humans as microdialysis studies can not be performed. However, any alterations in 5-HT levels do have a pronounced effect on behavior but also affect other physiological processes. The serotonin system is involved in numerous of vital developmental and physiological processes. Animal models with SERT modulations do show depressive characteristics such as anhedonia, anxiety-like behavior and anti-social behavior. However, due to the input from many other pathways and the involvement of the p38 MAPK in various other processes, the precise behavior induced solely by the p38 MAPK pathway can not be established (yet) (7). Although, a role for p38 MAPK in depression is definitely established, several question marks still need to be elucidated.

Role of proinflammatory cytokines in the proposed mechanism

Another route in which p38 MAPK is activated also involves the AR (figure 2). Activation of AR through stress exposure leads to the activation of p38 MAPK, ERK1 and ERK2 through cAMP. Next, ATF1 and ATF2 will be phosphorylated by the MAPKs increasing transcription of IL1 and IL6. The mean reason for the different route seems to be the cell in which it takes place. Activation through PKG is set in serotonin neurons while activation through cAMP is set in microglial cells. If these pathways can also be induced in microglia or serotonin neurons respectively still needs to be unravelled. Furthermore, proinflammatory cytokines seem to be a hallmark of major depression. However, if proinflammatory cytokines play a part in the mechanism involving p38 MAPK is uncertain. Activation of proinflammatory cytokines may be "just" a consequence instead of a cause in this case. Although, we have to bare in mind that the mechanism described here is not the complete mechanism in which p38 MAPK acts or how depression is evolving. Therefore, proinflammatory cytokines probably do contribute to the disease but by another pathway.

As mentioned before, although cytokines may not be the main cause of depression, elevated levels of proinflammatory cytokines may affect processes important for normal behavior using various methods. In line with this view, anti-TNFα does not resolve depression but may attenuate depressive symptoms (Raison et al., 2012). Elevations of proinflammatory cytokines such as IL1, IL6 and TNFα may contribute in the adrenergic-PKG-p38 MAPK pathway inducing changes in the SERT. It is well known that IL1 increases NE in the synapse (Kabiersch et al., 1988), leading to activation of adrenergic system and thereby PKG and p38 MAPK eventually resulting in an increased activity, affinity and density of the SERT. Several reports show similar effects for TNFα, although these effects might be the cause of TNFα-induced IL1. There is no evidence that IL6 influences this pathway (Dunn et al., 2005). Furthermore, inhibition of p38 MAPK abolishes IL1- or TNFα-induced SERT alterations (Zhu et al., 2006) – again indicating that proinflammatory cytokines do not have a direct influence on monoamine transporters. It is possible that proinflammatory cytokines enhance depressive symptoms even more through a positive feedback loop. It has been proposed that IL1β activate microglial cells through the adrenergic system. As mentioned before, activation of the adrenergic system in microglia cells also lead to increased levels of proinflammatory cytokines thereby creating a positive feedback loop (Blandino et al., 2006; Furuyashiki, 2012; Johnson et al., 2005; Lv et al., 2012; Tan et al., 2007). Therefore, chronic inflammation of the peripheral immune system caused by either stress (and preserved by the positive feedback loop) or by systemic inflammation may send continue signals to the CNS leading to extended activation of the adrenergic system providing a synergistic effect when proinflamamtory cytokines and chronic stress are present together. This will maintain an activated p38 MAPK with its interactors and thereby prolong the increased affinity and activity of the SERT.

New treatment insights for depression

Currently, the most used therapy for major depression is the blocking of the SERT with selective SSRIs. Alternatives for SSRIs are other drugs that block the SERT such as imipramine, fluoxetine, paroxetine, sertraline and citalopram. SSRIs rapidly inhibit the uptake of serotonin in the synapse resulting in normalized 5-HT levels. However, this acute effect does not resolve depression or the depressive symptoms (Jayanthi et al., 2005). Overall it will take 2-3 weeks before SSRIs will become of therapeutic value. This, as mentioned before, indicates that the elevation of 5-HT in the synapse induces compensatory or intracellular regulatory pathways, hence neuroplasticity, resulting in behavioral alterations relieving the patient from major depression. However, SSRI treatment is only successful in a third to a half of all depressed patients. The same is observed with other SERT blockers. For instance, paroxetine reduces depressive symptoms induced by IFNα treatment, but does not cure the patient from depression (Raison et al., 2007). Nevertheless, although SERT blockers might not completely resolve depression, modulation of the kinetics and number of the SERT will strongly influence the efficacy of antidepressants. In the last years other treatments, in addition to SSRIs, have become of interest to treat major depression. Inflammatory biomarkers such as proinflammatory cytokines, acute phase proteins and chemokines have been found to be upregulated in depressed patients. Importantly, patients with high levels of inflammatory cytokines have been reported to be less responsive to conventional antidepressants. In line with this view, treatment-resistant patients have been found to exhibit increased concentrations of IL6 compared to treatment-responsive patients (Miller et al., 2008; Lanquillon et al., 2000; Raison et al., 2007; Raison et al., 2012). Furthermore, successful antidepressant treatment has been associated with reduction in proinflammatory cytokine concentrations such as TNFα and IL6. These findings suggest that therapeutic efficacy might be increased when inflammatory signaling pathways are downregulated. It is possible that conventional antidepressants might only affect selected symptoms (e.g. depressed mood and anxiety) while other symptoms (e.g. fatigue and psychomotor slowing) are not affected and might be addressed by other pathways, possibly induced by proinflammatory cytokines (Miller et al., 2008). Furthermore, inflammatory cytokines can sabotage and circumvent the effects of antidepressant medication in several ways. Proinflammatory cytokines can modulate the expression of monoamine transporters, activate enzymes (e.g. indoleamine 2,3-dioxygenase) which reduce the concentration of the serotonin precursor tryptophan, inhibit enzyme cofactors to

Box 1

Future research possibilities

1. How chronic stress activates the adrenergic system is unknown. This can be elucidated in the blood of experimental animals who experienced chronic psychosocial stress. Blood platelets also take up 5HT in the same manner as in the CNS (Lau et al., 2012). Thus, in platelets the precise mechanism on which chronic stress act to activate the adrenergic system can be investigated.
2. If PKG activates p38 MAPK directly can be investigated using a coexpression immuno-histochemistry assay. Coexpression of PKG with P38 MAPK will indicate that they do interact with each other which will favor the direct phosphorylation of p38 MAPK by PKG.
3. To unravel if increases in surface density of the SERT occurs through PKG or p38 MAPK activation a surface/internal immunohistochemistry can be performed on platelets. The platelets should be incubated with either p38 MAPK agonist or PKG agonist and p38 MAPK inhibitor. First immunohistochemistry for the surface SERT should be performed using a buffer without damaging the cell's surface, next the platelets can be incubated with a buffer containing triton to allow internal immunohistochemistry of the SERT. Now the amount of surface expressed SERT can be quantified.
   Another possibility: the phosphorylated state of the SERT can be investigated after incubation with p38 MAPK agonist or PKG agonist by incubating anti-phosphate antibodies. However, these are unfortuanetly not available yet for the SERT. Therefore a more tradinational approach can be used. First, whole cells are incubated with radiolabeled ^{32}P-orthophosphate and cellular extracts are generated. The SERT proteins are separated by SDS-PAGE and exposed to film to measure the levels of phosphate (de Graauw et al., 2006).
4. Whether p38 MAPK acts directly on the SERT after chronic stress exposure can be investigated using a coexpression immunohistochemisty. Furthermore, using immunohistochemistry can elucidate whether p38 MAPK travels to the nucleus and if it is coexpressed with the interactors PP2A and syntaxin1A. If p38 MAPK is not coexpressed with PP2A, it might be possible that it will activate PP2A through slow upregulation via gene transcription. In that case the mRNA of PP2A can be blocked with miRNA to see if the activation of PP2A is blocked.
5. Inhibition of depolarization by syntaxin1A in serotonergic neurons can be investigated using electrophysiology. Brain slices incubated with syntaxin1A should depolarize less than control.
6. Whether depressed patients show altered SERT functioning can be investigated in blood samples of depressed patients. In platelets the activation of the SERT can be investigate to see if there is indeed an increase in the activity, affinity and surface density of the SERT compared to healthy subjects.
7. The precise behavioral changes induced by p38 MAPK activation are unknown. Administration of p38 MAPK agonist (e.g. PD169316) to experimental animals could unravel the behavioral changes induced by p38 MAPK.

reduce monoamine precursors and can block neurogenesis through the nuclear factor κB pathway (figure 1) (Raison et al., 2006, 2012). Therefore, treatment possibilities aimed to downregulate inflammatory cytokines might contribute to the antidepressant effect and will be especially useful in patients with treatment-resistant depression.

Anti-inflammation therapy – restoring immune balance

Recent data indeed indicate that treatment with anti-inflammatory cytokines or inflammatory antagonists can have, or contribute to, antidepressant effects. For instance, TNFα antagonist reduced depressive symptoms in treatment-resistant patients (McNutt et al., 2012; Miller et al., 2008; Raison et al., 2012). Similar results were obtained using an anti-inflammatory agent, celecoxib in combination with reboxetine (Miller et al., 2008). Animal studies are also consistent with the above findings. Mice with a TNFα receptor KO have been found to be resistant to anxiety-like behaviors induced by infection (Simen et al., 2006). In addition, cytokine-induced sickness behavior, which holds characteristics of depression, can be attenuated by cytokine antagonists or anti-inflammatory cytokines (Pugh et al., 1999). Bacterial probiotics who stimulate the CNS through gut microbiota has been found to protect rats from depression-like behaviors after maternal separation stress (Rook et al., 2012). Moreover, proinflammatory cytokine antagonists show antidepressant effects even in the absence of an immune challenge (Reynolds et al., 2004). These findings suggest that anti-inflammatory agents (e.g. IL10) or proinflammatory cytokine antagonists, in addition to conventional antidepressant therapy, might hold an improvement in treatment possibilities, especially for treatment-resistant patients. However, the field just started investigating the efficacy of pro-inflammatory antagonist or anti-inflammatory agonists treatment in depression. In addition to inflammatory antagonist or anti-inflammatory agents, it may be possible to induce T regulatory cells as a treatment for major depression. T regulatory cells are known to restore the balance between proinflammatory and anti-inflammatory immune states. Therefore, activating T regulatory cells might downregulate the chronic inflammation seen in treatment-resistant patients with major depression. Indeed, treatment with mycobacterium vaccae induced T regulatory cells and improved quality of life scores in patients with inflammatory diseases (Rook et al., 2012). Therefore it is postulated that T regulatory cells might activate anti-inflammatory mediators and thus prevent dysregulation of the serotonergic system eventually limiting depressive-like symptoms.

Exercise as a therapy for depression

Another treatment approach has been introduced for major depression. Exercise has been shown to be an effective treatment for major depression (Trivedi et al., 2011). It is believed that exercise normally reduces inflammation and therefore might be effective for major depression. Although this effect is not observed in all studies. Rethorst et al., (2012) did not find that exercise augmented treatment by lowering proinflammatory cytokines, where others did (Nicklas et al., 2010). However, due to the possibility of effectiveness, it is plausible that in depressed patients exercise could improve treatment.

Unconventional therapies

Overall, a combination of several treatments will probably have the greatest antidepressant effect in depressed patients, especially treatment-resistant patients. Most likely a combination with proinflammatory antagonists or anti-inflammatory agents would relieve the patient from depressive symptoms as treatment-resistant patients have high levels of proinflammatory cytokines (mostly TNFα and IL6) which can sabotage conventional therapy. However, other treatment options have not even been explored yet. Several studies used PKC agonists (e.g. β-PMA (Samuvel et al., 2005)) to examine the effect it has on the SERT (internalization by phosphorylation). These agonists might contain therapeutic value in major depression as it downregulates the SERT and consequently increases 5-HT levels in the synapse. Furthermore, as proinflammatory cytokines induce tryptophan breakdown, increases in tryptophan might be used as a treatment application. Higher levels of tryptophan will eventually lead to higher levels of 5-HT in the synapse. Unfortunately, these treatment possibilities first need thorough examination in animals models as well as in humans to investigate their treatment efficacy for depression.

Monitoring treatment effectiveness for depression

The effectiveness of additional treatment methods or conventional treatment methods for major depression are normally measured by questionnaires and quality of life scores. Naturally these forms of measurements can be quite ambiguous. Other methods to monitor the effectiveness of a treatment are therefore of great interest. One biomarker that has attracted great attention are proinflammatory cytokines. As IL6 and TNFα elevations are mostly observed in depressed patients and are implicated in treatment effectiveness, they can be of interest for monitoring the treatment (Bufalino et al., 2012; Miller et al., 2008; Raison et al., 2007, 2012). IL6 and TNFα can be easily

measured from blood samples and act as a marker for treatment effectiveness for major depression. These markers can be monitored during conventional antidepressant treatment or in addition to other treatments. Bloods samples collected from patients could provide useful information about possible treatment-resistance cases and could indicate whether a type of treatment is effective or if additional treatment is necessary (Maes et al., 2012; Pace et al., 2006; Pace et al., 2009; Raison et al., 2012). Another form of monitoring became available already in the 1980's where platelets were used to investigate 5-HT uptake. In recent years several studies has shed light on the intracellular processes in platelets and have shown that 5-HT uptake from plasma by platelets is dependent on the SERT (Mercado et al., 2010). The mechanisms that control the SERT in platelets seem to be similar to the mechanisms that are observed in the CNS (Lau et al., 2012). For instance, treatment of SSRIs will also inhibit 5-HT uptake by the SERT in platelets (Lau et al., 2012; Lawrence et al., 1997; Malison et al., 1998). For that reason, it seems plausible that platelets could be obtained from patients to investigate and monitor the effects of treatment on the SERT. If there is a decrease in 5-HT uptake, and therefore an increase in 5-HT concentrations levels, the treatment should be effective. Overall it is important to bare in mind that all monitoring options should be considered together. The questionnaire forms and quality of life scores should obviously not be discarded as they represent the feeling of the patient which can impossibly be monitored with any molecular technique. However, other monitoring options might contribute to information about treatment effectiveness in a patient or in matching a patient to a certain treatment. For those two reasons the above mentioned technique could represent novel and useful monitoring possibilities.

Conclusion

This review gives an overview of the involvement of the p38 MAPK pathway in depression, or depressive-like features. Previous studies provided interesting results about the mechanism by which p38 MAPK is activated and the effects activated p38 MAPK exerts on other proteins and pathways. Until now, results from studies investigating the precise mechanism in which chronic stress activates the p38 MAPK pathway and the kind of consequences this has on the SERT and eventually on behavior were unclear and sometimes even contradicting. In this review we have proposed a mechanism by which chronic stress can lead to the activation of p38 MAPK and eventually to depression, although certain steps are still unclear and need further examination. Furthermore, the role of proinflammatory cytokines were discussed in this review. Many studies indicate that high levels of proinflammatory cytokines will contribute to, and in some cases may even induce, major depression. However, proinflammatory cytokines do not seem to play a major role in the causation of p38 MAPK-induced depression. However, porinflammatory cytokines together with chronic psychosocial stress can have a synergistic effect on major depression symptoms. The p38 MAPK pathway is activated via NE in the adrenergic system which can be exarggerated by proinflammatory cytokines and chronic psychosocial stress. Furthermore, IL1 and IL6 can create a chronic state of inflammation through a postive feedback loop, increasing NE concentration levels. In addition, the proinflammatory cytokines can sabotage and undermine effective conventional treatment of major depression. Therefore several new treatment possibilities and monitor options were considered in this review. As major depression is becoming a world wide health issue, it is important to elucidate all the different pathways by which depression, or depressive-like features, can occur. We provided a more detailed description of one of these pathways involving p38 MAPK and the SERT. Dysregulation of the serotonergic system is implicated in many psychiatric disease, such as obsessive compulsive disorder, autism and schizophrenia. Therefore, the pathway described in this review together with the new research, therapeutic and monitor options is relevant not only for depression, but also for other psychiatric disorders.

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