Addendum | Summary

SUMMARY

Schizophrenia is a serious developmental psychiatric disorder affecting approximately 1% of the general population. It is characterized by a wide variety of symptoms, divided in positive, negative and cognitive aspects. Some factors that may contribute to the development are obstetric events, chronic social adversity and drug abuse. It is recognized that these adverse environmental effects will only result in the development of schizophrenia if they are combined with genetic predisposition. Genetic make-up accounts for 50% of the risk of schizophrenia development.

Numerous animal models have been developed in order to mimic aspects of schizophrenia. One of these models is the amphetamine sensitization paradigm. When animals are treated with amphetamine for several days and are injected again after a withdrawal period with a so-called challenge injection of the stimulant, this results in a sensitized state of the animal. This sensitized state has some similarities to the human situation of schizophrenia, reflected in a hyperactive dopamine system and the ability of dopamine antagonists to block this expression of sensitization.

Important brain areas playing a role in this sensitized state are the nucleus accumbens (NAc), the prefrontal cortex (PFC) and the hippocampus that all receive direct dopaminergic input from the ventral tegmental area. In **Chapter 2** we studied gene expression differences in these three dopaminergic brain areas in genetically identical DBA2/j mice that differ in their response to the amphetamine sensitization paradigm. The goal was to study the genes that might be responsible for the differences in sensitivity in order to identify potential psychosis susceptibility genes. We showed that of three brain areas, the CA1 area of the hippocampus harbored the most robust differences in gene expression between low or high responders to the amphetamine sensitization paradigm. Moreover, we found that the differentially expressed genes were common target genes of the Glucocorticoid Receptor (GR) and Mycocyte Enhancer Factor 2 (MEF2), suggesting a role for both transcription factors in generating the divergent phenotypes. The hippocampus is directly involved in inhibiting amphetamine-induced sensitivity, probably by controlling NAc neuronal activity and interfering with the firing of dopaminergic neurons form the ventral tegmental area. Furthermore, GR is highly expressed in the hippocampus and glucocorticoids are known to modulate amphetamine sensitivity.

At the time no reports existed on a possible interaction between GR and MEF2. **Chapter 3** therefore focused on studying whether such an interaction exists *in vitro* in neuronally differentiated PC-12 cells. Treating cells with the GR agonist dexamethasone (DEX), resulted in phosphorylation of MEF2

at serine 408, a modification known to convert MEF2 into a transcriptional repressor. Moreover, MEF2-DNA binding upstream of the MEF2 target gene c-JUN, was significantly enhanced by DEX. This suggests a state of active transcriptional repression which correlated with the downregulation of c-JUN. Finally, we showed that downregulation of MEF2 by means of lentiviral knockdown, resulted in inhibition of c-JUN expression to a similar extent as normally seen after DEX treatment, which could not be further reduced by DEX. These results suggest that GR-regulated downregulation of c-JUN is mediated by MEF2.

GR and MEF2 transcription factors are known to play an important role in synaptic plasticity, which is a crucial hallmark of psychostimulant sensitization. Neuronal depolarization is an important prerequisite to demonstrate synaptic plasticity and MEF2 is known to be activated in a depolarizing environment. In **Chapter 4** a study is described in which we examined how GR regulates MEF2 activity when neuronally differentiated PC-12 cells are depolarized.The results showed that expression of the MEF2 target gene NR4A1, which is known to be affected by depolarization, was attenuated when depolarization was combined with GR activation. Surprisingly, while GR activation or depolarization alone resulted in enhanced MEF2-DNA binding upstream of NR4A1, MEF2-DNA binding was completely reduced to vehicle levels when DEX was administered within a depolarized environment. The results demonstrate that glucocorticoids acting via GR can maintain the balance in depolarization-induced synaptic plasticity.

In Chapter 5 the previous results were used to design a study *in vivo* to ask the question whether phosphorylation of MEF2 in the hippocampus and striatum is modified by amphetamine sensitization and at what time point in the sensitization process this may happen. The results showed that, although locomotor activity is enhanced by amphetamine at all-time points measured, MEF2 phosphorylation is increased only after 5 consecutive days of amphetamine treatment. This effect was observed in the hippocampus but not in the striatum, where amphetamine treatment did not have any effect on MEF2 phosphorylation. We subsequently tried to manipulate MEF2 phosphorylation *in vivo* in order to study its effect on locomotor activity. For this purpose we used roscovitine, which is an inhibitor of the kinase CDK5, that is known for its effect on MEF2 phosphorylation as well as on amphetamine sensitization. Roscovitine administered intracerebroventricularly enhanced amphetamine-induced locomotor activity while it increased phosphorylation in the hippocampus. The results suggest that MEF2 phosphorylation in the hippocampus is transiently modulated by amphetamine treatment and that roscovitine-induced changes in locomotor activity correlate with enhanced MEF2 phosphorylation. We therefore propose

that MEF2 activity may play an important role in the process of psychostimulant sensitivity and psychosis susceptibility.

The results that were obtained in this thesis precipitated a model (Figure 7.2) in which stress and the subsequent rise in circulating glucocorticoid concentration activate GR and modulate the activity of MEF2. This altered MEF2 activity causes a change in the expression of its target genes that play a role in synaptic plasticity. Regulation of MEF2 is proposed as a new mechanism via which glucocorticoids via GR regulate synaptic plasticity. We suggest that this mechanism is implicated in behavioral sensitization. Interestingly, the *in* vivo results are only observed in the hippocampus making this brain area an exciting target for further studies to understand its involvement in behavioral sensitization, in particular with respect to the role MEF2 plays in this process. In conclusion, we have identified a GR-mediated pathway influencing MEF2 activity, that might give new insight in the mechanism of psychostimulant sensitization and psychosis susceptibility.