The consequences of exposure to stress on mood and substance-use regulation

A neurobiological and –epigenetic perspective

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1. INTRODUCTION

Substance-use disorders and mood disorders are among the most prevalent psychiatric disorders in Western countries. Interestingly, the comorbidity rates between substance use disorders and mood disorders are high (Grant, Stinson, Dawson, & Chou, 2004). As outcome of an American survey was reported that the lifetime odds of alcohol dependence were significantly elevated for both men (three-fold) and women (quadruple) with major depressive disorder (MDD). In contrast, subjects with alcohol dependence demonstrated a two-fold increase in the lifetime odds of depression (Trull, Waudby, & Sher, 2004). The disorders are likely physiologically related since an abrupt cessation of drugs in addicted individuals causes feelings of anxiety and depression (Pandey, 2004) and depressive-like behaviour in animal studies (Barr & Markou, 2005). Moreover, combat veterans suffering from post-traumatic stress disorder (PTSD), a mood disorder triggered by a traumatic experience, show significantly higher rates of drug abuse than veterans without the disorder (Donovan, Padin-Rivera, & Kowaliw, 2001; Ouimette, Coolhart, Funderburk, Wade, & Brown, 2007). Possibly the two disorders share a common ground in neurobiology and onset.

In accordance with PTSD, researchers have found that addictive and depressive (anhedonic) behaviour can be developed as a consequence of exposure to acute and chronic environmental stressors in different life phases, potentially reinforced by a genetic predisposition (Covington & Miczek, 2005; McEwen, 2008). For example, animal studies have shown that maternal stress during prenatal and early postnatal periods (Meaney, Szyf, & Seckl, 2007; Campbell, Szumlinski, & Kippin, 2009), and exposure to acute and chronic stress during adult life increase vulnerability to addicted and depressive behaviour later in life (Dewart, Frank, & Schmeidler, 2006; Brown & Erb, 2007; Briand & Blendy, 2010). Supportively, a human study reported that childhood trauma, such as sexual abuse and neglect, was associated with a higher propensity to use addictive substances and an elevated risk for psychiatric disorders later in life (Walker et al., 1999). So which are the neural mechanisms that can explain a causal relation between exposure to stress and mood and substance disorders?

The neural mechanisms involved in the regulation of stress, mood and addiction have been extensively studied as separate processes. In general, we can state that emotional and physiological stress result in changes in homeostasis and activate the neuroendocrine stress system, also known as the limbic-hypothalamic-pituitary-adrenal (LHPA) axis. Drug addiction, characterised by compulsive seeking and taking of drugs despite adverse consequences, is known to affect the brain's natural reward centres, including the mesocorticolimbic dopamine (DA) system (Koob & Kreek, 2007; Tsankova et al., 2007) and mood disorders, characterised by i.e. low self-esteem, loss of

interest and depressed feelings (Curtis & Valentino, 1991), are often ascribed to a disturbed serotonergic (5-HT) system and norepinephrine (NE) system, because the most important antidepressants act on these systems (Eric J Nestler et al., 2002). However, pharmacological, electrophysiological and lesion studies have revealed that these depicted stress, mood and addiction systems interact at different sites through different classes of neurotransmitters in the brain (see reviews: L. A. Briand & Blendy, 2010; Holsboer, 2000; G. F. Koob, 2008). These neural interactions may underpin the development of an addictive or depressive disorder after exposure to stress.

Nonetheless, the impact that stressful factors have varies among individuals and can be liable to e.g. genetic background and/or gender. For instance, Van der Veen and colleagues (2008) selected low (AKR) and high (C3H) maternal care mouse strains. Pups from two unrelated strains (DBA/2J and C57BL/6J mice) were cross-fostered to these high and low caring mother strains and the authors found that adult DBA mice, raised by a low caring mother strain, significantly differed from DBA mice raised by high caring mother strain in terms of cocaine intake and immobility response in the forced swim test, while C57BL/6J mice showed no differences. This finding illustrates that C57BL/6J mice seem to have a resistant genetic background and are resilient to the effects of reduced levels of motherly care, while DBA mice show a sensitivity to this particular environmental factor early in life (Van der Veen et al., 2008). Also in humans, specific genetic isomorphisms have been associated with increased sensitivity to stressful life events and their adverse consequences (Bethea et al., 2005; Charney & Manji, 2004). Interestingly, in a few studies it was however not possible to link stress sensitivity or psychiatry to specific isomorphisms, although behavioural characteristics were significantly associated with experiences or drug-use in the previous generation (Moss, Vanyukov Yao & Kirillova, 1999). These findings suggests that the effects of stress and related disorders can be inherited, but that factors other than genetic make-up, play a role.

Indeed, molecular studies have revealed that environmental factors can alter genetic expression, without changing the genetic make-up. Moreover, these alterations in genetic expression can be passed on to the next generation (Bagot & Meaney, 2010; Cottrell & Seckl, 2009; Lupien, McEwen, Gunnar, & Heim, 2009; Weinstock, 2008). These alterations are regulated through epigenetic mechanisms (Jiang et al., 2008). Several epigenetic mechanisms have been identified, also in the context of stress exposure, depression and drug use. However, how epigenetic alterations due to stress are inherited is an area of research still in its infancy.

The objective of this literature review is to investigate, on a neurobiological and cellular level, how stress can change the brain in such a way that it is more prone to depression and addiction. Some of these changes have already been associated with an epigenetic cause, however little with inherited epigenetic alterations. The first part of this review will cover some of the stress-induced neurobiological processes that are associated with synaptic and morpholoigcal changes linked to major depressive disorder and substance abuse. The second part will focus on the epigenetic mechanisms involved in these alterations and discuss the value of epigenetic research.

2. STRESS REGULATION AND THE CONSEQUENCES OF CHRONIC EXPOSURE TO STRESS

2.1 Stress and the limbic-hypothalamic-pituitary-adrenal system

Stress responses in both humans and rodents are regulated by the limbic-hypothalamic-pituitary-adrenal (LHPA) system (Lopez, Akil, & Watson, 1999). Therefore, understanding the LHPA system, its input and projection sites and its elements vulnerable to stress-induced alterations contribute to understanding the risks of chronic exposure to stress.

Psychogenic stress is followed by the activation of the LHPA axis, through increased levels of neurotransmitters in response to external stimuli that stimulate the expression of the neuropeptide corticotropin-releasing hormone (CRH) secreted from the parvocellular neurons of the paraventricular nucleus (PVN) in the hypothalamus together with the secretion of vasopressin. CRH and vasopressin stimulate the secretion of adrenocorticotropic hormone (ACTH) in the anterior lobe of the pituitary gland. In response to ACTH, glucocorticoids, like cortisol (corticosterone in rodents), are secreted from the adrenal cortex and close the negative feedback cycle by acting on the PVN and pituitary gland and inhibiting the stress response (Majzoub, 2006; Swanson, 1984).

Chronic stress, in prenatal, early or adult life, typically results in a desensitization of the negative feedback loop and hyperactivity of the LHPA system, through increased secretion of CRH or altered regulation of the receptors (*see §2.3*, Majzoub, 2006). Animal studies have revealed that chronic stress can result in morphological changes in the brain. Chronic stress has been associated with atrophy of pyramidal neurons and a decrease in dendritic spine density in the dentate gyrus in the hippocampus (Mcewen, 1999) and medial prefrontal cortex (mPFC, J J Radley et al., 2004; Jason J Radley & Morrison, 2005). However, opposite effects, stress-induced increases in dendritic length and spine density, were observed in the amygdala (Vyas, Mitra, Rao, & Chattarji, 2002). In addition, enhanced stress-induced corticosterone secretion as a consequence of chronic exposure to stress was observed. Confirmatively, the morphological changes in the hippocampus, amygdala and PFC can be replicated by chronic administration of glucocorticoids (Gray, Milner, & McEwen, 2012; Mcewen, 1999). Interestingly, the elevated corticosterone levels in rats were subsequently associated with an enhanced locomotor response to a novel environment, which can be interpreted as increased anxious behaviour (Campbell et al., 2009; Fujioka et al., 1999). Supportively, behaviour observed in the social defeat paradigm (BOX 1), a well-used animal model to induce stress and at the same time distinguish between stress-sensitive and stress-resilient animals, was correlated with both anhedonic and addictive behaviour. Defeated mice, the ones that interact less or not at all during the social interaction test, display an elevated stress response, characterised by an increased glucocorticoid activation (Covington & Miczek, 2005; Krishnan et al., 2007). In addition, sensitivity to

the social defeat paradigm was correlated with depressive-like behaviour in the forced swim test and tail suspension test, as well as elevated drug taking behaviour (Kippin, Szumlinski, Kapasova, Rezner, & See, 2009; Campbell et al., 2009).

BOX 1

Social defeat paradigm

The social defeat paradigm alters the motivation for social interactions in rodents. Mice are subjected to daily bouts of social defeat for 8 (Covington et al., 2011) – 10 days . The mice are exposed to a different aggressor every day for 5 min and then separated from the aggressor behind a perforated protective barrier for the reminder of the day. Finally, during the social interaction test, social approach towards an unfamiliar mouse is measured. Undefeated control mice spent most of their time interacting socially when presented to an unfamiliar target mouse, while defeated mice displayed intense aversive responses and spent less time in close proximity to the target mouse (Berton et al., 2006; Covington et al., 2011).

Moreover, atrophy, similar to the stress-induced decrease in neurons, was found post mortem in the hippocampi of individuals that suffered from chronic depression (Warner-Schmidt & Duman, 2006). Magnetic resonance imaging studies have confirmed this characteristic (Dranovsky & Hen, 2006) and showed that chronic antidepressant treatment up-regulates hippocampal neurogenesis (Warner-Schmidt & Duman, 2006). Thus, both physiological and behavioural studies have provided outcome to suggest that shared stress-induced mechanisms can underlie the development of sensitivity to stress, depressive symptoms and drug dependence characterised by morphological changes in the brain. Next, will be explained how stress can induce the morphological changes that are generally linked to depression. For this reason, first a brief summary on major depressive disorder.

2.2 Major depressive disorder, alcohol intake and the action of antidepressants

Major depressive disorder (MDD) is characterised with a range of symptoms, including disturbed mood and sleeping patterns and feelings of worthlessness and hopelessness (Curtis & Valentino, 1991). The therapeutic effect of a variety of antidepressants including tricyclic agents, selective serotonin re-uptake inhibitors, monoamine oxidase inhibitors, atypical antidepressant compounds, and electroconvulsive therapy, involves adaptations in serotonin (5-HT) and norepinephrine (NE) (Celada, Puig, Amargós-bosch, Adell, & Artigas, 2004). Different genetic features, such as genetic variants of the 5-HT transporter have been associated with variations in vulnerability to mood disorders as well as differences in therapeutic response to antidepressant treatment (Peters, Slager, McGrath, Knowles, & Hamilton, 2004). The 5-HT transporter gene (SLC6A4) has been linked to stress too. A specific polymorphism, the short (s) 5-HT transporter gene-linked promoter region (5-HTTLPR) of SLC6A4 is shown to be associated with psychiatric disorders, like MDD, and elevated CRH concentrations after stress experiences (Coplan et al., 2011; Ressler et al., 2011). The short variant of the 5-HTTLPR was further related to significant less 5-HT transporter mRNA expression (Canli & Lesch, 2007). Interestingly, SSRIs seem less effective in MDD patients with the short 5-HTTLPR

variant (Illi et al., 2011). Possibly, because this variant already has a reuptake inhibitory effect, due to lesser 5-HT expression and the depressive symptoms are caused by a different mechanism in these patients. Other studies have, for example, reported a significant lower number of the norepinephrine transporter (NET) in post mortem locus coeruleus (LC) tissue, the centre of norepinephrine secretion, of subjects diagnosed with MDD compared to healthy controls (Klimek, Zak-Knapik, & Mackowiak, 1994). The drug of abuse mainly associated with MDD and other mood disorders, like PTSD is alcohol. Alcohol dependence has been linked to alterations in the LHPA axis, increased cortisol levels after induction, and 5-HT function that regulates the LHPA system (Athenelli, Maxwell, Geraciot and Hauger, 2006), which may precede depressive symptoms. In contrast, alcohol is suggested to relieve depression and anxiety (Bolton, Robinson, & Sareen, 2009), possibly due to the increase in inhibitory GABA activity that counteracts the LHPA system. In general, the action of alcohol is difficult to pinpoint (Carta, Mameli, & Valenzuela, 2004).

2.3 Altered expression levels in response to stress

Alterations in morphology, that may underlie MDD and other disorders, can be explained by an interplay of stress hormones, peptides and neurotransmitters. Manipulations in a complex neural network, including the LHPA system and its glutamatergic and GABAergic input that is being modulated by the neuromodulators, such as norepinephrine, dopamine and serotonin have been linked to different symptomatology. The changes in expression of ligands and their receptors can mainly be explained through changes in the involved second messenger pathways.

Chronic psychogenic stress can affect the LHPA system at different sites. Chronic exposure to stress is mainly associated with increased basal levels of CRH expression and down-regulation of the glucocorticoid receptors in the PVN (Herman, Ostrander, Mueller, & Figueiredo, 2005). In general, psychogenic stressors trigger pathways from the sensory organs, brain stem and mid brain nuclei, like the raphe nuclei and ventral tegmentum area (VTA), to the peri-PVN area and PVN via the PFC, bed nucleus of the stria terminalis (BNST), basolateral amygdala (BLA) and the LC (Aguilera & Liu, 2012). The secretion of CRH in the PVN is regulated by neurotransmitters, including NE afferents from the LC and glutamatergic (Glut) afferents from the BLA, and the signaling transduction systems coupled to their receptors. Norepinephrine acts on alpha adrenergic receptors which are coupled to the guanyl nucleotide binding protein (G-protein) Gq11 and phospolipase C (PLC). Binding results in an increase in intracellular calcium (Ca^{2+}) and protein kinase C (PKC) activity. Simultaneously, glutamate interacts with NMDA and mGluR5 receptors also leading to increases in intracellular Ca^{2+}.

These two mechanisms are suggested to mediate rapid CRH release within seconds (Huang & Reichardt, 2003).

Conversely, CRH transcription is activated within minutes and is regulated through a process that requires the second messenger cyclic adenosine monophosphate (cAMP) activated by the G-protein coupling and binding of brain derived neurotrophic factor (BDNF) to tropomyosin-related kinase B (TrkB) receptors (Huang & Reichardt, 2003; Jeanneteau et al., 2012). After the increase in intracellular calcium levels, cAMP and consequently calcium calmodulin dependent protein kinase (CaMK), PKC, and protein kinase A (PKA) are activated. The activation of PKC and PKA transactivates the intracellular signalling mitogen activated protein kinase (MAPK) pathway. MAPK and CaMK subsequently activate cAMP responsive element binding protein (CREB) by phosphorylation. Phosphorylated CREB (pCREB) binds to the cAMP response element (CRE) region of the CRH gene, together with CREB-binding protein (CBP) and would activate the transcription process (Aguilera & Liu, 2012; Jeanneteau et al., 2012). However, phosphorylation of CREB alone is not sufficient to stimulate the CRH promoter. The CREB regulated coactivator 2 (CRTC2, initially called TORC2) must be actively transported to the nucleus to allow CREB-dependent transcription of CRH. Interestingly, glucocorticoids, via glucocorticoid receptor signaling, deactivate CREB-mediated CRH expression by neutralizing the function of CRTC2 (Jeanneteau et al., 2012). Perhaps, through this mechanism the secretion of CRH is inhibited by glucocorticoids and does the negative feedback loop exist. However, it has been shown that after prolonged stress and chronic corticosterone exposure the expression of glucocorticoid receptors in the hippocampus reduces. For instance, reduced levels of glucocorticoid receptor mRNA were found post mortem in the brains of individuals with a history of child abuse. This reduction was associated with a decreased level of the nerve growth factor-inducible protein A (NGFI-A, also known as e.g. egr1, zif268 and d2) transcription factor binding and NGFI-A-inducible gene transcription (McGowan et al., 2009). Binding of NGFI-A induces the activity of the exon 1(7) on the GR (NR3C1) promoter (Weaver et al., 2007), through the MEK-ERK1/2 signaling pathway that is linked to the TrkB receptor.

In contrast, postnatal augmented maternal care in animals has shown to up-regulate glucocorticoid receptors in the hippocampus and maternal care is therefore suggested to have a protective (resilient) effect (Liu, 1997; Meaney & Szyf, 2005). This augmented maternal care, expressed in licking, grooming and arched-back nursing (LG ABN) was linked to an increased expression of NGFI-A in the gene promoter of Nr3c1 in the rat hippocampus (Weaver, Diorio, Seckl, Szyf, & Meaney, 2004). In addition, Meaney and Szyf (2005) reported that the offspring of high LG-ABN mothers also

were less anxious and had attenuated corticosterone responses to stress when compared to pups of low LG-ABN mothers (Meaney & Szyf, 2005). The down- and up-regulation of glucocorticoid receptors through fluctuating levels of the neurogrowth factor NGFI-A can possibly be explained by an epigenetic mechanism (*Chapter 3*). Thus, we now know that chronic stress and glucocorticoid administration can result in a reduced number of glucocorticoid receptors in the hippocampus. Additionally, has been reported that chronic stress and glucocorticoid administration is linked to atrophy of the hippocampus. Could these two processes be possibly linked to each other?

2.4 Implications supporting disrupted hippocampal neurogenesis under the influence of stress

Adult neurogenesis (AN), is the process of generating new neurons that integrate existing circuits after fetal and postnatal development. AN is observed in the hippocampus of humans and rodents (Zhao, Deng, & Gage, 2008). In brief, new hippocampal granule cells arise from neural progenitor cells in the subgranular zone (SGZ) of the dentate gyrus (DG) and become integrated into the local neuronal network. The maturation and integration of the neurons into the local network of adult neurons is highly GABA (local and synaptic) dependent in the first few weeks when synapses and dendrites are formed. By 3 weeks, axons (mossy fibers) are formed connecting to hilar neurons and CA3 pyramidal cells. In the last developmental stage until 2 months, the newborn neurons will receive glutamatergic input and their spines develop further. However, the neurons are still characterised by o.a. increased neuronal activity from efferents, presumably similar to hippocampal long term potentiation (LTP), compared to mature neurons (Herold, Jagasia, Merz, Wassmer, & Lie, 2011). Hippocampal LTP is apparent as an increase in non-NMDA receptor-mediated glutamate transmission (Kauer, 2004) More specifically, LTP depends critically upon expression of the AMPA receptor subunit, GluR1 (Zamanillo, 1999), and an increase in AMPA receptors at synaptic sites (Kauer, 2004). Overall, both GABA and glutamatergic input control newborn neuron survival.

In support with these observations, GABA synthesizing enzyme glutamic acid decarboxylase isoform 67 (GAD67) mRNA expression was reduced in the hippocampus (Thompson Ray, Weickert, Wyatt, & Webster, 2011). However, the levels of GAD67 mRNA were unchanged in subjects treated with antidepressants (Iyo et al., 2010). This may suggest that in patients with major depressive disorder hippocampal neurogenesis is affected through reduced levels of GAD67, and consequently GABA. However, more factors have been reported to be crucial in hippocampal neurogenesis, including the cyclin-dependent kinase 5 (Cdk5). Cre-recombinase-mediated conditional knock out of Cdk5 in neural progenitor cells in the dentate gyrus prevented maturation of new neurons. Additionally, selective KO of Cdk5 in mature neurons in the hippocampus, and Cdk5 gene deletion specifically in DG granule neurons reduced the number of new born neurons. Moreover, this effect was activator-

specific, because only the Cdk5 activator p35 KO, but not p39 (cJun) KO mice showed fewer newborn neurons (Lagace et al., 2008). The expression of p35 is induced by BDNF and NGFI-A has demonstrated to mediate the induction of p35. Interestingly, Cdk5 also phosphorylates the glucocorticoid receptor and hereby up-regulates its transcriptional activity (Jessberger et al., 2008).

Indeed, another important factor in neurogenesis was demonstrated to be the MEK-ERK1/2 signaling pathway activated by TrkB-BDNF binding. TrkB-deficient (cre-induced) adult-born neurons in $TrkB^{lox/lox}$ mice showed impaired synaptic plasticity. Furthermore, a substantial population of these neurons died at the immature-to-mature neuronal transition. Behaviourally, the impairment in TrkB signaling in newborn neurons was associated with increased anxiety-like behaviour in the mutant mice (Bergami, Rimondini, Santi, Blum, & Go, 2008). Consistently, reduced mRNA expression of BDNF and the TrkB receptor was found post mortem in the hippocampus of individuals with major depressive disorder (Thompson Ray et al., 2011). A decrease in BDNF levels was also reported in the hippocampus after exposure to chronic stress (Lakshminarasimhan & Chattarji, 2012). More specifically, endogenous corticosterone levels showed to have a biphasic effect on the BDNF secretion in a medium: stimulating BDNF secretion at lower concentrations and suppressing it at higher concentrations (Kino et al., 2010). In contrast, in a different study BDNF levels were increased in the amygdala (Lakshminarasimhan & Chattarji, 2012). This is consistent with the opposite morphological changes that have been reported in the amygdala after exposure to stress. Confirmatively, reversed morphological changes after antidepressant treatment were reported, as well as opposite BDNF levels in the hippocampus and amygdala (Kino et al., 2010; Lakshminarasimhan & Chattarji, 2012). In general, antidepressants increase intracellular levels of cAMP (Nestler et al., 1989) and therefore promote intracellular pathways. Even when the subtypes of NE or 5-HT receptors the antidepressant act on are not coupled to the cAMP pathway, CREB phosphorylation will be induced through mobilization of intracellular calcium or membrane depolarization and subsequent calcium influx, and still lead to activation of gene expression (Blendy, 2006) of for example BDNF (Conti, Cryan, Dalvi, Lucki, & Blendy, 2002) and TrkB receptor genes (Iyo et al., 2010).

In conclusion, the down-regulation of glucocorticoid receptors and the disrupted neurogenesis may hypothetically be linked. Both the expression of glucocorticoid receptors, and the expression of the p35 activator are dependent on the MEK-ERK1/2 signaling pathway (Kim, Shin, Yoon, & Kim, 2011) and thus the availability of BDNF and TrkB receptors. Furthermore was demonstrated that antidepressants promote neurogenesis through an up-regulation of TrkB and a normalisation of

GABA, through GAD67 expression, that promotes the first stages of neurogenesis (Iyo et al., 2010; Kino et al., 2010). In light of addiction, synaptic plasticity similar to the processes observed in the hippocampus, has also been reported in the mesocorticolimbic system in response to stress and drugs (Daftary, Panksepp, Dong, & Saal, 2009). The mesolimbic system projects to and receives projections from the hippocampus, amygdala, BNST and PFC (Wise, 1996) and these interactions may be a key in the comorbidity MDD and drug abuse.

2.5 Mesolimbic dopamine system in relation to drug use and stress exposure

The mesolimbic dopamine system is composed of different afferents and efferents of the nucleus accumbens (NAc). The NAc exists for 95% of medium spiny GABA-ergic neurons (MSNs). Other neuron types that are found in the NAc are cholinergic and GABA-ergic interneurons (Belujon & Grace, 2011). Dopamine-releasing neurons from the ventral tegmentum area (VTA) innervate the dendritic spines of the dopaminoceptive MSNs in the NAc. Furthermore, the NAc receives glutamatergic input from the hippocampus, amygdala and prefrontal cortex (Wise, 1996; Adinoff, 2004) that excite all types of NAc neurons. Dopamine modulates this glutamatergic input (Robison & Nestler, 2011).

The mesolimbic dopamine system is associated with processing natural and addictive reward. For example, repeated administration of drugs of abuse results in drug-induced modifications in synaptic plasticity in the mesolimbic dopamine system. These synaptic alterations are suggested to drive addiction (Russo et al., 2010). Moreover, stress cross-sensitizes with psychostimulants. Thus, an animal exposed to a stressor will show a heightened responsivity to e.g. cocaine and vice versa. For instance, chronic cocaine treatment made mice more susceptible to chronic social defeat, with social avoidance as result (Vialou et al., 2010). Different classes of drugs affect the mesolimbic dopamine system through different mechanisms. The psychostimulants cocaine and amphetamine bind to the dopamine transporters (DATs) on the VTA neuron that re-uptake DA from the synaptic cleft (Kauer, 2004). By blocking DATs cocaine directly prolongs the effect of the VTA DA signals on the MSNs. These MSNs express either high levels of D1 or D2 dopamine receptors (D1DR & D2DR, Lee et al., 2006; Robbins & Everitt, 1999). Dopaminergic receptors are co-expressed on MSNs with different glutamate (NMDA, AMPA and metabotropic) receptors that modulate Ca^{2+} entry and second signaling pathways (Kelz et al., 1999; Eric J Nestler, 2008). Chronic cocaine treatment has shown to increase the spine density of the MSNs of both dopamine Drd1-EGFP and Drd2-EGFP positive neurons in transgenic mice. However, this increase was only maintained in the Drd1-EGFP positive

neurons after 30 days of drug withdrawal, suggesting that sensitization is regulated through D1 dopamine receptor signaling (Lee et al., 2006).

Dopamine receptors are also co-expressed with glucocorticoid receptors (GRs) in the NAc, which have shown to modulate dopamine activity elicited by drug reward. Inactivation of the GR gene (Nr3c1) in GR^{D1Cre} mutant mice was associated with reduced firing rate and frequency of burst events in the VTA (Ambroggi et al., 2009) and reduced cocaine-elicited (acute) DA release in NAc. These findings were linked to reduced levels of the immediate early genes c-Fos and NGFI-A and a reduction of phosphorylation of ERK1/2 in response to cocaine in the NAc (Barik et al., 2010). Furthermore, genes implicated in the glutamatergic transmission (Grin2b and Nlgn3) were down-regulated, which could be a consequence of less mesocortical DA and an explanation for a decrease in excitatory input to the VTA. GR^{D1Cre} mice showed reduced cocaine self-administration, reduced cocaine-induced conditioned place preference and locomotor sensitization. Moreover, mutants stressed in the social defeat paradigm showed no social avoidance (Barik et al., 2013). These results implicate the relevance of glucocorticoid binding, and the LHPA axis, in reward and stress processing.

Supportively, mice that displayed social avoidance showed increased DA bursting firing in the VTA ex vivo (Feder, Nestler, & Charney, 2009; Krishnan et al., 2007). Furthermore, a rat study demonstrated that adrenalectomized rats showed lower drug intake compared to controls. Subsequent administration of corticosterone resulted in increased self-administration of alcohol and cocaine (Piazza and Le Moal, 1998). Additionally, adrenalectomy reduced the extracellular concentrations of dopamine in the shell of the NAc (Piazza et al., 1996, 2000). Moreover, the selective knock down of CREB in the NAc has been associated with resilience to chronic stress (Vialou, Robison, et al., 2010), which can possibly be linked by reduced gene expression of CREB-related genes, such as Fos, BDNF, CRH and GluR1. Indeed, in contrast increased BDNF-TrkB signaling was observed in the NAc after chronic social defeat in mice displaying social avoidance (Krishnan et al., 2007; Covington et al., 2011). Furthermore, ΔFosB and the activity-dependent transcription factor, serum response factor (SRF) and the AMPA receptor subunit GluR2 were down-regulated in the NAc in sensitive mice, compared to resilient mice after chronic stress. Supportively, NAc tissue from depressed patients revealed decreased GluR2 levels and SRF levels compared to controls (Vialou, Maze, et al., 2010; Vialou, Robison, et al., 2010). GluR2-lacking AMPA receptors are Ca^{2+}-permeable with increased medium spiny neuron excitability in response to glutamate as result. Increased glutamatergic acitivty could compensate for the increase in DA release and this ratio may underlie sensitivity and resilience. Similarly, overexpression of ΔcJun (splice variant of cJun), antagonizing ΔFosB activity, resulted in a reduction of GluR2 and made mice more susceptible to chronic social stress (Vialou,

Robison, et al., 2010). However, SRF manipulation did not play at all a role in the response to chronic cocaine exposure and ΔFosB was up-regulated after cocaine treatment. Therefore, stress and cocaine may be regulated through different pathways (Vialou, Maze, et al., 2010).

2.6 Synaptic plasticity and sensitization in the mesocorticolimbic dopamine system

Responses to stress and addictive substances are generally expressed in increased DA release in the VTA and the consequences in receptor expression and further neurotransmission in the NAc. Thus, this suggests that afferents of the VTA play a significant role in processes such as behavioural sensitization. Activity in the VTA is regulated by different input. The primary excitatory afferents to the VTA include direct glutamatergic input from prefrontal cortex. The VTA also receives excitatory input from the amygdala, laterodorsal tegmental nucleus, and BNST. Dopamine neurons in the VTA express metabotropic and ionotropic glutamate receptors, including both NMDA and AMPA subtypes (NMDARs and AMPARs) (Kauer, 2004). Indeed, local injections of D1DR antagonists and NMDAR agonists in the VTA directly blocked the development of behavioural sensitization to repeated administration of amphetamine (Rougé-Pont, Moal, Vincenzo, & Cedex, 1995). Similarly to hippocampal LTP associated with neurogenesis, exposure to an addictive drug may elicit NMDA receptor dependent LTP in the mesolimbic DA system (Hyman & Malenka, 2001; E J Nestler, 2001). However, NMDAR antagonists that block behavioural sensitization to psychostimulants are ineffective once sensitization is established (E. Wolf & White, 1994). Therefore, mainly the early development of sensitization may be a consequence of enhanced glutamatergic synaptic transmission in the VTA (Kauer, 2004). Another measure of LTP is the AMPA/NMDA ratio. The ratio between AMPAR-mediated excitatory postsynaptic current (EPSC) and NMDAR-mediated EPSC was increased in brain slices of cocaine-treated rodents, which indicates that cocaine increases AMPAR-mediated glutamatergic transmission (Malenka & Bear, 2004). Interestingly, AMPAR/NMDAR ratios in VTA GABAergic neurons were unchanged, which indicates that drug treatment increases excitation, but not inhibition (Williams, 1994). Importantly, also repeated stress elevated the AMPAR/NMDAR ratio in VTA DA neurons. This increase was modulated through the activation of GRs, since Dexamethasone (Dex), a potent synthetic GR agonist, significantly increased AMPAR/NMDAR ratios in VTA DA neurons in the same magnitude as stress (Saal, Dong, Bonci, & Malenka, 2003). Furthermore, was shown that activation of GRs up-regulates postsynaptic AMPARs within the same neuron, which appeared to be mediated by a GR-mediated transcriptional process (Cho and Little; Karst & Joels, 2005). Confirmatively, up-regulation of GluR1 levels in the VTA were reported (Fitzgerald, Hamedani, & Nestler, 1996). This up-regulation was subsequently blocked by treatment with a glucocorticoid receptor antagonist (Saal et al., 2003). GRs are present on dopamine

(VTA) and dopaminoceptive (NAc) neurons. However, work of Barik and colleagues (2010; 2013) have demonstrated that only inactivation of GRs in the dopaminoceptive neurons affected stress and drug responses. No effects were found in GR^{DATCre} mice (Barik et al., 2013; Barik et al., 2010). Indeed, the increased AMPAR/NMDAR ratio caused by stress was blocked when stress followed administration of an NMDAR antagonist, suggesting that NMDAR activation is downstream from glucocorticoid action (Saal et al., 2003).

Another possible link between VTA dopamine release and increased NMDA receptor-dependent plasticity is that long-term depression (LTD) is blocked by drugs of abuse, making it easier to induce LTP. LTD is considered to be a normal brake mechanism preventing enhanced LTP and is characterised by a reduction in the efficacy of synapses (Kauer, 2004). Indeed, psychostimulants effectively prevent LTD at VTA synapses. This block of LTD was shown to be mediated by dopamine acting on D2 auto receptors on dopamine neurons in the VTA. LTD can also be blocked by PKA inhibitors or by inactivation (chelation) of intracellular Ca^{2+} (Thomas & Malenka, 2003).

2.7 MDD and drug-use interactions explained by glutamatergic hippocampus-NAc projections

Previous paragraphs have depicted that chronic exposure to stress could make the brain more prone to the effects of drugs (Vialou et al., 2010) and precede the morphological changes that are associated with major depressive disorder (Warner-Schmidt & Duman, 2006). Cross-sensitization that was previously discussed concerning psychostimulants is also demonstrated for alcohol (ethanol) (Phillips, Roberts, & Lessov, 1997), nicotine (Shim, Javaid, Wirtshafter, & Jang, 2001) and morphine (Tzschentke & Schmidt, 1997). This supports that chronic stress can increase the rewarding properties of these addictive substances. However, the question is if the synaptic and morphological changes associated with depression could hypothetically make the brain more sensitive to the abuse of addictive substances too. First of all, we have discussed both hippocampal neurogenesis and LHPA hyperactivity are associated with MDD. The hippocampus also projects to the PVN and its altered action may affect the LHPA system (Haarst, Oitzl, & Kloet, 1997). Therefore, hippocampal atrophy on itself could promote sensitization by increased levels of glucocorticoids. This was supported by the lack of behavioural sensitization after inactivation and blocking of glucocorticoid receptors in the NAc (Saal et al., 2003; Barik et al., 2010; 2013). However, the hippocampus also provides glutamatergic input for the VTA trough the ventral subiculum (vSub). This projection has been implicated in the reinstatement of cocaine use. The vSubs of rats were stimulated after extinction of cocaine self-administration. The stimulation elicited significantly increased cocaine-seeking behaviour. Confirmatively, this behaviour was blocked when

glutamatergic receptors in the VTA were pharmacologically blocked (Kauer, 2004; Vorel et al., 2001). In addition, chemical stimulation of the vSub by infusions of NMDA also increased spontaneous firing of DA neurons in the VTA. Interestingly, this increase in firing was abolished when a glutamate receptor antagonist was locally infused in the NAc (Floresco, Todd, & Grace, 2001). The vSub (hippocampus) has also been suggested to drive craving, stimulated by stress, through context-specific stimuli that further triggers hippocampal activity (Belujon and Grace, 2011).

Conversely, was shown that not hippocampal, but PFC lesions, that provide glutamatergic input to the VTA block behavioural sensitization to amphetamine (M. E. Wolf, Xue, Li, & Wavak, 2000). Moreover, repeated electrical stimulation of glutamatergic prefrontal projections sensitized animals to cocaine administration, but stimulation of the hippocampus did not (Kauer, 2004; Schenk & Davidson, 1992). The PFC also excites the VTA through a glutamatergic projection, and receives not only input from the hippocampus, but also from the amygdala. The amygdala input might explain why hippocampal stimulation on its own was not sufficient for behavioural sensitization. Indeed, behavioural sensitization was found to be dependent on amygdala activation (Kalivas & Aledatter, 1993). Morphologically, MDD has been significant increases in amygdala size and increased activity in response to emotional cues (Gray, Milner, & McEwen, 2012; Mcewen, 1999). Therefore, an altered glutamatergic amygdala-PFC projection towards the NAc could underlie drug sensitivty in MDD patients. The synaptic and morphological changes discussed so far have been explained through altered second messenger pathways. However, the modifications in these cascades have furhter been linked to altered gene expression levels through processes that might give insight in the causal mechanims stimulated by chronic exposure to stress.

3. STRESS AND EPIGENETICS

As mentioned briefly, some seemingly inherited neural or behavioural characteristics cannot solely be explained by a genetic predisposition, but do correlate with gene expression levels and seem to be a consequence of parental behaviour (e.g. drug intake) or experiences (e.g. exposure to stress). For example, preadolescent boys whose biological fathers had substance abuse problems showed structural lower event-related potential in response to an anticipated stressor compared to sons of non-addicted men. This hypothetical inherited effect was linked to increased levels of substance-use and asocial behaviour (Moss, Vanyukov Yao & Kirillova, 1999). Epigenetic processes could explain this observation.

BOX 2

Chromatin immunoprecipitation (ChIP) techniques

Chromatin immunoprecipitation (ChIP) is a method used to map transcription factor (TF) binding regions. The principle underpinning this assay is that DNA-binding proteins, like transcription factors and histones, can be cross-linked to the DNA that they are binding. By using an antibody that is specific to a putative DNA binding protein, one can immunoprecipitate the protein–DNA complex out of cellular lysates (Lee, 2003; Lee et al., 2006). ChIP-on-chip combines ChIP with DNA microarray technology and allows to identify all binding sites at a genome. A more recent technique ChIP-Sequencing (ChIP-PET), also combines ChIP with DNA sequencing at a larger level (Lee, 2003; Lee et al., 2006). Lastly, the Serial Analysis of Chromatin Occupancy (SACO) technique combines ChIP with serial analysis of gene expression (SAGE) and enables the identification of transcription factor target sites or epigenetic marks across entire mammalian genomes (Impey et al., 2004).

In contrast to genetic makeup, the epigenome, is dynamic and epigenetic modifications can be induced in response to a variety of factors, including the use of drugs, social interactions, and stress without changing the genetic make-up (Dudley, Li, Kobor, Kippin, & Bredy, 2011; Feinberg, 2007). Epigenetic modifications induce long-lasting changes in gene transcription that result in biological and behavioural adaptations (Feinberg, 2007). An example of an important epigenetic process during prenatal development, and relevant for inherited effects of drug use, is genomic imprinting. Genomic imprinting refers to a mechanism whereby only one of the two parental alleles is expressed while the other parental allele in not transcribed (monoallelic expression). DNA methylation, histone acetylation and methylation can be the cause of the expression or silencing of these alleles. Monoallelic expression has been associated with different inheritable diseases (Yang et al., 2003). Genomic imprinting could underlie the inherited effects of drug use, since for instance, animal studies have demonstrated that chronic paternal alcohol consumption has a disadvantageous effect on the development of offspring even in the absence of in utero alcohol exposure (Ouko et al., 2009).

As mentioned before, epigenetic modifications include acetylation, phosphorylation and methylation of histones and methylation of DNA and respectively function through chromatin

remodelling or act at DNA directly (Kumar et al., 2005). Chromatin is the combination of DNA wrapped around histones, and other proteins associated with DNA. During chromatin remodelling, histone modifications affect the binding affinity between histones and DNA, by loosening or tightening the DNA wrapped around the histones, with changes in gene transcription and expression as result (Kumar et al., 2005). Loosening the DNA wrapped around the histones positively influences transcription, since the DNA binding sites are better accessible for the transcription machinery (Kumar et al., 2005; N. Tsankova et al., 2007). The histone modifications made possible by the combination of histone modification enzymes at the assembling of histone tails is referred to as the histone code. This histone code can change over time and defines the parts of the genome that are accessible to transcription at that time (Jenuwein & Allis, 2001). Epigenetic modifications can be monitored with chromatin immunoprecipitation (ChIP) techniques (BOX 2, Impey et al., 2004). Since, epigenetic modifications are identified as key regulators of important developmental events, the risk that external factors like stress affect the epigenetic processes is more present when a brain area is in a developmental stage (Lupien et al., 2009).However, exposure to addictive substances and stress later in life have also shown to result in epigenetic alterations in the brain areas discussed in the previous chapter, linked to the behavioural alterations associated with drug use and depression (Pascual, Boix, Felipo, & Guerri, 2009; Coe et al., 2003; Eric J Nestler et al., 2002; Weaver, Cervoni, et al., 2004).

3.1 Histone methylation and alterations in BDNF-TrkB signaling

First of all, researchers have studied the epigenetic mechanisms involved in the sensitivity (and resilience) to social defeat (BOX 1) (Krishnan et al., 2007; Maze et al., 2010). As discussed before increased BDNF-TrkB signaling was observed in the NAc of mice after chronic social defeat (Krishnan et al., 2007; Vialou et al., 2010). The researchers expected epigenetic regulations to underlie this induced stress responsivity. Indeed, defeated mice, susceptible to the social defeat paradigm, displayed reduced dimethylation of histone 3 lysine 9 (H3K9me2) of NAc neurons through the repression of the lysine 9 histone H3 methyltransferase G9a, compared to undefeated and control mice (Krishnan et al., 2007; Maze et al., 2010). Interestingly, similar results were found after repeated (not acute) cocaine treatment (Covington et al., 2011; Maze et al., 2010).

Methyltranferases are important in the process of histone methylation. In this epigenetic process the methyltranferases transfer a methyl group to the ε-amino groups of lysine and arginine residues. Methyltranferases use a reactive methyl group bound to sulfur in S-adenosyl methionine (SAM) as methyl donor (William Renthal et al., 2007; N. Tsankova et al., 2007). Each methylation process is controlled by specific histone methyltransferases (HMTs), like G9a. These transferase enzymes

determine whether transcription of a gene promoter is activated or repressed (Maze & Nestler, 2011). For instance, trimethylation of H3 lysines 4 (H3K4me3) and 36 (H3K36me3) often correlated with increased levels of transcriptional activity (Maze & Nestler, 2011). In support of the results of the study of Maze, Covington and colleagues (2010, 2011), di- and tri-methylation on H3 lysines 9 (H3K9me2/3) and 27 (H3K27me2/3) are generally associated with transcriptional repression (Rice & Allis, 2001). Also exposure to a hostile postnatal environment down-regulated BDNF through hypermethylation of the exon IV on its promotor in the PFC of rats (Roth & Sweatt, 2010). Interestingly, both the increased levels of anxiety and stress responsivity, as well as the methylated promoter were inherited by the offspring of mothers that had been subjected to low maternal care, even when the offspring were cross-fostered at birth to normal caring mothers. Hypothetically, through genomic imprinting (Roth & Sweatt, 2010). Interestingly, inducing H3K9me2 (through HSV injections expressing wild-type G9a-GFP) in the NAc of mice sensitive to social defeat protected these mice from the deficits in social interaction, compared to defeated controls. This suggests that increased H3K9me2 can promote resilience to chronic social stress (Covington et al., 2011). Confirmatively, increased susceptibility to social stress was reported when G9a was knocked down in the NAc through Cre-GFP before the mice were subjected to the social defeat paradigm (Covington et al., 2011). Repression of BDNF transcription as consequence of the histone modification was also reported after repeated cocaine treatment and lead to increased dendritic spine density on MSNs, and enhanced cocaine-induced reward behaviour (Maze et al., 2010).

Wilkonson and colleagues (2009) have shown that imipramine, a tricyclic antidepressant can reverse the repression of H3K9me2 and H3K27me2 and increase in phospho-CREB levels (phosphorylated state of CREB) induced by social defeat stress (Wilkinson et al., 2009). Phospho-CREB binding to gene promoters indicates repression of gene expression and was especially present in genes that encode proteins that control gene activation and expression in the NAc. Additionally, fluoxetine has shown to reverse the effects of H3K9me3 in the hippocampus as a consequence of acute restraint stress and reduce the social avoidance after chronic social defeat (Kumar et al., 2005). Furthermore, chronic fluoxetine treatment also decreased acetylation of H3 in the caudate putamen and frontal cortex in healthy control rats (Cassel et al., 2006). The H3 dimethylation levels in the NAc of mice that did not develop depression-like behaviour strongly resembled the dimethylation profile of mice that received chronic imipramine treatment after social defeat (Kumar et al., 2005). This may suggest that antidepressant treatment could potentially reduce stress susceptibility through induction of H3 dimethylation in the NAc (Boks et al., 2012; Wilkinson et al., 2009; Hunter, McCarthy, Milne, Pfaff, & McEwen, 2009; Covington et al., 2011). Intriguingly, epigenetic regulations have not only been reported after pharmacological treatments, but also after a non-pharmacological

intervention for depression, chronic electroconvulsive seizures (ECS). ECS up-regulates the expression of BDNF and CREB in the hippocampus and such up-regulations have been shown to mediate antidepressant activity in animal models (Berton et al., 2006; Tsankova, Kumar, & Nestler, 2004).

3.2 Histone (phospho)acetylation in the BDNF-TrkB pathway in response to stress

Cocaine and stress exposure have also been associated with cFos and BDNF induction in the NAc. Conversely, cFos was significantly decreased in GR^{D1Cre} mice compared to controls after social defeat and cocaine treatment. The induction of gene expression is frequently linked to the process of histone acetylation. The induction of immediate early genes, like cFos and cJun is mainly regulated through phosphoacetylation (Brami-Cherrier, Lavaur, Pagès, Arthur, & Caboche, 2007). Histone acetylation is mostly associated with promoting gene transcription through loosening the DNA around the histones. Acetylation removes the positive charge on the histones. The neutralization of the electrostatic interaction between the negatively charged phosphate groups of DNA and positively charged ε-amino groups on lysines located on histone N-terminal tails reduces contact between DNA and histones proteins, which results in a more relaxed chromatin (euchromatin) structure to allow greater access to DNA for the transcriptional machinery (Kouzarides, 2007). This process is tightly regulated through the actions of many histone acetyltransferases (HATs) and can be reversed by histone deacetylases (HDACs), which respectively catalyse the addition or removal of acetyl groups on histone proteins. Genome-wide analyses have indicated a positive correlation between levels of histone acetylation at gene promoters and transcriptional activity (Pokholok et al., 2005). Indeed, the increase in cFos after a single cocaine injection was linked to H4 hyper-acetylation at the cFos promoter in the striatum. Conversely, the acetylation was augmented by systemic administration of the HDAC inhibitors sodium butyrate (SB) and Trichostatin A (TSA). Administration of the HDAC inhibitors in conjunction with cocaine led to increased levels of H3 phosphoacetylation, as well as cFos mRNA expression in the striatum compared to cocaine alone, which implicates cross-sensitization. H3 hyperacetylation was reported at the BDNF promoter in the striatum after chronic cocaine treatment (McClung et al., 2004; E J Nestler, Barrot, & Self, 2001; Kumar et al., 2005; McClung & Nestler, 2003).

3.3 DNA methylation of CRH, GRs and monoamergic transporters in response to stress

Lastly, we earlier discussed the altered expression levels of CRH, glucorticorticoids and their receptors in response to chronic stress. These alterations subsequently influenced the LHPA action

with consequences for the monoamergic systems. Also the regulation of stress-related ligands and receptors have shown to be regulated through epigenetic processes. For example, Oberlander and colleagues (2008) demonstrated that a stressful prenatal environment (E-PS) during the early stages of gestation led to reductions in methylation at specific cytosines within the CRH promoter CpG island in both the hypothalamus and central amygdala. In this case, the process of methylation is considered DNA methylation since the gene transcription is influenced directly by blocking the DNA binding sites itself, instead of indirectly by facilitating chromatin remodelling (Tsankova et al., 2007). DNA methylation is often found at cytosine residues (5-methylcytosine; 5mC), which in mammals primarily occur in the context of the CpG dinucleotide (Lister et al., 2010; Maze & Nestler, 2011; Suzuki & Bird, 2008). CpG sequences throughout the genome are usually heavily methylated. However, at the promoter regions of genes groups of unmethylated CpGs, so called CpG islands, are found. The amount of DNA methylations at a promoter site correlates with the extent of gene inactivation (N. Tsankova et al., 2007). DNA methylation is thought to inactivate genes in two ways. Firstly, the methylation of DNA itself physically obstructs the binding of transcriptional proteins to the gene. This may occur by transfer of a methyl group to cytosine residues at the dinucleotide sequence CpG and is catalysed by DNA methyltransferases (DNMTs) (Hake, Xiao, & Allis, 2004; Lachner & Jenuwein, 2002). Secondly, methylated DNA may be bound by proteins known as methyl-CpG-binding domain proteins (MBDs), like methyl-CpG-binding protein 2 (MeCP2). MBD proteins alter transcription efficiency by recruiting additional proteins to the locus, such as histone deacetylases (HDACs) and other chromatin remodelling proteins that can modify histones, which results in more condensed chromatin, where DNA is tightly wrapped around the histones, which obstruct the DNA binding sites and represses gene transcription (Chahrour et al., 2008; Murgatroyd et al., 2009; Skene et al., 2010).

Thus, the reduction in CRH methylation after E-PS results in increased levels of CRH. This alteration was indeed associated with increased HPA reactivity (higher corticosterone levels and decreased hippocampal GRs), reduced 5-HT transporter levels in the hippocampus and a trend for increased tryptophan hydroxylase-2 (TPH2) expression in the serotonergic neurons of the dorsal raphe of male mice. Moreover, mice displayed depression-like phenotypes (i.e. tail suspension and the forced swim tests and stress-induced bingeing responses) that were partly diminished after treatment with the anti-depressant citalopram (Mueller & Bale, 2008). The location of methylation on the 5-HT transporter (SLC6A4) gene were in another study identified in the CpG island on the CpG residues, cg22584138 and cg05951817. This methylation was mostly found for the splice variant containing Exon 1A and 1B of SLC6A4 (Vijayendran, Beach, Plume, Brody, & Philibert, 2012). Variants that were

earlier associated with psychiatry. Interestingly, also adverse childhood experiences such as child sex abuse were related to this methylation (Beach et al., 2011). Methylation of cg22584138 was influenced by both genotype (splice variant) and sex abuse, whereas methylation of cg05016953 was influenced only by sex abuse history (Vijayendran et al., 2012).

Additionally, increased methylation of the NGFI-A region was observed in E-PS mice, which obstructs transcription of the GR promotor. Supportively, also in the human homolog of the GR gene increased methylation at the NGFI-A binding site was associated with prenatal stress and increased cortisol levels (Oberlander et al., 2008). Moreover, decreased GR mRNA expression together with GR promoter DNA methylation was found in the hippocampus of suicide victims with a history of child abuse (McGowan et al., 2009). Previously was discussed that augmented maternal care (LG ABN) in rodents could be linked to increased levels of NGFI-A in the $GR1_7$, once again differences in maternal care were significantly related to methylation levels (Weaver, Diorio, et al., 2004; Weaver et al., 2007), similarly to the effects of E-PS. The methylation related to low maternal care persisted in adulthood and was correlated with higher responsivity to stress. Although the methylation was long-lasting, the effects could be reversed by the class I and II HDAC inhibitor Trichostatin A (TSA). TSA increased levels of H3K9 acetylation, cytosine demethylation and the binding of NGFI-A at the $GR1_7$ promoter in the low LG ABN raised offspring. Interestingly, cross-fostering with high-caring also seem to decrease the methylation levels (Weaver, Diorio, et al., 2004; Weaver et al., 2007).

Besides epigenetic regulation of the glucocorticoid receptor, there are also more indications of epigenetic changes that affect CRH. For example, high maternal care in rats enhanced the expression levels of neuron-restrictive silencing factor (NRSF), which is an epigenetic regulator of transcription known to negatively regulate the expression of CRH in the PVN (Korosi et al., 2010). Furthermore, Elliott and colleagues (2010) studied the effects of chronic stress on the expression levels of CRH with the social defeat paradigm (BOX 1) in adult mice. Indeed, increased CRH mRNA levels in the PVN and demethylation at four CpGs in the promoter of CRH in the subset of defeated mice were reported. In contrast, stress resilient adult mice were characterised with methylation in the CRH promoter. Additionally, the antidepressant imipramine attenuated both the social avoidance behaviour and the promoter methylation levels of the previously defeated mice, which supports that also methylation of CRH is reversible and directly linked to stress (Elliott, Ezra-Nevo, Regev, Neufeld-Cohen, & Chen, 2010). In addition, expression of the methyl-binding proteins MeCP2 and MBD1 was increased after chronic fluoxetine treatment, accompanied by increased Hdac2 expression, which both indicate inhibition of transcriptional activity in these brain regions (Cassel et al., 2006).

Interestingly, also gender specific differences were observed regarding methylation of the CRH gene after exposure to stress. Chronic variable mild stress (CVMS) induced gender specific changes in CRH gene methylation in the PVN, oval (BNSTov) and fusiform (BNSTfu) parts of the BNST, PVN and central amygdala. The HAT CREB-binding protein (CBP) was increased in the female BNST and HDAC5 was decreased in the male central amygdala. These changes were accompanied by an increased amount of cFos in the PVN, fusiform part of the BNST and central amygdala in males, and of FosB in the PVN of both sexes and in the male BNSTov and BNSTfu. In the PVN, CVMS increased CRH mRNA in males and CRH peptide decreased in females (Sterrenburg et al., 2011). The origin of these differences is unknown, but possibly gender-specific sex-hormones play a role. Moreover, in the placenta of E-PS females a stress-induced elevation of DNMT1 was reported compared to controls. However, this difference was not found between the male groups. A stress-induced elevation of DNMT1 may indicate that females are able to circumvent the effects of stress by strengthening the maintenance of methylation during prenatal stress exposure (Leonhardt, Page, Weier, & Bestor, 1992; Mueller & Bale, 2008). Also, in human studies gene expression levels in the placenta were suggested to be an indicator of the potential to inherit a disorder. SLC6A4 mRNA expression was found to be significantly increased in placentas of women with MDD (untreated and treated with SSRIs), compared to controls. The finding that maternal depression and anxiety affects gene expression of placental SLC6A4 suggests a possible mechanism for the effect of maternal mood on fetal neurodevelopmental programming (Ponder, Saisbury, McGonnigal, Laliberte, Lester and Padbury, 2011). Until now only behavioural correlates and stress hormone levels in animal and human studies have indicated that for example the offspring of stressed parents indeed show increased stress responsivity (Grossman, Morris, & Bierer, 2007; Yehuda & Bierer, 2009). However, the exact mechanisms still have to explored and studied.

4. DISCUSSION

The major aim of this literature study was to explore the morphological, synaptic and epigenetic changes in the brain in response to exposure to stress that could hypothetically increase vulnerability for the effects of addictive drugs and/or precede major depressive disorder. Animal studies have significantly demonstrated relations between exposure to stress in different life phases, physiological changes in the brain and affected behavioural outcome (Meaney, Szyf, & Seckl, 2007; Campbell, Szumlinski, & Kippin, 2009). Confirmatively, these findings mirrored symptoms and observations in human tissue of MDD patients (Warner-Schmidt & Duman, 2006). However, causality in the development of these synaptic changes are difficult to pinpoint. As discussed in the first part, many physiological variables in either hippocampal neurogenesis or behavioural sensitization after psychostimulant treatment are correlated and dependent on each other (Jessberger et al., 2008; Kino et al., 2010; Lakshminarasimhan & Chattarji, 2012). Thus, after manipulation with external stimuli the causality in the observed alterations is difficult to reveal. Signalling cascades provide more information on which level gene expression is altered. However, the important transcription factors and signalling proteins are involved in the expression of again other common transcription factors. Although research has discovered many cellular mechanisms, isolating the function and role of specific transcription factors within one pathway or mechanism in relation to behavioural correlates might be the next step.

Several synaptic changes associated with exposure to stress were also linked to underlying epigenetic mechanisms. For now, the presence of altered epigenetic factors, as well as alterations in other transcription factors in response to stress support the hypothesis that the brain interacts with its environment (Kubota, Miyake, & Hirasawa, 2012; Rosenfeld, 2010). However, since research is conducted in terms of correlations it is difficult to define the epigenetic modifications as alterations at the very end of the ladder underlying all other stress-induced alterations, or that the epigenetic modifications are just a consequence of synaptic, cellular or extra-cellular mechanisms, like for instance glial interactions changing gene expression (Weaver, Cervoni, et al., 2004). Once further insight is obtained on the importance of the epigenetic modifications in the cycle of alterations in response to stress exposure one could discuss whether these processes are an important potential pharmacological target (Crepaldi & Riccio, 2009).

Further identifications of the factors, like transferase enzymes, involved in epigenetic processes may support discovering the onset of epigenetic alterations. A great deal is discovered about the

functions of different transferase enzymes, since different transferases were shown to be linked to specific epigenetic outcome and gene transcription levels (Riccio, 2010). However, further research on the effects that stress or addictive substances have on these enzymes and the tranferase genes that are responsible for chromatin remodelling could provide more knowledge on the potential importance of epigenetic research in psychiatric disorders regarding to treatment. There is no doubt that research on epigenetics changed and will change our view on behaviour and the influence of external factors on the human system and the next generation (Boks et al., 2012). Epigenetic research can make individuals aware of their health style and make one take responsibility for habits that have negative effects on the next generation, such as nicotine and alcohol-use during pregnancy (Haycock, 2009; Launay et al., 2009; W Renthal & Nestler, 2008) However, learning how adverse effects of stress and substance-use are transferred to the next generation, may be more accessible once the stress-induced processes in one life time are fully understood.

Overall, this literature review provided a wide insight, discussing morphological, cellular, synaptic levels and DNA expression, on which chronic stress exposure can have its effects. A few possibilities were discussed that could link the stress-induced effects associated with addiction and depression. However, this review mostly focused specifically on major depressive disorder and psychostimulant-use. MDD already knows different symptom subgroups and the drugs used among these groups are also scattered (Bolton et al., 2009; Eric J Nestler & Carlezon, 2006; Ponomarev, Rau, Eger, Harris, & Fanselow, 2010). Nonetheless, this review highlights the importance of glucocorticoid receptors in the mesocorticolimbic dopamine system, including the amygdala, hippocampus and prefrontal cortex. Further research will have to demonstrate if more subtle individuals differences can explain sensitivity or resilience to the effects of stress.

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