Translational regulation in the adult brain

mediated by BDNF signalling, stress, and chronic antidepressant treatment

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Dissertation for the degree philosophiae doctor (PhD)

University of Bergen

2010

Acknowledgements

The work described in this thesis was carried out at the Department of Biomedicine, University of Bergen during the period 2004-2009. The financial support was provided by the University of Bergen.

First of all I would like to thank my supervisor, Prof Clive Bramham, for introducing me to the field of neuroscience, for his enthusiasm, encouragement, and ability to always look at the bright side of things.

I also owe my colleagues in the group many thanks for contributing to a very good environment, by being such helpful and wonderful people which they all are. Thanks to both those who have left the lab during these years (Bjarte Håvik, Kjetil Bårdsen, Elhoucine Messaoudi, Margret Kjellingland, Åse-Marit Kristiansen, Anita Røyneberg, Girstaute Dagyte, Lars Rune Sæterdal, Jelena Mrdalj, Birgitte Berentsen) and those who have come afterwards (Karin Wibrand, Sjoukje Kuipers, Jonathan Soulé, Adrian Tiron, Maria Alme, Debabrata Panja, Martin Pilskog, May Lillian Ofte, Rajeevkumar Nair, Ashraf Pakzad Fathpour, Henriette Aksnes, Sue Olsen, Manja Schubert, Balagopal Pai, Margarethe Bittins, Craig Myrum). You have all contributed to make these years a really good time for me. Specially thanks to Tambudzai Kanhema, who has always been here. Thanks for teaching me the basic methods, but most of all thanks for your care and encouragement during these years. I also want to thank you, as well as Jonathan Soulé, May Lillian Ofte and Debabrata Panja, for constructive feedback on one of my manuscripts.

Many thanks also to all the other people in the 7th floor for contributing to a nice and friendly working environment.

Thanks to Torhild Fjordheim Sunde, Jelena Mrdalj and Debabrata Panja for excellent technical help with perfusion and sectioning of brains.

Also many thanks to Janne Grønli for the collaboration you initiated, thanks for valuable discussions and support. Thanks also to the co-authors for helpful comments on the manuscript.

Many thanks also to my friends and family for their support. A special thank to my parents for all the care and practical help. Without all your help with babysitting and much more, this work would have been much harder to accomplish.

Finally, thanks to Jostein for all the extra work you have taken on, without complaining, these years I have spend so much time away from home. Thanks for your patience and for listening to my frustrations.

I also thank my two daughters, Kaia and Alva, for making life wonderful!

Bergen, May 2010

Grethe Dagestad

List of abbreviations

AMPA	Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
Arc	Activity-regulated cytoskeleton-associated protein
BDNF	Brain-derived neurotrophic factor
CA	Cornu Ammonis
CaMKII	Calcium and calmodulin-dependent protein kinase II
CPEB	Cytoplasmic polyadenylation element-binding protein
eEF2	Eukaryotic elongation factor 2
eIF4E	Eukaryotic initiation factor 4E
ERK	Extracellular signal-regulated protein kinase
GABA	Gamma amino-butyric acid
HFS	High-frequency stimulation
IEG	Immediate early gene
KO	Knock-out
LTP	Long-term potentiation
LTD	Long-term depression
MAPK	Mitogen-activated protein kinase
MEK	Mitogen and extracellular signal regulated protein kinase
MPP	Medial perforant path
mTOR	Mammalian target of rapamycin
NGF	Nerve growth factor
NMDA	N-methyl-D-aspartate
NT-3	Neurotrophin-3
NT-4	Neurotrophin-4
PCR	Polymerase chain reaction
PSD	Postsynaptic density
SD	Synaptodendrosome
TIEG1	TGF -beta-induced immediate early gene-1
TrkB	Tropomyosin-like receptor kinase B

Abstract

One of the most amazing features of the brain is its plasticity. This is a fundamental process by which the brain acquires information and makes the appropriate adaptive changes, a process that makes us able to think, feel, and remember. Synaptic plasticity refers to the ability of connections between neurons, the synapses, to change in strength following activity. Dysfunctional synaptic plasticity is recognized as a key factor in several neurological and psychiatric disorders, including major depressive disorder. The consolidation of long-term changes in synaptic connectivity typically requires gene expression and protein synthesis. Recent work shows that post-transcriptional regulation of mRNA translation is critical for consolidation of synaptic plasticity and long-term memory. The secretory peptide, brain-derived neurotrophic factor (BDNF) has been identified as a possible common mediator of translational regulation and synaptic plasticity. However, the role of post-transcriptional mechanisms in stress and depression are little understood.

This thesis examines post-transcriptional regulation by BDNF during synaptic plasticity, and how translation is regulated in response to antidepressant treatment and stress.

First we examined the effects of BDNF on translational regulation when BDNF was infused into the dentate gyrus in vivo, and following directly application to isolated synapses in vitro (synaptodendrosomes). The results indicate a compartment-specific regulation of translation in the dentate gyrus in response to BDNF. In synaptodendrosomes, BDNF rapidly enhances phosphorylation of the eukaryotic initiation factor 4E (eIF4E) and enhances synaptic protein expression. In vivo, local infusion of BDNF induces long-term potentiation (BDNF-LTP) of synaptic transmission at perforant path granule cell synapses, associated with phosphorylation of eIF4E and eukaryotic elongation factor 2 (eEF2). BDNF-LTP and changes in translation factor phosphorylation state are dependent on activation of the extracellular signal-regulated kinase (ERK).

Next, we examined the effect of systemic treatment with the selective serotonin reuptake inhibitor (SSRI) fluoxetine. We show that chronic, but not acute, injections with fluoxetine induces region-specific effects on translation factor activity. Interestingly, chronic fluoxetine, like BDNF-infusion, increased phosphorylation of eIF4E and eEF2 specifically in the dentate gyrus. The RNA-binding protein cytoplasmic polyadenylation element-binding protein (CPEB), which regulates translation of specific mRNAs, was hyperphosphorylated in the prefrontal cortex and hippocampus proper following chronic fluoxetine treatment. This

effect was sustained in the hippocampus proper up to one week after cessation of treatment. In addition, chronic fluoxetine induced brain region-specific expression of two atypical CPEB isoforms, CPEB3 and CPEB4. Together, these findings support a role for post-transcriptional regulation in the delayed action of antidepressant drugs.

Finally, we examined the effect of chronic mild stress (CMS) as well as acute sleep deprivation, a potential antidepressant treatment in humans, on post-transcriptional mechanisms of gene expression in the prefrontal cortex, hippocampus proper and dentate gyrus. Groups of rats received either: 1) ordinary daily care, 2) 4 weeks of chronic mild stress, 3) a single 8 hour session of total sleep deprivation, or 4) four weeks of CMS followed by sleep deprivation. The results demonstrate region-specific post-transcriptional regulation in response to CMS and sleep deprivation. In the dentate gyrus and hippocampus proper, eIF4E and eEF2 phosphorylation were selectively modulated by SD, indicating a suppression of general protein synthesis in these structures. In the prefrontal cortex, phosphorylation of eIF4E is enhanced following both CMS and sleep deprivation, indicating enhancement of translational initiation and protein synthesis. These regional differences were further supported by differential regulation of genes (Arc and BDNF) previously coupled to synaptic plasticity and antidepressant responses.

We conclude that post-transcriptional regulation is affected not only during BDNF-LTP, but also in a brain region-specific manner following stress and antidepressant treatment.

List of original papers

This thesis is based on the following papers, referred to by their roman numerals in the text.

I Kanhema T, **Dagestad G**, Panja D, Tiron A, Messaoudi E, Havik B, Ying SW, Nairn AC, Sonenberg N, Bramham CR. Dual regulation of translation initiation and peptide chain elongation during BDNF-induced LTP in vivo: evidence for compartment-specific translation control. *J Neurochem.* 2006;19:1328–1337.

II **Dagestad G**, Kuipers SD, Messaoudi E, Bramham CR. Chronic fluoxetine induces region-specific changes in translation factor eIF4E and eEF2 activity in the rat brain. *Eur J Neurosci.* 2006;23:2814–2818.

III **Dagestad G**, Bramham CR. Chronic fluoxetine induces phosphorylation of CPEB1 and brain region-specific expression of CPEB3 and CPEB4. *Manuscript*

IV **Dagestad G**, Grønli J, Milde AM, Murison R, Portas CM, Bramham CR. Chronic mild stress and sleep deprivation acts at the post-transcriptional level: brain region-specific regulation of translation factor activity and CPEB phosphorylation. *Manuscript*

Content

1 Introduction

1.1 Synaptic plasticity

In 1894, the neuroanatomist Ramón y Cajal proposed that memory is stored as an anatomical change in the strength of neuronal connections. The idea was incorporated into a formal hypothesis for learning and memory processing by Donald Hebb in 1949. He proposed that cells may grow new connections or undergo metabolic changes that enhance their ability to communicate (Hebb's postulate). Their theories were solely based on theoretical ground. However, later experimental investigations proved they were right, and today it is widely accepted that neurons can undergo lasting changes. The connection between neurons, the synapse, is the most fundamental unit for information transmission in the nervous system. Synaptic plasticity refers to the ability of synaptic connections to change in strength following activity. Long-lasting changes in synaptic plasticity is considered to underlie learning and long-term memory (Malenka and Nicoll, 1999). Much work in the field of neuroscience has focused on understanding the molecular mechanisms regulating synaptic plasticity.

1.1.1 Long-term potentiation (LTP)

Although conceptualized by Hebb in the 1940s, it was not until the 1970s the first publications appeared that clearly demonstrated that synapses could sustain long-lasting changes in synaptic efficacy. It was then reported that high.-frequency stimulation of afferent fibers of the perforant path in the hippocampus could induce a long-lasting increase in synaptic strength, a phenomenon which was termed long-term potentiation (LTP) (Bliss and Lomo, 1973). Together with long-term depression (LTD), which refers to long-lasting decrease in synaptic strength, these are the most studied forms of long lasting synaptic plasticity in mammals (Malenka and Bear, 2004).

Most of the work on LTP and LTD has been performed in the hippocampal formation, a bilateral cortical structure, located in the medial temporal lobe. The hippocampal formation belongs to the limbic system, and is required for different forms of memory (Squire et al., 2004). On the basis of structural differences, the hippocampal formation can be divided into the dentate gyrus (DG), the hippocampus proper (Cornu Ammonis fields 1-3 or CA1-CA3) and the subiculum (Amaral and Witter, 1989) (Figure1).

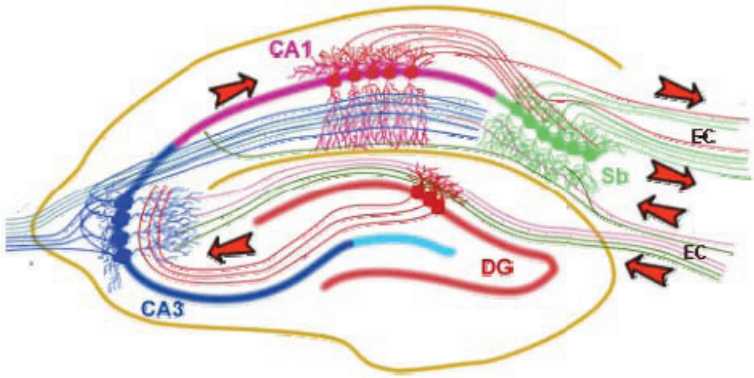

Figure 1 The hippocampal dentate gyrus (DG) receives the major afferent input from the entorhinal cortex (EC) via the perforant path. The granule cells of the dentate gyrus in turn send their axons (the mossy fibers) to innervate the CA3 region. The pyramidal neurons of theCA3 region project via the Schaffer collaterals to the CA1 pyramidal neurons. The principal output of the hippocampus finally forms the connection of the CA1 to the subiculum (Sb) and on to the entorhinal cortex (From Morris and Johnston, 1995).

LTP has been the object of intense investigations over the last three decades, as it is believed to provide an important key to understand some of the cellular and molecular mechanisms that underlies memory formation (Pastalkova et al., 2006; Whitlock et al., 2006). LTP seems to be an appropriate cellular model for memory as there is good agreement between results from LTP experiments and memory tests (Lynch, 2004).

Induction of LTP is mediated by activation of the excitatory neurotransmitter glutamate and regulation of intracellular signalling cascades. Glutamate, which is the major excitatory neurotransmitter in the brain, causes neuronal depolarization via activation of postsynaptic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors that increase intracellular Na^{+}. This leads to the subsequent activation of N-methyl-D-aspartate (NMDA) receptors and results in influx of Ca^{2+} through the receptor channel. Similar to memory, LTP can be divided into two distinct phases which involves different molecular mechanisms; early phase LTP (E-LTP), which lasts only minutes to few hours, and late phase LTP (L-LTP), which can persist for many hours, days or months . E-LTP, like short-term memory, does not require new gene expression, but requires modification of pre-existing proteins. This is typically phosphorylation of protein kinases that are sensitive to the increase in intracellular Ca^{2+}, like α- Ca^{2+}/ calmodulin-dependent protein kinase II (α-CaMKII) and protein kinase C (PKC). L-LTP, like long-term memory, requires new gene transcription and

protein synthesis (Davis and Squire, 1984; Bliss and Collingridge, 1993; Nguyen et al., 1994; Kandel, 2001). Activation of protein kinases is a crucial event in signal transduction leading to gene transcription. α-CaMKII, PKC, protein kinase A (PKA), and extracellular signal-related protein kinase (ERK) have been suggested to have important roles in LTP (Soderling and Derkach, 2000; Sweatt, 2001; Rosenblum et al., 2002). When ERK is activated it can translocate to the nucleus where it activates the transcription factor cAMP-response-element binding protein (CREB) and transcription of immediate early genes (IEGs) (Patterson et al. 2001; Rosenblum et al. 2002). One of the IEGs is Arc, which encodes an mRNA that rapidly transports to dendrites of dentate granule cells and accumulates near stimulated synapses where it is translated locally (Lyford et al. 1995; Link et al. 1996; Steward et al. 1998; Steward and Worley, 2001; Rodriguez et al. 2005). L-LTP is expressed through several morphological and molecular changes of the synapse. Dendritic spines, which are the primary recipients of excitatory input, can undergo rapid changes. This can include altered shape or enlargement of spines, increased number of AMPA receptors in the spines, as well as formation of new spines (Isaac et al., 1995; Liao et al., 1995; Toni et al., 1999; Bourne and Harris, 2008). Dendritic spines provide a local biochemical compartment where ions and signalling molecules become concentrated following synaptic activation. The process of spine enlargement is dependent on sustained synthesis of Arc protein (Bramham et al., 2010). Another protein critically involved in LTP, and which can activate Arc-dependent LTP consolidation, is brain-derived neurotrophic factor (BDNF).

1.1.2 BDNF

The neurotrophin family consists of secretory peptides that were originally identified as promoters of neuronal survival. The mammalian neurotrophins consist of nerve growth factor (NGF), neurotrophin-3 (NT-3), neurotrophin-4 (NT-4) and brain-derived neurotrophic factor (BDNF) (Lewin and Barde, 1996). Each of them activate one or more of the three members of the tropomyosin-related kinase (Trk) family of receptor tyrosine kinases (TrkA, TrkB and TrkC), while all of them activate the p75 neurotrophin receptor (p75NTR), a member of the tumour necrosis factor receptor superfamily. In addition to promote survival, the neurotrophins also have important roles in development and differentiation of the nervous system (Ernfors, 2001). BDNF, which is the most abundant neurotrophin in the mammalian brain (Murer et al., 2001), has received a lot of attention because of its critical role in synaptic

plasticity (Bramham and Messaoudi, 2005). BDNF is widely distributed in the mammalian brain, with the highest expression levels in the hippocampus, neocortex, amygdala, cerebellum, septum, and several hypothalamic nuclei (Ernfors et al., 1990; Hofer et al., 1990; Phillips et al., 1990; Dugich-Djordjevic et al., 1995; Castren et al., 1995). BDNF binds the TrkB receptor with high affinity to induce its dimerization and autophosphorylation of tyrosine residues in the cytoplasmic kinase domain. When phosphorylated, these residues serve as docking sites for scaffolds/adaptor proteins that are coupled to three main signalling pathways: the extracellular signal-regulated protein kinase (ERK)-, the phospholipase C-γ (PLC-γ)-, and the phosphatidylinositol-3-kinase (PI3K) pathways (Kaplan and Miller, 2000; Reichardt, 2006). By activating these signalling pathways, BDNF regulate gene expression, through modulation of transcription factors (Finkbeiner et al., 1997) and translation factors (Soule et al., 2006).

Signalling through the ERK pathway is critical for activation of the transcription factor CREB (Shaywitz and Greenberg, 1999; Adams et al., 2000). ERK also plays a critical role in protein synthesis dependent plasticity by increasing phosphorylation of eIF4E (Klann and Dever, 2004; Kelleher, III et al., 2004). Activation of the PLC-γ pathway leads to an increase of intracellular Ca^{2+}, and activation of Ca^{2+}-sensitive kinases like α−CaMKII**.** The activity-induced expression of BDNF has also been shown to depend on Ca^{2+}-influx and α−CaMKII activation (Du et al., 2000). The PI3K pathway activates translation via a cascade driving the mammalian target of rapamycin (mTOR), a major regulator of protein synthesis (Sarbassov et al., 2005). BDNF stimulates translation through activation of mammalian target of rapamycin (mTOR) in this pathway (Schratt et al., 2004). The PI3K pathway also regulates the trafficking of synaptic proteins (Yoshii and Constantine-Paton, 2007).

BDNF is critically involved in LTP. First of all, induction of LTP induces expression of BDNF mRNA in the hippocampus (Castren et al., 1993; Bramham et al., 1996). Release of BDNF has been shown to be activity-dependent (Goodman et al., 1996; Canossa et al., 1997; Hartmann et al., 2001; Aicardi et al., 2004), and activity-dependent release of BDNF plays a critical role in LTP (Bramham and Messaoudi, 2005). Studies with transgenic mice have shown that LTP is impaired in mice lacking BDNF, but that this impairment is reversed when BDNF is re-expressed (Korte et al., 1995;Korte et al., 1996). In addition, a weak synaptic activation, below the LTP threshold, induces LTP when BDNF is simultaneously applied (Figurov et al., 1996; Kovalchuk et al., 2002). It is thought that the potentiating effect of BDNF on E-LTP is achieved, at least in part, by enhancing synaptic responses to high

frequency stimulation and by presynaptically facilitating synaptic vesicle docking (Pozzo-Miller et al., 1999; Jovanovic et al., 2000; Tyler and Pozzo-Miller, 2001). Moreover, exogenously applied BDNF to hippocampal slices can induce a long-lasting increase in synaptic efficacy in the CA1 region (Kang and Schuman, 1995). Also, BDNF infusion into the dentate gyrus of intact rats induces a long-lasting potentiation (Messaoudi et al., 1998), an effect termed BDNF-LTP. It has been suggested that BDNF is necessary for, and can directly activate the transcription- and translation dependent late phase of LTP (Korte et al., 1998; Gartner and Staiger, 2002; Messaoudi et al., 2002; Barco et al., 2005). BDNF-LTP is dependent on activation of the ERK pathway, and increased transcription and translation of activity-regulated cytoskeletal-associated protein (Arc) (Ying et al., 2002; Messaoudi et al., 2007). A microarray study has identified nine genes that are strongly upregulated with Arc during BDNF-LTP and LTP induced by high-frequency stimulation (HFS-LTP), in the dentate gyrus (Wibrand et al., 2006).

1.1.3 Dendritic protein synthesis

As mentioned, long-lasting forms of synaptic plasticity and memory are dependent on synthesis of new proteins. The classical view is that proteins are synthesized in the cell body and delivered to potentiated synapses. The possibility that proteins could be synthesized at other sites then in the cell body was first suggested by the observations of mRNAs and polyribosomes in dendrites (Bodian, 1965; Steward and Levy, 1982). Since this discovery, several studies have shown that dendritic protein plays a key role in synaptic plasticity (Sutton and Schuman, 2006). Dendritic protein synthesis enables synapses to control synaptic strength independent of the cell body via rapid protein production from pre-existing mRNA. It also enables specificity in that proteins are made available only at the stimulated synapses, which is where new proteins are required.

Many mRNAs have been found to be localized to dendrites (Crino and Eberwine, 1996; Steward and Schuman, 2001; Zhong et al., 2006). Some of these have been shown to be locally translated in response to extracellular stimulation, e.g. α-CaMKII (Ouyang et al., 1997; Ouyang et al., 1999; Scheetz et al., 2000), Arc (Steward et al., 1998), and CREB (Crino et al., 1998). However, little is known about the mechanisms that regulate translation in dendrites, and causal roles for specific dendritically synthesized proteins in stable forms of synaptic plasticity have not been clearly defined.

Several studies have demonstrated that BDNF can regulate the local synthesis of synaptic proteins (Kang and Schuman, 1996; Huber et al., 2000; Aakalu et al., 2001; Zhang and Poo, 2002; Yin et al., 2002).

1.2 Translational regulation

Regulation of gene expression at the level of mRNA translation plays a key role in regulation of long-lasting synaptic changes. Translational regulation may effectively dictate the time, place, and amount of protein synthesis in order to meet the dynamics demands of the active neuron.

Translational regulatory mechanisms fall into two categories: general mechanisms, which apply to translation of many or all mRNAs, and mRNA-specific mechanisms, which apply to the translation of a particular subset of mRNAs based on recognition of RNA binding proteins or miRNA to specific sequences in the target mRNA. Neurons employ both general and mRNA specific translation in the context of long-term synaptic plasticity.

Translation is generally divided into three steps: initiation, elongation, and termination. Initiation involves recruitment of mRNA and $tRNA_i^{Met}$ to a ribosome. During elongation, aminoacyl-tRNAs are sequentially recruited and the peptid-chain lengthens incrementally as amino acids are covalently attached via peptide bonds. Finally, the polypeptide chain is released from the ribosome. Each step is regulated by several translation factors; eukaryotic initiation factors (eIF), eukaryotic elongation factors (eEF) and eukaryotic release factors (eRF). In eukaryotic cells, initiation is usually the rate-limiting step in the translation process, and therefore also a primary target for regulation (Gingras et al., 1999). However, translation may also be regulated at the elongation step (Browne and Proud, 2002). Regulation of the activity of translation factors by phosphorylation appears to be a general mechanism for the regulation of general protein synthesis.

In this thesis, I have focused on general mechanisms of translational regulation, mediated by two key translation factors, eIF4E and eEF2, as well as mRNA-specific mechanisms, mediated by CPEB1, which binds to and regulate the translation of specific mRNAs.

1.2.1 The role of eIF4E in translation initiation

Translation initiation can be subdivided into three steps: 1) formation of the 43S ribosomal preinitiation complex, 2) binding of the mRNA to the 43S ribosomal complex, and 3) formation of the 80S ribosomal complex (Figure 2).

Recruitment of the ribosome to the mRNA usually occurs by a cap-dependent mechanism, in which ribosome binding is facilitated by the 7-methylguanosine 5-triphosphate cap structure (m7GpppX) in the 5' end of the mRNA. eIF4E binds with high affinity to the 5'-mRNA cap structure and forms the eIF4F complex (Matsuo et al., 1997). In addition to eIF4E, the eIF4F complex also contains a RNA scaffolding protein (eIF4G) and eIF4A that functions to identify and unwind the mRNA for subsequent 43S ribosome association (Gingras et al., 1999). In addition to binding eIF4E and eIF4A, eIF4G also binds the translation factor eIF3 and the poly(A)- binding protein (PABP), which binds to the poly(A) tail on the 3'-end of the mRNA (Sonenberg and Dever, 2003). eIF4E may also mediate 5' mRNA interactions with its 3'-terminus which result in circular mRNA conformations with altered rates of translation (Stebbins-Boaz et al., 1999; Cao and Richter, 2002). Binding of eIF4E to the 5'-mRNA cap can be regulated by proteins that bind to and sequester eIF4E and prevents eIF4E from binding to eIF4G, or by phosphorylation of eIF4E.

The eIF4E-binding proteins (4E-BPs) are regulated by phosphorylation as well; when 4E-BP is phosphorylated, its binding to eIF4E is inhibited so that eIF4E is free to bind the cap and eIF4G and initiate translation (Gingras et al., 1999). Several protein kinases have been shown to phosphorylate 4E-BP, including mammalian target of rapamycin (mTOR), whose activity is blocked by rapamycin (Beretta et al. 1996), and the mitogen-activated protein kinases ERK1 and ERK2 (Lin et al. 1994).

Binding of eIF4E to the 5'-mRNA cap can also be regulated via phosphorylation of eIF4E itself. Mammalian eIF4E is phosphorylated on a single residue, Ser209, by the mitogen-activated protein kinase-interacting kinase 1 (Mnk1) (Wang et al., 1998; Waskiewicz et al., 1999). Mnk1 is phosphorylated and activated by the mitogen-activated protein kinases ERK and p38 (Waskiewicz et al., 1997), kinases that are known to be critical for various forms of synaptic plasticity (Bolshakov et al., 2000; Sweatt, 2001; Thiels and Klann, 2001; Gallagher et al., 2004). Several studies have demonstrated that eIF4E phosphorylation is positively correlated with enhanced translation of mRNA (Scheper and Proud, 2002; Senthil et al., 2002; Duncan et al., 2003; Gingras et al., 2004). It has been indicated that phosphorylation of eIF4E reduces its cap-binding affinity (Scheper and Proud, 2002; Zuberek

et al., 2003) and that this reduced affinity for the cap structure results in release of eIF4E, permitting reinitiation on the same mRNA or on another message (Scheper and Proud, 2002), thereby resulting in increased translation activity. Phosphorylation of both 4E-BP and eIF4E are increased by ERK activation in the hippocampus, thereby synaptic activity that activates the ERK pathway can stimulate translation initiation (Kelleher et al. 2004; Banko et al. 2005).

Enhanced phosphorylation of Mnk1 and eIF4E has been shown to occur in cultured neurons treated with BDNF, and the increased eIF4E phosphorylation was shown to be ERK dependent (Takei et al. 2001; Kelleher III et al. 2004). It has also been shown that NMDA receptor activation enhances the phosphorylation of Mnk1 and eIF4E via ERK in hippocampal area CA1 (Banko et al. 2004) and L-LTP-inducing stimulation has been shown to result in an ERK-dependent increase in eIF4E phosphorylation (Kelleher III et al. 2004). Thus, phosphorylation of eIF4E by Mnk1 via activation of either ERK or p38 is another way in which translation initiation is likely to be regulated during synaptic plasticity. BDNF-induced increase in translation is dependent on both the ERK and PI3K pathways (Takei et al., 2001; Takei et al., 2004). Activation of eIF4E can be mediated through both the ERK and PI3K pathways. Activated ERK phosphorylates its substrate MAP kinase-interacting kinase1 (Mnk1), which again phosphorylates eIF4E.

Once the eIF4F complex has bound to the 5'-mRNA cap structure, the 43S ribosomal preinitiation complex is recruited to the 5'-end of the mRNA and scans the 5´-UTR until it reaches the start codon (AUG). eIF4G is bridging the mRNA to this complex through interactions with eIF3, which is bound to the 40S ribosomal subunit (Gingras et al., 1999). At the start codon, the 60S ribosomal subunit joins the 40S subunit to form a translationally competent 80S ribosome. The next step is now elongation.

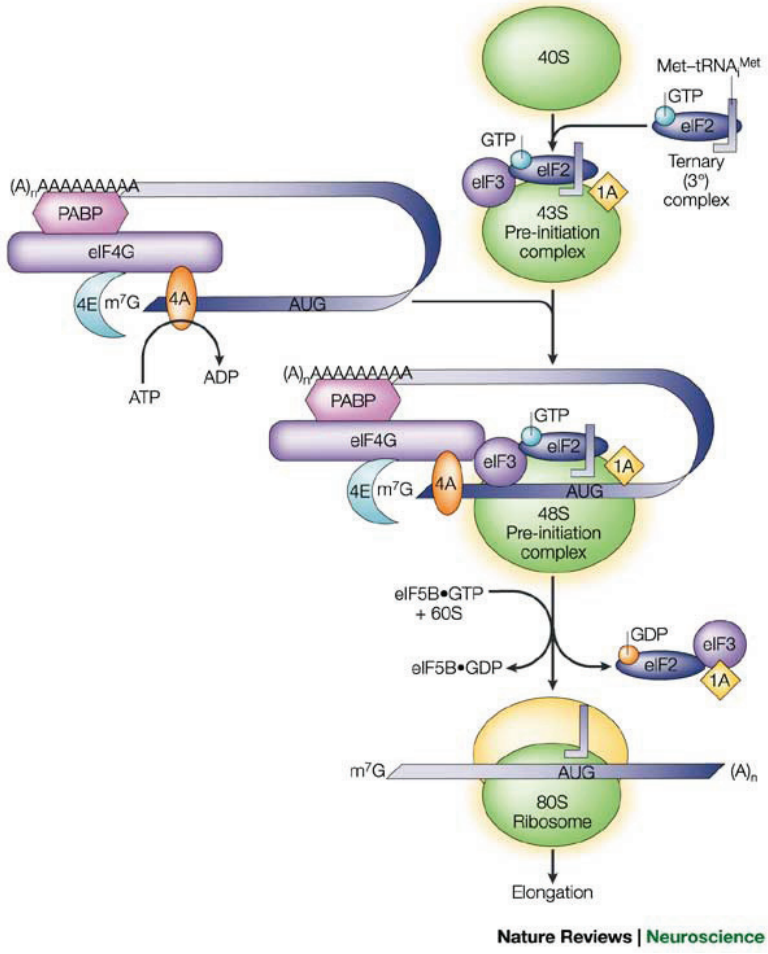

Figure 2 Pathway of translation initiation (From Klann and Dever, 2004)

1.2.2 The role of eEF2 in translation elongation

Elongation is the process of adding amino acids to the growing polypeptide chain. One amino acid is added at a time, and peptide bonds are formed between the incoming amino acid and the growing polypeptide (Figure 3).

The process of peptide-chain elongation involves recruitment of the amino acyl-tRNAs to the A-site of the ribosome and translocation, in which the ribosome moves relative to the mRNA by the equivalent of one codon. In eukaryotes, the factors involved in amino acyl-tRNA recruitment are eEF1A and eEF1B, while translocation requires eEF2. The major mechanism for regulating elongation is through phosphorylation of eEF2 (Herbert and proud 2007). eEF2 is a GTP-binding protein that mediates translocation of peptidyl-tRNA from the A-site to the P-site on the ribosome by hydrolyzing GTP (Moldave, 1985). The movement of the ribosome along the mRNA is repeated until a stop codon is reached, at which point elongation is terminated and the ribosomal subunits are released from the mRNA. Phosphorylation of eEF2 at threonine-56 (Thr56) inhibits its activity by preventing it from binding to the ribosome, leading to reduced rate of elongation and decreased general translation (Ryazanov and Davydova, 1989; Carlberg et al., 1990; Redpath and Proud, 1993). However, some mRNAs which are critically involved in long-lasting synaptic plasticity (like Arc and α-CaMKII), undergo enhanced translation under conditions of eEF2 phosphorylation and decreased general translation and (Scheetz et al., 2000; Chotiner et al., 2003; Kelleher, III et al., 2004; Belelovsky et al., 2005).

eEF2 is phosphorylated by eEF2 kinase. This kinase is regulated by Ca^{2+}/calmodulin, and thus by neuronal activity (Herbert and Proud, 2007). eEF2 kinase can also be phosphorylated by cAMP-dependent protein kinase (PKA), which results in that eEF2 kinase becomes partially independent of Ca^{2+}/calmodulin for activity (Redpath and Proud, 1993). eEF2 kinase has also been shown to be a substrate for p70 S6 kinase and p90RSK1 (Wang et al., 2001). p70 S6 kinase is activated in an mTOR dependent manner, whereas p90RSK1 is activated by ERK. The ERK pathway can be activated by a variety of stimuli including different agonists, and this signalling connection then potentially allow these different agonists to modulate protein synthesis via the ERK pathway (Browne and Proud, 2002).

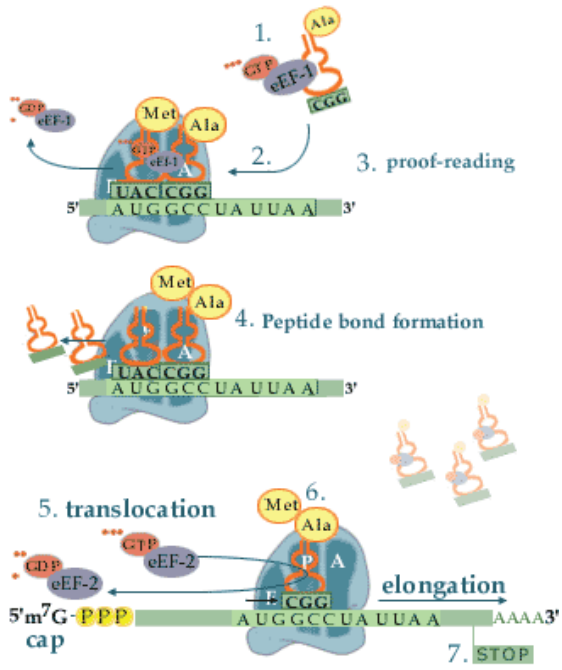

Figure 3 Simplified model for the translation elongation process (From *www.nobelprize.org*)

1.2.3 The role of CPEB in mRNA-specific translational regulation

CPEB1 is a member of an evolutionarily conserved family of RNA binding proteins (CPEB1-4 in vertebrates). CPEB1 targets mRNAs containing one or more cytoplasmic polyadenylation elements (CPEs), a U-rich structure in the 3´ UTR (Hake et al., 1998).

CPEB was first described in *Xenopus* oocytes (Hake and Richter, 1994) and this is also where most of the mechanisms regarding CPEB have been elucidated. In its non-phosphorylated form, CPEB functions indirectly as a translational repressor. CPEB binds to CPE in the target mRNA and inhibits translation initiation. CPEB binds to the protein Maskin, which in turn is bound to eIF4E. The binding of Maskin to eIF4E precludes the binding of eIF4G to eIF4E, thus inhibiting the formation of the initiation complex (Figure 4). Repressing translation may be of importance when CPEB1 is contributing to transport of its targets along microtubules, another important function of this protein (Huang et al., 2003).

Cytoplasmic polyadenylation is a widely used mechanism to activate translation, and CPEB1 is the key protein that controls this process. Polyadenylation requires both the CPE

and a nearby structure, the hexanucleotide sequence (AAUAAA). Phosphorylation of CPEB initiates polyadenylation (Mendez et al., 2000), which is the addition of adenine bases (A) to extend the poly(A) tail in the 3´end of the mRNA. Extension of the poly(A)- tail length is linked to 5´ capping of the mRNA and enhanced translation (Kuge and Richter, 1995); phosphorylation causes CPEB to bind and recruit the cleavage and polyadenylation specificity factor (CPSF) into an active polyadenylation complex, and helping it to associate with the hexanucleotide sequence. CPSF recruits poly(A) polymerase (PAP) to the end of the mRNA, were it catalyzes addition of poly(A) (Barnard et al., 2004). The newly elongated poly(A)-tail is then bound by poly(A)-binding protein (PABP), which in turn associates with eIF4G. This association will displace Maskin, and eIF4G can bind to eIF4E, and initiating translation (Stebbins-Boaz et al. 1999; Cao and Richter, 2002). CPEB1 contains two critical residues (T171 and S177) which can be phosphorylated by either Aurora A kinase or α-CaMKII in hippocampal neurons in response to NMDA receptor activation (Huang et al., 2002; Atkins et al., 2004).

CPEB1 is present in many regions of the brain, most abundantly in the hippocampus and cerebral cortex, were it localizes to synapses and particular the postsynaptic density (Wu et al., 1998). At synapses, many of the factors involved in polyadenylation-induced translation have been detected (Huang et al. 2002).

Only a few targets have been shown to undergo polyadenylation-induced translation in response to synaptic activity; α-CaMKII, tissue plasminogen activator (tPA), MAP2 and AMPA receptor binding protein (ABP), (Wu et al., 1998; Huang et al., 2002; Shin et al., 2004; Du and Richter, 2005). The identified CPEB target mRNAs encode proteins that have important roles in synaptic plasticity (Du and Richter, 2005; Pique et al., 2008). Studies with CPEB knock-out mice have also demonstrated that CPEB is important for some forms of synaptic plasticity (Alarcon et al., 2004), as well as for extinction of hippocampal-dependent memories (Berger-Sweeney et al., 2006).

The other CPEB isoforms, CPEB2-4, have been shown to constitute a different class of RNA binding proteins. Huang and colleagues demonstrated that CPEB2–4 do not interact with the CPE, but bind another element in the mRNA. Moreover, CPEB3, unlike CPEB1, does not require the hexanucleotide AAUAAA to regulate translation, neither does it bind CPSF, indicating that it regulates translation in a polyadenylation-independent manner (Huang et al., 2006). CPEB3 and -4 are expressed in only partially overlapping regions of the brain, but both are expressed in dendrites and synapses of hippocampal neurons (Huang et al. 2006). While CPEB3 has been found to repress translation of AMPA receptor GluR2 mRNA

in neurons (Huang et al., 2006) and also to be associated with human episodic memory (Vogler et al., 2009), a function of CPEB4 has still not been characterized.

The CPEB family are very attractive proteins considering that CPEB in the sea slug *Aplysia Californica* (ApCPEB) has been shown to have prion-like properties, caused by an N-terminal domain with high glutamine content and conformational flexibility (Si et al., 2003). When in the prion state, CPEB induces other normally shaped CPEB proteins to also enter the prion state. Unlike other prion proteins, it is the dominant, self-perpetuating state of the protein which is the active form. By having such prion-like properties, CPEB might be able to maintain its activity for a long time after stimulation and therefore have an important role in maintenance of long-term synaptic plasticity (Shorter and Lindquist, 2005; Si et al., 2010). The CPEB3 protein is the most likely candidate for being a functional homologue of the ApCPEB as they share some common features, like the glutamine-rich region in the N-terminal (Theis et al., 2003).

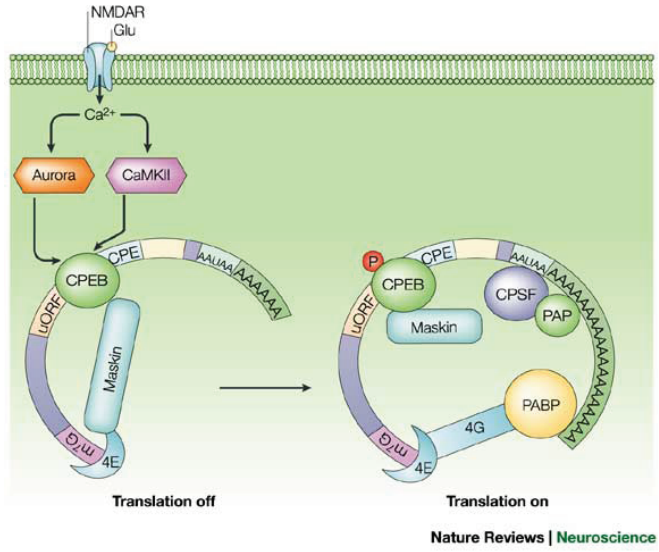

Figure 4 Regulation of translation initiation by CPEB (from Klann and Dever, 2004)

1.3 Depression

Mood disorders are generally classified as either major depressive disorder (MD) or bipolar disorder (BD). The focus in this thesis will be major depressive disorder, which is termed depression in the following text. Depression is a serious condition that affects both the mental and physical health. Up to 20% of the population worldwide is affected, women almost twice as often as men (Wong and Licinio, 2001). Based on a survey by the World Health Organization depression is one of the top ten causes of morbidity and mortality worldwide. Formal classification as depression requires that at least five of the following symptoms last for at least two weeks:

- Depressed or irritable mood
- Decreased interest in pleasurable activities and ability to experience pleasure (anhedonia)
- Significant weight gain or loss (>5% change/month)
- Sleep disturbance (Insomnia or hypersomnia)
- Psychomotor agitation or retardation
- Fatigue or loss of energy
- Feelings of worthlessness or excessive guilt
- Diminished ability to think or concentrate
- Recurrent thoughts of death or suicide

Depressed mood or anhedonia must be one of the symptoms (Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR) (American Psychiatric Press; 2000). Diagnosing can be complicated as subtypes of depression may differ not only in aetiology and clinical picture, but also in clinical response to medical treatments (Antonijevic, 2006). It has been indicated that depression subsumes numerous disease states of distinct aetiology, and perhaps distinct pathophysiology (Berton and Nestler, 2006).

Some individuals have a higher genetic risk for being depressed than other. The heritability of depression is likely to be in the range of 40-50%. There has been identified a number of genetic loci associated with vulnerability to depression or antidepressant efficacy. For example, there seem to be an association between combined polymorphisms in the 5-HT1A and BDNF genes and the risk of treatment-resistant depression (Anttila et al., 2007). Genetic predispositions are thought to interact with environmental risk factors for the

development of depression. Recent interest in epigenetic regulation of gene expression in the brain might lead to new, important information about depression. A review from Tsankova et al., 2007 summarizes recent evidence for how epigenetic mechanisms of gene regulation in neurons can be implicated in depression (Tsankova et al., 2007).

Left untreated, depression can be life-threatening due to risk of suicide. Treatment usually consists of psychotherapy and/or antidepressant medications, while electroconvulsive treatment (ECT) has for a long time been used to treat depression in patients who do not respond to other treatments (Paul et al., 1981). Although very effective, the use of ECT can result in memory loss and other cognitive deficits (Frasca et al., 2003). The primary medical treatment of depression has been through drugs that increase the concentration of biogenic monoamines (serotonin, noradrenalin and/or dopamine) in the brain.

In the 1950s it was discovered that a drug which was prescribed against tuberculosis elevated mood in some patients who were depressed. It was then revealed that the drug inhibited the neuronal breakdown of monoamines by the enzyme monoamine oxidase, which led to the statement of the monoamine hypothesis of depression (Bunney, Jr. and Davis, 1965; Schildkraut, 1965). The hypothesis proposes that depression is caused by a deficiency of monoamines in the brain, and that targeting this with antidepressants would tend to restore normal function in depressed patients (Nestler et al., 2002). Based on this, monoamine oxidase inhibitors were developed as the first class of antidepressants. In the late 1980s, Prozac (fluoxetine) and other drugs that block serotonin reuptake transporters without affecting other monoamines were approved for commercial use. These selecitve serotonin reuptake inhibitors (SSRI`s) became known as "novel antidepressants" along with other newer drugs such as serotonin-noradrenaline reuptake inhibitors (SNRIs) and noradrenaline reuptake inhibitors (NRIs). These, and also newer types of drugs designed to increase monoamine levels in the brain, are more efficient and produce less side effects than the older drugs do. However, more than half of all depressed individuals do not respond adequately to antidepressant drugs, and side-effects are still problematic to many (Trivedi et al., 2006; Sonnenberg et al., 2008; Papakostas, 2010). This raises a requirement for new and more efficient treatment. After the discovery of the SSRIs in the 1980s, development of newer drugs has seen little progress. The reason for this is that still today the precise molecular and cellular mechanisms underlying the action of these drugs or depression are not clearly understood.

1.3.1 The neurobiological basis of depression

Depression is a complex and highly heterogeneous disorder that is influenced by genetic, environmental and neurobiological factors. Despite years of intensive research, the neurobiological basis of depression and the mechanisms underlying antidepressant action remains poorly understood. Although antidepressant drugs raises the monoamine levels in the brain shortly after they are taken, these drugs have to be taken for at least two weeks until a therapeutic response appear. This long delay in clinical response indicates that the monoamine hypothesis does not provide a complete explanation for the actions of antidepressant drugs or for the pathophysiology of depression. The monoamine hypothesis evolved over the years to include, for example, adaptive changes in 5-HT receptors to explain the delayed clinical response to antidepressant treatment. Still, the monoamine hypothesis does not address key issues, like why all drugs that enhance serotonergic or noradrenergic transmission are not necessarily effective in depression. Despite the limitations, development of the monoamine hypothesis has been of great importance in understanding depression and in the development of safe and effective antidepressants (Hirschfeld, 2000).

The delayed clinical response constitutes a major problem in the treatment of depression. A better understanding of how the drugs work and also of the aetiological and pathophysiological factors in depression is needed to develop more efficient and faster acting treatment. Today, it is becoming more and more evident that certain aspects of depression result from maladaptive neuroplastic changes in specific neural circuits (Krishnan and Nestler, 2008), whereas antidepressants produce secondary changes that are on a longer timescale and mediate molecular and cellular plasticity (Nestler et al., 2002; Pittenger and Duman, 2008).

1.3.2 Stress and depression

Stress is probably the most important environmental factor that can trigger depression in some vulnerable individuals (Kendler et al., 1999; Caspi et al., 2003). Severe stress, either physical or psychological, often precedes the first or second episode of depression, but plays a minor role in the onset of subsequent episodes. Studies in twins have shown a clear interaction between genetic predisposition and a recent stressful life event in the precipitation of a depressive episode (Kendler, 1998). Stressful life events in childhood have been shown to predispose the individual for development of depression in adulthood (Kendler et al., 1992; McCauley et al., 1997; Agid et al., 2000). Individuals who have both a genetic risk of being

depressed and a traumatic childhood seem to be unusual prone to developing depression (Nemeroff and Vale, 2005).

The relationship between stress and depression remains to be fully established. The reduced neuronal plasticity, which is suggested as an important link, may be explained by dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis as well as structural and physiological alterations in the hippocampus and prefrontal cortex. At the molecular level, it has been suggested that cellular deficiencies in these brain regions may partly result from a decrease in the expression BDNF.

1.3.2.1 Dysregulation of the HPA axis

Stress is defined as an event or events that are interpreted as uncontrollable or threatening to an individual, and which elicit physiological and behavioural responses. Some of these responses are caused by activation of the HPA axis, a feedback loop by which signals from the brain trigger the release of hormones needed to respond to the stress. In response to stress, the hypothalamus releases corticotropin-releasing hormone (CRH). The CRH in turn causes the pituitary gland to release adrenocorticotropic hormone (ACTH) into the bloodstream, from which it enters the adrenal glands and causes them to secretre glucocorticoids (cortisol in humans, corticosterone in rats). Glucocorticoids also exerts a negative feedback effect to shut down the stress response after the threat has passed, acting upon the hypothalamus and causing it to stop producing CRH. This stress circuit affects systems throughout the body. Under stressful conditions, there is a strong increase in the glucocorticoids secretion achieved within 10 to 30 minutes (Paris et al., 1987). Glucocorticoids readily enter the brain and their actions are mediated by two receptors, the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR). In the rat brain, corticosterone binds to mineralocorticoid receptors (MRs) with a 10-fold higher affinity than to glucocorticoid receptors (GRs) (Reul and de Kloet, 1985; Reul et al., 1998). As a consequence, these MRs are close to saturated under basal resting conditions, while high corticosterone concentrations during stress occupy both MRs and GRs (Reul and de Kloet, 1985). MRs are predominantly localized in limbic brain regions (including the hippocampus) while GRs are widely distributed in the brain.

Glucocorticoids typically have adaptive effects in the short run. Acute, mild stress can have a positive effect in turns of enhanced learning and memory (Luine et al., 1996). It is also

found that low levels of glucocorticoids amplify LTP (de Kloet, 2004). Conversely, high levels or severe stress impair LTP (Kim and Diamond, 2002; de Kloet, 2004) and enhance LTD (Xu et al., 1997) in the hippocampus.

Prolonged stress may create abnormalities in the HPA axis, which include a hypersecretion of glucocorticoids. This is thought to be an important factor in the pathogenesis of depression (Holsboer, 2000; Ising et al., 2005). Treatment with SSRI`s normalizes the HPA axis, but the mechanisms behind are unknown. When treatment stops, the HPA axis abnormalities returns, indicating that treatment have to be continued to avoid recurrences of depression (Plotsky et al., 1998). The HPA axis regulates arousal, sleep, appetite and the capacity to experience and enjoy pleasure factors that also are known to be affected in depression.

There exist large individual differences in how to cope with stress, and it is expected that differences in coping styles reflects differences in individuals vulnerability to stress related disorders (Koolhaas et al., 2007). Coping strategies involves behavioural, physiological and neurobiological tools that helps to diminish the impact of the stressors. Proactive coping is associated with high sympathetic reactivity to stressors whereas the more passive or reactive coping style generally has a higher HPA axis reactivity (Koolhaas, 2008).

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1.3.2.2 Structural changes in the hippocampus and prefrontal cortex

The consequences of stress are different in different brain regions, for example are the effects in amygdala completely opposite to what is seen in the hippocampus and prefrontal cortex. Here, the focus will be on the two latter structures.

Since the hippocampal formation has the highest expression of GC receptors in the brain (de Kloet et al., 1998) it is especially vulnerable to increasing levels of glucocorticoids/stress. Several forms of stress lead to atrophy and retraction of apical dendrites in the CA3 region (Watanabe et al., 1992; McEwen, 1999), reduced extent of neurogenesis in the dentate gyrus (Gould et al., 1997; Gould et al., 1998), and reduced complexity of neuronal processes in the hippocampus (Alfarez et al., 2009). Since the hippocampus has afferents to, and regulates the function of, the prefrontal cortex, reduced hippocampal function could also have consequences for the prefrontal cortex. The hippocampus also innervates and regulates the nucleus accumbens and ventral tegmental area (VTA), and impaired hippocampal function might contribute to the symptoms of anhedonia

(Warner-Schmidt and Duman, 2006), since the nucleus accumbens is involved in the mechanisms of natural reward (Nestler and Carlezon, Jr., 2006). The hippocampus also has projections to the hypothalamus, and another important function of the hippocampus is its negative modulation of the HPA axis. Hippocampal dysfunction is thought to contribute to the dysfunction of the stress response that is seen in depression.

Chronic stress leads to regression of the apical dendrites of pyramidal cells in the medial prefrontal cortex of rats (Cook and Wellman, 2004). Chronic stress (Banasr et al., 2007) or exposure to glucocorticoids (Alonso, 2000) also results in a reduction in the proliferation of glia in the PFC. A reduction in the number of glia is also observed in depressed patients (Ongur et al., 1998; Rajkowska, 2000). Loss of glia is expected to have negative impact on the neuronal plasticity, as glia provides metabolic support for the neurons, in addition to having an important role in the synthesis and inactivation of glutamate. Stress and glucocorticoids lead to elevations in extracellular glutamate concentrations in the hippocampus and prefrontal cortex, which may contribute to the deleterious effects of stress in these brain regions (Lowy et al., 1993; Sapolsky, 2000).

Hippocampal volume has been found to be decreased after chronic stress in rats (Lee et al. 2009), as well as in depressed humans (Sheline et al., 1996; Campbell et al., 2004; Lee et al., 2009). Frequency of depressive episodes and the duration for which depression is untreated correlate with magnitude of reduction in hippocampal volume (MacQueen et al., 2003; Sheline et al., 2003). Reduced hippocampal volume in depression is most likely a result of depression rather than a cause. But a smaller hippocampus is thought to be a predisposing factor for, rather than a consequence of, post-traumatic stress disorder (Gilbertson et al., 2002).

Since hippocampus has a critical role in learning and memory, and the prefrontal cortex is important for working memory and executive function, the damaging effects of stress seen in these regions also affect these functions, in both animals (Sapolsky, 2003) and humans (Shors, 2006). Clinical studies have found that depressed patients also often have difficulties with concentration and attention, diminished cognitive flexibility, and deficits in explicit memory (Austin et al., 2001; Fossati et al., 2001; Fossati et al., 2002; Hammar et al., 2009).

Antidepressants protect against hippocampal volume loss in humans (Sheline et al., 2003) and reverse stress-induced atrophic changes in the hippocampus of animals (Czeh et al., 2001), thus supporting the hypothesis that the pathophysiology of stress-related disorders such as depression involves reductions in neuronal connectivity and that this effect is reversible by antidepressant treatment. Results suggest that antidepressant treatment increases synaptic

plasticity and connectivity in brain regions associated with mood disorders (Sairanen et al., 2007). In addition, most antidepressants increase hippocampal neurogenesis, which is also required for the behavioural response to antidepressants (Santarelli et al., 2003).

1.3.3 The neurotrophic hypothesis of depression

The damaging effects of stress to the hippocampus and prefrontal cortex can be explained at least in part by lack of neurotrophic support. BDNF is the most studied neurotrophic factor in this regard. As described previously, BDNF is a member of the neurotrophin family and plays an important role in the development and plasticity of the brain by promoting neurogenesis, synaptic plasticity and cell survival. Several lines of evidence also points out BDNF to have an important role in depression and in mediating the effects of antidepressants. These observations have led to the neurotrophic hypothesis of depression, which proposes that depression is caused by reduced levels of neurotrophic factors and subsequently decreased plasticity in critical neuronal networks, whereas enhancing this neuronal plasticity induces the antidepressant effect (Duman, 2004). The neurotrophic factors themselves do not control mood, but they act as necessary tools in the activity-dependent modulation of networks (Castren et al., 2007).

BDNF is highly regulated by different stimuli, including stress and antidepressant drugs. Both acute and chronic stress, as well as chronic exposure to corticosterone, has been shown to decrease BDNF mRNA and protein levels in the hippocampus of rats (Nibuya et al., 1995; Smith et al., 1995; Schaaf et al., 2000; Gronli et al., 2006; Jacobsen and Mork, 2006) and this reduction can be prevented by antidepressant drug treatment (Tsankova et al., 2006). Stress-induced downreglation of BDNF-signalling, and neurogenesis, as well as atrophy and death of neurons has been suggested to contribute to the reduction in hippocampal volume (Sapolsky, 1996; Sheline, 1996; Duman et al., 1999; Sapolsky, 2000).

Also, levels of brain and serum BDNF has been found to be significantly lower in untreated patients with depression compared with treated patients or healthy controls (Shimizu et al., 2003; Karege et al., 2005; Monteleone et al., 2008), while the BDNF levels are increased in patients receiving antidepressant treatment (Chen et al., 2001; Shimizu et al., 2003; Huang et al., 2008). As previously described, BDNF is strongly implicated in the regulation of hippocampal long-term potentiation (LTP) (Bramham and Messaoudi, 2005). Reduced hippocampal BDNF levels have furthermore been shown to impair memory

performance in a number of animal studies (Schaaf et al., 2001; Gorski et al., 2003; Monteggia et al., 2004). Low plasma BDNF levels have also been associated with memory impairment associated with depression (Grassi-Oliveira et al., 2008).

Interestingly, most antidepressant treatments increase BDNF synthesis and signaling in the hippocampus and prefrontal cortex (Castren, 2004; Castren and Rantamaki, 2010). In contrast, other classes of psychotrophic drugs, such as opiates or psychostimulants do not stimulate BDNF expression in these regions (Chlan-Fourney et al., 2002). Increased BDNF signalling in response to chronic antidepressants can reverse the stress-induced reduction of hippocampal function and synaptic connections (Nestler et al., 2002; Duman and Monteggia, 2006). BDNF-mediated signalling appears to be both sufficient and necessary for antidepressant action in rodents (Saarelainen et al., 2003).

It is thought that induction of BDNF expression in response to chronic antidepressant treatment is mediated by the cAMP-CREB pathway (Conti et al., 2002). Repeated, but not acute, antidepressant treatment upregulates, via β-adrenergic - or 5-HT receptors, the coupling of the stimulatory G protein (Gs) to adenylyl cyclase (AC), increases levels of cAMP-dependent protein kinase (PKA). PKA, Ca^{2+}-dependent protein kinases and also ERK can phosphorylate and activate CREB. CREB, which is an transcription factor, regulates expression of BDNF. The cAMP-CREB pathway has been found to be upregulated in response to antidepressant treatment (Nibuya et al., 1996; Tiraboschi et al., 2004) (Figure 5).

Further evidence for the neurotrophic hypothesis of depression are displayed through several findings which demonstrate signalling pathways involved in BDNF-regulated synaptic plasticity to be involved in stress, depression and antidepressant mechanisms. Chronic stress, when induced depressive-like behaviour in animal models, has been shown to decrease the activity and level of ERK and CREB in the hippocampus and prefrontal cortex (Gronli et al., 2006; Qi et al., 2008). The depressive-like behaviour and decreased activity of ERK and CREB was reversed by fluoxetine treatment (Qi et al. 2008).

In rodents, antidepressant effects in two depression models (the learned helplessness and forced swim test) have also been observed on direct infusion of BDNF into the hippocampus (Shirayama et al., 2002) and midbrain (Siuciak et al., 1997). Such effects were blocked on the conditional or inducible knockout of the gene encoding BDNF from forebrain regions or with expression of truncated TrkB receptors (Monteggia et al., 2004; Monteggia et al., 2007). An opposite role of BDNF is suggested in the ventral tegmental area-nucleus accumbens pathway, where over-expression of BDNF or truncated TrkB receptors induces depression- or antidepressant-like effects, respectively (Eisch et al., 2003).

The neurotrophic hypothesis of depression may explain the delayed clinical response to antidepressant drug, as it is thought that a clinical response depend on physical growth and reorgnaization in the brain, which are responses that are mediated by BDNF signalling (Castren, 2005). However, depression may not be triggered by deficits in BDNF alone, but may require impairments in multiple pathways. Elucidation of the molecular mechanisms, including signalling pathways and factors regulating gene expression, that underlie the actions of stress, depression and antidepressants could reveal targets for the development of novel, faster-acting antidepressant agents.

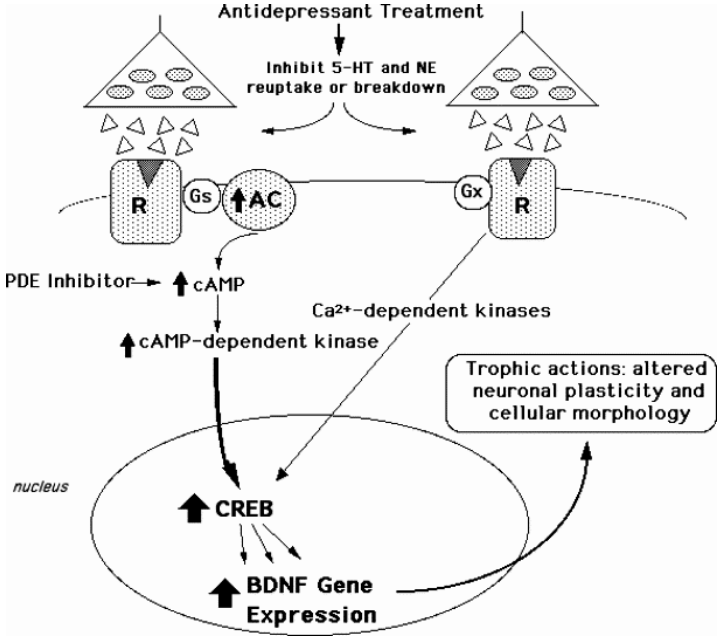

Figure 5 Regulation of the cAMP-CREB cascade and expression of BDNF by antidepressant treatment (From Duman et al., 2002).

1.3.4 Sleep in depression

Sleep disturbances is a common feature in patients with depression (Berger and Riemann, 1993), and it has been indicated to be an important aetiological factor in the development of the disorder (Lustberg and Reynolds, 2000; Srinivasan et al., 2009). As mentioned previously, altered sleep is also a part of the diagnostic criteria for depression. It has been suggested that abnormalities of circadian rhythms, which have been described in depressed patients might be a precipitating factor in the disorder (Souetre et al., 1989; Avery et al., 1999; Bunney and Bunney, 2000). However, the relationship between sleep disturbances and depression is not completely understood.

Up to 90% of depressed patients have complaints of insomnia, which involves difficulties in falling asleep, frequent awakenings during the night (sleep fragmentation), and early morning awakenings (Cajochen et al., 2000; Almeida and Pfaff, 2005). The internal sleep organization is also impaired, with a reduced latency to enter the first REM sleep episode, increased total percentage of REM sleep, a reduction of deep SWS (Benca et al., 1992). Since most antidepressant drugs inhibit REM sleep, it has been suggested that REM sleep pressure is enhanced in depressed patients (Vogel et al., 1990). A small group of depressed patients (6% to 29 %) show the opposite, having complaints of hypersomnia (Roberts et al., 2000).

Although most depressed patients have disturbed sleep, paradoxically, 50-60 % report improved mood following one night of sleep deprivation (Wu and Bunney, 1990; Riemann et al., 1999; Giedke and Schwarzler, 2002). The antidepressant effect of sleep deprivation is usually only transient, and in most cases relapse occurs after the first episode of recovery sleep (Southmayd et al., 1990). Therefore it is rarely used clinically. However, an understanding of the neurobiological mechanisms underlying the antidepressant effect of sleep deprivation should help to develop new rapidly acting antidepressant strategies.

2 Aims

Paper I

To determine whether BDNF regulates intitation and translation factor activity during BDNF-LTP in vivo and in isolated synapses from the adult dentate gyrus in vitro.

Paper II and III

To determine whether post-transcriptional mechanisms, mediated by translation factor and CPEB activity, contribute to the antidepressant (fluoxetine) action.

Paper IV

To examine the effect of stress and sleep deprivation on post-transcriptional regulation by translation factors and CPEB.

3 Methods

Detailed explanations of the methods are found in the “Materials and methods” section in the attached papers.

4 Summary and discussion of results

4.1 Paper I

Brain-derived neurotrophic factor (BDNF) is critically involved in long-lasting forms of synaptic plasticity which requires new protein synthesis. Previously, we have shown that local infusion of BDNF into the dentate gyrus of intact rats triggers LTP (BDNF-LTP), and that this depends on new gene expression and protein synthesis (Ying et al., 2002; Messaoudi et al., 2002; Messaoudi et al., 2007). Protein synthesis can be regulated at the level of initiation and elongation, and two of the key regulators of these events are eIF4E and eEF2. However, these two have never been studied together in the context of synaptic plasticity. In this study we investigated the regulation of these factors in response to exogenously applied BDNF in vivo, and also to isolate the synaptic effects of BDNF application to synapses in vitro.

LTP was induced in the dentate gyrus of anesthetized rats by local infusion of BDNF. The brains were collected at 15 min and 3 h after the end of infusion. CA1, CA3 and dentate gyrus were microdissected and homogenized. Western blotting was used to determine phosphorylation- and total level of the translation factors eIF4E and eEF2 in the homogenate. BDNF-LTP did not induce any significant effects in the CA1 or CA3 regions. In the dentate gyrus, there was increased phosphorylation of eIF4E, paralleled by increased levels of total eIF4E, 15 min after end of the infusion. At this timepoint there was also an increase in eEF2 phosphorylation, but total levels of eEF2 were unchanged. 3 h after end of infusion, phosphorylation levels of eIF4E and eEF2 and total levels of eIF4E had returned to control. The effects of BDNF-LTP on phosphorylation and total levels of eIF4E were confirmed and further localized by immunohistochemistry. Enhanced staining was observed in the granule cell layer of the BDNF-infused dentate gyrus, compared to the contralateral side. Infusion of cytochrome C, which has similar molecular weight and charge as BDNF, had no effect on the translation factors.

Previous studies from our laboratory have reported that BDNF-LTP requires activation of the ERK pathway (Ying et al., 2002; Messaoudi et al., 2002). It has also been shown that BDNF-induced signalling through this pathway regulates phosphorylation of eIF4E in vitro (Takei et al., 2001; Kelleher, III et al., 2004). We therefore examined a possible role of ERK activation in regulation of eIF4E and eEF2 during BDNF-LTP. The ERK inhibitor UO126 blocked BDNF-LTP and the increases in phosphorylation of eIF4E and eEF2 and total eIF4E.

Next, we wanted to examine the possibility of spatially different effects of BDNF on translation factor regulation. In order to investigate the effect of BDNF in dendritic spines, we used a synaptodendrosome (SD) preparation, a subcellular fraction containing pinched-off dendrites. SDs were fractionated from dentate gyrus of naïve rats, and treated with BDNF for 5, 15 and 30 min. After 5 min of BDNF-treatment there was a significant increase in eIF4E-phosphorylation, but no change in eEF2 phosphorylation. Paralleling this was an enhanced expression and phosphorylation of α-CaMKII, as well as enhanced expression of total eEF2. No significant changes were observed after 15 or 30 min treatment with BDNF.

Taken together, these findings demonstrate that induction of BDNF-LTP in vivo induces a transient phosphorylation of eIF4E and eEF2 that requires ERK signalling. The demonstration of ERK-dependent regulation of translation factor eIF4E and eEF2 during BDNF-LTP indicates that ERK has a central role in BDNF-induced synaptic plasticity. A coincident increase in phosphorylation of eIF4E and eEF2 indicates enhanced general protein synthesis. Phosphorylation of eIF4E at Ser209 is generally associated with increased rates of translation (Gingras et al. 2004). This phosphorylation reduces eIF4Es affinity for the 5′ cap, which promotes reinitiation on the same mRNA or on another message (Scheper and Proud 2002). eIF4E is phosphorylated by the mitogen-activated protein kinase-interacting kinase 1 (Mnk1), which is a substrate of ERK (Wang et al. 1998; Waskiewicz et al. 1999). The ERK pathway is required for BDNF-induced enhancement of protein synthesis (Kelleher et al. 2004). The elongation step is also a target for regulation, which can be mediated by phosphorylation of eEF2 (Browne and Proud, 2002). Phosphorylation of eEF2 at Thr56 inhibits its activity, which thereby leads to decreased elongation and decreased rates of general translation (Redpath and Proud, 1993). However, some mRNAs which are critically involved in long-lasting synaptic plasticity undergo enhanced translation under conditions of eEF2 phosphorylation and decreased general translation (Scheetz et al., 2000; Chotiner et al., 2003; Belelovsky et al., 2005; Park et al., 2008). When elongation becomes the rate-limiting step in translation, it has been suggested that eEF2 phosphorylation may selectively favour translation of poor initiators, or inefficiently translated mRNAs, such as Arc and α-CaMKII (Walden and Thach, 1986; Belelovsky et al., 2005).

In the present study we observed that in vivo induced BDNF-LTP is associated with a transient ERK-dependent phosphorylation of eEF2 in whole dentate gyrus. Surprisingly, BDNF stimulation of synaptodendrosomes did not alter eEF2 phosphorylation state, but led to increased eIF4E phosphorylation and enhanced ecpression of α-CaMKII. Thus, BDNF appears to affect translation in a compartment-specific manner, enhancing initiation at

synapses while promoting elongation arrest at non-synaptic sites. In contrast, NMDA treatment of synaptoneurosomes enhances eEF2 phosphorylation and suppresses general protein synthesis while increasing α-CaMKII expression (Scheetz et al. 2000). Further work is needed to clarify the role of eEF2 phosphorylation in different forms of synaptic plasticity.The enhanced expression of α-CaMKII following BDNF stimulation of synaptodendrosomes, as observed in the present study, can not be due to protein transport, but might result from the translation of dendritically localized α-CaMKII mRNAs. Previous studies have shown that BDNF can regulate the local synthesis of synaptic proteins (Kang and Schuman, 1996; Huber et al., 2000; Aakalu et al., 2001; Zhang and Poo, 2002; Yin et al., 2002). It has been suggested that BDNF regulates local translation through activation of the ERK- and PI3K pathways (Schratt et al., 2004). An illustration of neurotransmitter-coupled pathways that have been implicated in the control of dendritic protein synthesis is given in figure 6.

4.2 Paper II and III

Antidepressant drugs must be taken for several weeks until a therapeutic effect occur, even if these drugs raise the monoamine levels in the brain immediately. This suggests that increasing monoamine levels is not enough, but that a secondary, delayed response is what eventually mediates a mood-enhancing response. Since depression is characterized by impaired neuronal plasticity, it is suggested that antidepressants are able to normalize such effects, and that the delayed effect of antidepressants reflects the time required to produce neuroadaptive mechanisms that may enhance neuronal plasticity (Pittenger and Duman, 2008). Characterizing the mechanisms that underlies this delayed response might reveal new targets for more fast acting drugs. While most attention has been given to characterize the effects on gene transcription, possible effects on post-transcriptional regulation have received almost no attention. In paper II and III we investigated the effect of the selective serotonin reuptake inhibitor fluoxetine on post-transcriptional regulation. In paper II, the effect on two factors critically involved in the regulation of general translation, eIF4E and eEF2, was examined. In paper III, we examined the effect of fluoxetine on a RNA-binding protein that serves mRNA specific translation, CPEB1.

Rats received either one injection (acute treatment) or daily injections of fluoxetine for 21 days, before the dentate gyrus, hippocampus proper and prefrontal cortex were

microdissected and further analysed. Western blotting was used to determine the level of phosphorylation and total level of eIF4E, eEF2 and CPEB. While acute treatment with fluoxetine had no significant effects, chronic treatment induced region-specific changes in translation factor and CPEB phosphorylation state. In all three regions, chronic fluoxetine induced increased phosphorylation of eEF2 (paper II) and CPEB (paper III), while eIF4E was phosphorylated specifically in the dentate gyrus (paper II). In addition, in paper III we demonstrated that the increased activity of CPEB was sustained in the hippocampus proper up to one week after treatment. Expression of two other CPEB isoforms was also regulated in a region-specific manner in response to chronic fluoxetine; in the dentate gyrus, levels of CPEB3 were increased, whereas levels of CPEB4 were decreased. Conversely, in the prefrontal cortex, levels of CPEB3 were decreased, whereas levels of CPEB4 were increased one week after treatment was ended.

The impact of enhanced translation factor and CPEB activity in response to fluoxetine remains to be determined. Several studies have shown that antidepressant drugs produces secondary effects similar to those induced by neuronal plasticity, through increased synthesis and signalling of BDNF (Castren et al. 2004). Most antidepressant drugs increases the expression of BDNF. BDNF signalling is also suggested to be necessary for the effect of antidepressants, as these drugs fail to induce behavioural responses in mice with reduced BDNF levels or TrkB signalling (Saarelainen et al. 2003; Monteggia et al. 2004). Previously, we have shown that BDNF affects translational regulation through phosphorylation of eIF4E and eEF2 in the dentate gyrus, similar to what was seen after chronic fluoxetine. It is therefore tempting to consider that BDNF couples antidepressant treatment to enhanced translation factor activity. BDNF has been found to regulate translation through activation of the ERK- and PI3K-mTORC1 pathways (Schratt et al., 2004; Kelleher, III et al., 2004; Klann et al., 2004). Enhanced phosphorylation of eEF2 is associated with decreased rates of general translation (Redpath and Proud, 1993). In line with that, BDNF-induced dephosphorylation has been shown to increase the total rate of protein synthesis in cortical neurons (Inamura et al., 2005; Takei et al., 2009). However, other studies have shown that under conditions with enhanced eEF2 phosphorylation and decreased rate of general protein synthesis, some specific mRNAs undergo enhanced translation (Scheetz et al., 2000; Ying et al., 2002; Chotiner et al., 2003; Belelovsky et al., 2005; Kanhema et al., 2006). Thereby, increased phosphorylation of both eEF2 and eIF4E in response to chronic fluoxetine could favour translation of some mRNAs. The identity of these mRNAs and whether their protein products are involved in mediating an antidepressant response are issues that need to be addressed.

The enhanced phosphorylation of CPEB after chronic treatment with fluoxetine indicates that CPE-containing mRNAs might be involved in the antidepressant action. Many of CPEBs potential targets encode proteins that are important in mediating synaptic plasticity. Among these are BDNF and tPA, which are also implicated in antidepressant drug action. BDNF has been identified as a target as it contains both the CPE and hexanucleotide sequence, which are necessary for CPEB-induced polyadenylation. Importantly, no studies have demonstrated CPEB-mediated polyadenylation-induced translation of BDNF. tPA has been shown to be bound by CPEB and translated in the synaptic region in response to glutamate, dependent on metabotropic glutamate receptor (mGluR) activation (Shin et al., 2004). It remains to be examined whether CPEB regulates translation of these mRNAs in response to chronic fluoxetine. If so, CPEB might be an important factor linking chronic antidepressant treatment to upregulation of BDNF levels and signalling.

The dentate gyrus seems to have a particularly important role in the action of antidepressants. The ability to produce new granule cells throughout life (neurogenesis) is one of the unique features of this region. Chronic treatment with fluoxetine and several other antidepressant drugs has been shown to increase neurogenesis in the dentate gyrus (Malberg et al., 2000; Santarelli et al., 2003; Chen et al., 2006) which is also important for the behavioural effect of these drugs (Santarelli et al., 2003). BDNF signalling plays a role in the differentiation and survival of the newborn neurons (Lee et al., 2002; Sairanen et al., 2005; Bergami et al., 2008). In addition, BDNF specifically in the dentate gyrus (and not CA1) might be essential in mediating the therapeutic effect of antidepressants (Adachi et al., 2008). In the present study, localization of phosphorylated eIF4E is different after chronic fluoxetine treatment compared to what was observed after BDNF-LTP (Kanhema et al. 2006). While enhanced p-eIF4E was observed in the granule cell layer in the dentate gyrus after BDNF-LTP, increased staining was observed specifically in the subgranular zone after chronic fluoxetine. As the subgranular zone is where neurogenesis occurs, it might be that the increased staining with p-eIF4E was specific to antidepressant-induced new neurons.

One side-effect of intraperitoneal fluoxetine injections is skin irritations. This was observed in the present study and has also been reported by others (Fisher et al., 1999; Perrone et al., 2004). However, injection is a more reliable and controllable form of administration than oral delivery, but it is possible that the skin irritation is a confounding factor in studies of chronic intraperitoneal administration.

4.3 Paper IV

Chronic stress, which represents an important precipitating factor in depression, has shown to induce cellular and structural changes in the brain that might leads to dysfunctional synaptic plasticity. The molecular mechanisms underlying the response to stress are little understood.

In this study we examined the effects of chronic mild stress (CMS) and sleep deprivation (SD) in rats on translational regulation in the prefrontal cortex and hippocampal formation, brain regions that are strongly affected by stress (McEwen, 2001; Kim and Diamond, 2002; Pittenger and Duman, 2008). Four weeks of CMS treatment induced phosphorylation of eIF4E and eEF2 specifically in the prefrontal cortex; translation factor activity was unaltered in the hippocampus proper and dentate gyrus. Next we assessed the effects of 8 hours total SD, a treatment linked to rapid and transient relief from depression in humans, (Wu and Bunney, 1990). SD was given alone or following CMS (CMS+SD). Healthy rats subjected to SD alone exhibited enhanced phosphorylation of eEF2 in all brain regions, whereas eIF4E phosphorylation was elevated in prefrontal cortex but decreased in the dentate gyrus. When chronically stressed rats were sleep deprived (CMS+SD group), changes in translation factor phosphorylation in the dentate gyrus and hippocampus proper were similar to those induced by SD alone. No additional changes in eIF4E and eEF2 activity were detected in the prefrontal cortex in rats subjected to SD after CMS. CPEB phosphorylation state or total CPEB levels were unaffected, except of CPEB dephosphorylation in the hippocampus proper after SD. We also examined the expression of genes (Arc and BDNF) which are known to be upregulated during long-lasting forms of synaptic plasticity or chronic antidepressant treatment (Pei et al., 2003; Wibrand et al., 2006; Alme et al., 2007). In the prefrontal cortex, Arc and BDNF mRNA levels were increased following SD of both naïve and CMS treated rats. Levels of plasma corticosterone were elevated in a similar pattern. In the dentate gyrus and hippocampus proper, Arc mRNA levels increased only when treatments were combined (CMS+SD), accomponied by a decrease in BDNF mRNA expression in the dentate gyrus.

These findings demonstrate brain region-specific regulation of translation factor and CPEB phosphorylation state associated with CMS and with SD in naïve and CMS exposed rats. Prefrontal cortex was specifically affected by CMS, which induced increased phosphorylation of both eIF4E and eEF2 in this region. This change indicates enhanced translation. Phosphorylation of eIF4E is generally correlated with enhanced rates of translation (Scheper and Proud 2002; Senthil et al. 2002; Duncan et al. 2003). The

phosphorylation event decreases the affinity of eIF4E binding to the cap structure, which may promote scanning of the initiation complex to the startsite or facilitate recruitment of new complexes (ribosomal loading). Phosphorylation of eEF2 acts to slow the peptide chain elongation and inhibit general protein synthesis, while simultaneously promoting the translation of mRNAs that are poor initiators, typically mRNAs with extensive secondary structure in their 5´ leader sequence (Walden and Tchah 1986; Scheetz et al. 2000; Belelovsky et al. 2005). Thus, the parallel increase in eEF2 and eIF4E phosphorylation in the prefrontal cortex indicate enhanced translation in response to chronic stress. SD of naïve rats induced the same pattern of translation factor activity in the prefrontal cortex. This may indicate that prefrontal cortex is generally more sensitive to both acute and chronic stress, and the possible enhancement of translation may constitute a compensatory mechanism to the stress. In the dentate gyrus and hippocampus proper, translational control is selectively modulated by SD, and not affected by chronic stress. The dephosphorylation of eIF4E combined with enhanced phosphorylation of eEF2 in the dentate gyrus constitutes a double mechanism for decreasing overall protein synthesis. In the hippocampus proper, CPEB was dephosphorylated in response to SD in naïve rats, paralleled by increased eEF2 phosphorylation, which may indicate that sleep deprivation inhibits general protein synthesis in this region through decreasing elongation rate, as well as decreasing polyadenylation-dependent translation. Based on these findings we suggest that sleep deprivation selectively favours translation of certain mRNAs in the prefrontal cortex, while decreasing the capacity of general protein synthesis in the hippocampal formation. However, use of 2D gels and proteomic methods are needed to verify effects on protein synthesis.

When rats were exposed to sleep deprivation following CMS exposure, the responses in translation factor activity in the hippocampal formation were similar to those observed following sleep deprivation alone. This could indicate that this region is sensitive to sleep loss per se. However, the dephosphorylation of eIF4E in the dentate gyrus correlated with corticosterone levels, which indicate that at least this change was a response to stress. Since no change was observed following CMS, the hippocampal formation might have adapted to this prolonged stress. This is in contrast to prefrontal cortex, were similar responses in translation factor activity were observed following both CMS and sleep deprivation. Surprisingly, when sleep deprivation was given following CMS exposure, no additional response in translation factor activity was observed in the prefrontal cortex. This may indicate that chronic stress triggers a maximum translational response (a ceiling effect) in the prefrontal cortex that is not further enhanced by SD.

In this study we used a rat model for chronic stress based on subjecting rats to daily hassles. Exposure to the CMS protocol has been established and validated as an animal model of depression in our laboratory with e.g. less consumption of sucrose (anhedonic response) and sleep alterations (Gronli et al., 2004; Gronli et al., 2005). In the present study, rats exposed to CMS reduced their bodyweight, and showed sleep alterations typically observed in depressed patients (e.g. fragmented sleep, less deep sleep). However, no anhedonic behaviour (less preference for sucrose) in rats exposed to CMS was observed, mostly due to a high inter-individual variability. In addition, housing conditions might also be a factor that influenced the outcome. All rats were single-housed in individually ventilated cages, and could not smell each other. It has been demonstrated that isolated rats (single-housed) show a significant reduction in BDNF protein concentrations in the hippocampus compared to rats housed two per cage, and the authors suggested social isolation as a model for the study of antidepressant treatments (Scaccianoce et al., 2006).

CMS induced few changes in translation factor activity and mRNA expression. When these rats were exposed to sleep deprivation they had larger responses in eEF2 phosohorylation, although not significantly larger compared to sleep deprived naïve rats. However, a striking difference in mRNA expression was observed between CMS and naïve rats that were sleep deprived; Arc mRNA was upregulated in the dentate gyrus and hippocampus proper only after sleep deprivation of CMS rats, not after sleep deprivation of naive rats or after CMS alone. This effect was most prominent in the dentate gyrus, as there was a trend of increased Arc mRNA in the hippocampus proper following sleep deprivation alone. Another mRNA, TGF -beta-induced immediate early gene-1 (TIEG1), was also upregulated in a similar manner as Arc mRNA in both these regions (data not shown), significantly increased expression following sleep deprivation of CMS rats only. Coincident upregulation of Arc and TIEG1 mRNA has previously been shown in the dentate gyrus in response to both BDNF-LTP and HFS-LTP (Wibrand et al., 2006) and in the hippocampus proper in response to chronic, but not acute, treatment with the SSRI fluoxetine (Alme et al., 2006).

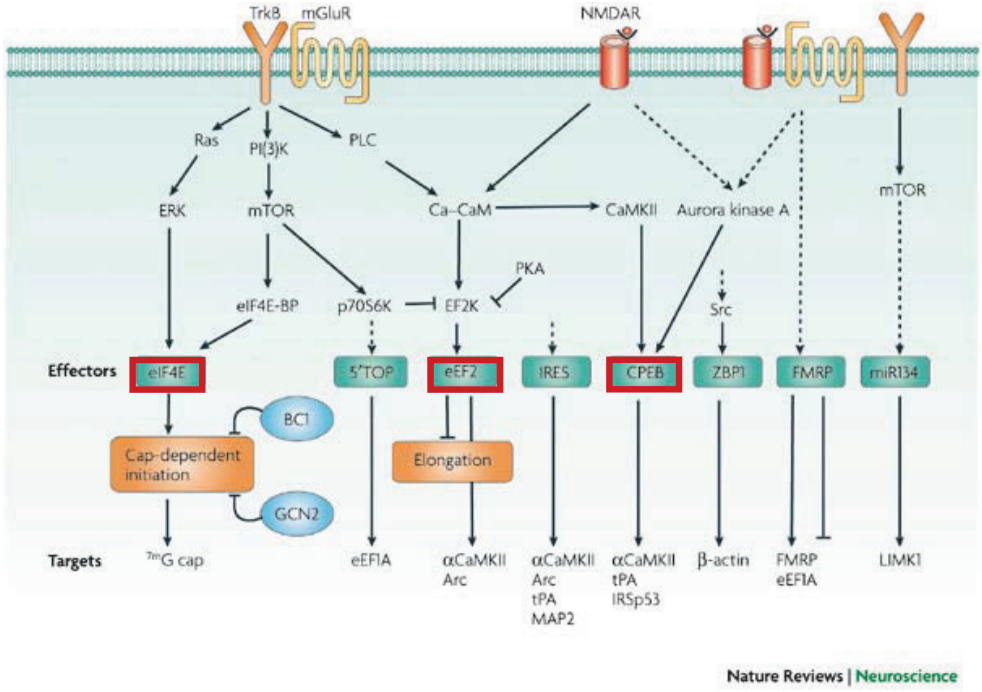

Figure 6 Illistration of neurotransmitter-coupled pathways that have been implicated in the control of protein synthesis at excitatory synapses in the mammalian brain (From Bramham and Wells, 2007).

5 Conclusions and future perspectives

Stress can induce molecular and cellular changes in the brain, and when stress is prolonged these changes may lead to dysfunctional synaptic plasticity. This is suggested to be a risk factor in the development of depression. Chronic treatment with antidepressant drugs is suggested to enhance synaptic plasticity, and thereby oppose the damaging effects of stress. The molecular mechanisms for how stress and antidepressants influence synaptic plasticity are not fully understood. Long-lasting synaptic plasticity is dependent on new protein synthesis, and regulation of translation plays a key role in regulation of synaptic plasticity.

In the present studies we examined the translational regulating mechanisms involved in a long-lasting form of synaptic plasticity, in response to acute and chronic antidepressant drug treatment, and in response to chronic stress and sleep deprivation, the latter which can act as an acute stressor, but which also has been shown to induce a rapid antidepressant effect.

In paper I we show that BDNF, when exogenously applied to induce LTP in the dentate gyrus, exerts rapid and transient modulation of both initiation and elongation as a general effect. This regulation is dependent on ERK signalling. BDNF-mediated regulation of translation is compartment-specific, as BDNF appears to selectively facilitate initiation at synapses. In paper II we show that chronic, but not acute, treatment with the antidepressant fluoxetine induces region-specific regulation of translation factor activity. In the dentate gyrus, regulation of initiation and elongation was similar to what we observed in response to BDNF-induced LTP in paper I. In paper III we demonstrate that chronic fluoxetine also affects other mechanisms of translational regulation, through phosphorylation of the RNA binding protein CPEB. From paper II and III we suggest that post-transcriptional regulation contributes to the delayed effects of antidepressant drugs. In paper IV we show that chronic stress affects translational regulation specifically in the prefrontal cortex, while having no effects in the hippocampus proper and dentate gyrus. Sleep deprivation has a more widespread effect on translational regulation; while elongation was similarly regulated in all regions, initiation was regulated in a region-specific manner.

Taken together, this work identifies translation as a common control point in the context of synaptic plasticity, stress, and antidepressant drug actions. This is important because it implies that stress mechanisms, like memory, may involve rapid synapse-specific control of protein synthesis. The functional impact of translational regulation associated with synaptic plasticity, stress and antidepressant treatment has to be elucidated. Identifying the subpopulations of mRNAs that are regulated, their localization, and the time-

course of the effects are the first steps to take. Ultimately, this could pave the way for the development of better antidepressants and cognition-enhancing drugs.

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