Gene expression profiling of morphine-induced neuronal adaptations
Siard Houtzager

In this thesis we investigated the molecular changes underlying morphine-induced long-lasting behavioral sensitization.

Daily morphine injections will induce a sensitization towards morphine, which can be measured long after the last injection. It is suggested that a ‘drug-memory’ has been established in the rat brain after repeated exposure. This ‘drug-memory’ is believed to play a crucial role in the addition process and to be the major drive for relapse of addictive behavior.

Neuronal substrate
The mesolimbic dopamine system plays an important role in the addiction process. Different addictive substances influence the dopamine neurotransmission in this system. Cocaine and amphetamine act directly on the dopamine neurotransmission, whereas morphine and heroin act indirect via the μ-opiate receptor present on the different neurons in the mesolimbic dopamine system. Activation of the μ-opiate receptor will lead to an increased dopamine release in an important nucleus in the mesolimbic dopamine system; the nucleus accumbens in the striatum. Due to the central role of the nucleus accumbens in the dopamine system, we investigated the molecular changes in the nucleus accumbens after morphine exposure. We have studied this by measuring gene expression of a large number of genes during development of sensitization to morphine. In particular, temporal gene expression measurements, as performed in this thesis, may indicate the type and timing of molecular and cellular responses (in casu the development and maintenance of a morphine-induced sensitized state).

In chapter 2 we have used an open screening method in order to search for transcripts regulated in the nucleus accumbens long-term (3 weeks) after repeated morphine exposure. Using the ‘differential display PCR’ technique, we aimed to reveal genes, which through persistent alteration in expression, might contribute to the long-lasting neuronal plasticity that is necessary for sensitization to occur. Although this technique generates a large number of false positives, we have identified a differentially expressed transcript coding for b-hemoglobin, a protein known to be present in erythrocytes. We show that it is also expressed in non-neuronal cells surrounding bloodvessels. Although the change in expression might play a role in the long-lasting changes in neuronal transmission, it is more likely that there is a change in blood-circulation in morphine treated animals, a phenomenon also seen in addictive humans.

In chapter 3 and 4 we have taken a different approach. We have investigated the dynamics of gene expression of a limited set of transcripts (160 genes) using quantitative PCR’. This approach aimed to reveal the contribution of particular types of responses over time, by choosing genes that might be markers for certain physiological processes.

First we measured the gene expression during the development of sensitization; at different days during the exposure to morphine (at 1, 2, 4 8 and 14 days) and during
the morphine abstinence period (1, 2, 4, 8, 12 and 18 days after the last morphine injection). We show that both exposure to morphine and subsequent abstinence from the drug induces distinct phase-specific temporal gene expression of functional groups of genes. In particular, we show that withdrawal of morphine is a new stimulus in terms of gene expression, and that this genomic response is long-lasting, with changes in gene expression evident even after a drug-free period of 2-3 weeks. Based on these results we could formulate a new hypothesis about the enduring drug-adapted state of the accumbal gene network.

In chapter 4 we have investigated gene expression profiles in the nucleus accumbens in morphine sensitive rats, which are re-exposed to morphine; compared with rats that are exposed to morphine for the first time. We have shown that the gene expression response of ‘morphine pretreated’ animals differs from the gene expression profiles of the morphine naive animals. This indicates that existing gene trajectories, and hence the wiring rules of the genomic (and maybe the cellular) network, show long-term adaptation and are prone to drug exposure. Morphine-induced long-term neuroadaptation leads to profoundly altered genomic reactivity at renewed drug exposure that may well subserve the maintenance of the drug-adapted state and concomitant behavioral sensitization.

Taken together, we observed that one or more of the four mechanisms (i.e. pre- and postsynaptic modification, glial regulation of neurotransmission, and rewiring of neuronal connections) proposed could play (simultaneously) a role in the development of drug-induced behavioral changes, among which is behavioral sensitization. We find ample evidence for long-term regulation of gene expression during and also after exposure to morphine. We find indications for molecular adaptation at various levels of integration in the system. These changes in gene expression result in neuroadaptation of the system, which can be observed by its typical sensitized behavioral response to re-exposure of the drug, as well as in terms of an adapted activity of gene expression. Future experiments will have to determine how particular changes observed contribute to the change in physiology of the system. Finally, based on the knowledge gained, new keys for therapies in the treatment of addiction might be developed.