English summary

*Molecular mechanisms controlling relapse to drug seeking in rodent models of addiction*

Drug addiction is a psychiatric disorder that is associated with the loss of control over the intake of drugs of abuse. This disorder is a major source of personal suffering and societal problems. Currently, a limited number of addiction therapies are available, but these only have minor preventive effects on relapse to the use of drugs of abuse.

During the last decades, brain imaging studies in humans have provided insight into the brain circuitry that is involved in the development of addiction and drug craving. In parallel, animal models have been developed to study the neurobiology of addiction in more detail and to test potential therapies. Repeated exposure to drugs induces adaptations in the brain that persist even after extended abstinence. These neuroadaptations are thought to underlie the chronically relapsing nature of drug addiction. It follows that a better understanding of the molecular mechanisms controlling relapse might contribute to the development of more efficient addiction therapies. The research described in this thesis makes use of different animal models of addiction to increase our knowledge of these mechanisms.

Chapter 2 describes the role of the extracellular matrix (ECM) in relapse to heroin seeking in rats. After heroin self-administration and a subsequent period of extinction or abstinence, the synaptic levels of the matrix proteins brevican and tenascin-R were reduced in the medial prefrontal cortex (mPFC), a brain area executing higher cognitive functions, of which the involvement in relapse has been demonstrated in humans and animals. Also in the nucleus accumbens, a brain area with an important role in reward and motivated behavior, these protein levels were reduced after extinction. Subsequently, relapse was induced by presenting the audiovisual stimuli that were paired with injections of heroin during the self-administration phase (“cue-induced relapse”). During relapse, ECM protein levels were partially normalized. Furthermore, heroin seeking could be attenuated by normalizing these protein levels before the relapse test. This indicates that reduced ECM levels might underlie a higher vulnerability for addictive behavior.

The role of the ECM component brevican is further explored in chapter 3. To do this, the formation of an appetitive cocaine memory was investigated in a mouse that expresses reduced levels of brevican (the *Bcan*<sup>−/−</sup> mouse) using the conditioned place preference paradigm. *Bcan*<sup>−/−</sup> mice showed a stronger preference for the compartment that was paired with injections of cocaine than wild-type mice. Increasing the expression level of brevican in the dorsal hippocampus, by local infusion of a viral vector with the *Bcan* gene, reduced the conditioned cocaine memory. The dorsal hippocampus is a brain area that is important for contextual memory. These findings indicate that reduced brevican levels in the dorsal hippocampus underlie a higher sensitivity for the rewarding effects of cocaine.

These studies show an important role of the ECM, and specifically brevican, in regulating addictive behavior. This is consistent with available literature that shows that drugs affect the expression or activity of ECM components, as well as the proteases that mediate their de-
Grade. Lower levels of ECM might result in increased plasticity in the brain circuitry that is involved in addiction, and by this contribute to the formation of drug-induced neuroadaptations and the persistent vulnerability to relapse. Compounds that can modulate ECM dynamics might therefore be employed to prevent relapse in the future.

Whereas our knowledge of the neurobiology of relapse to cocaine and heroin seeking is increasing, little is currently known about relapse to nicotine, the neuro-active component in tobacco. To gain insight into the acute changes that take place in the mPFC during relapse to nicotine seeking in rats, the relapse-associated regulation of proteins that are known to have an important role in synaptic plasticity was explored in chapter 4. Cue-induced relapse, after nicotine self-administration and extinction, was associated with an increase in the levels of the α1 and γ2 subunits of the GABA_A receptor, but not of subunits of glutamate receptors. Blocking membrane insertion of GABA_A receptors in the dorsal, but not the ventral, mPFC, resulted in augmented responding during relapse. In contrast, nicotine seeking was attenuated after infusion of the GABA_A receptor agonist muscimol in the dorsal or ventral mPFC. This study shows that cue-induced relapse to nicotine seeking is paralleled by acute GABAergic plasticity in the mPFC. From the observation that relapse in rats can be modulated by intervening at the level of GABA_A receptors, it can be concluded that these receptors are potential targets for the development of new, more effective therapies to support smoking cessation.

Chapter 5 describes a proteomics study that explores nicotine relapse-associated acute protein regulation in synaptic membranes of the mPFC and the insular cortex. The insula is a brain area that is involved in the monitoring of interoceptive states and is activated during nicotine cravings in humans. Whereas relapse was not paralleled by molecular changes in the insula, respectively 3 and 51 proteins were regulated during relapse in the mPFC of rats that underwent a period of extinction or abstinence after nicotine self-administration, respectively. A reduced level of the protein Src homology 2 domain-containing protein tyrosine phosphatase substrate-1 (SHPS-1), a transmembrane protein involved in intercellular communication, was validated in an independent group of animals that relapsed after extinction training. These experiments show that the mPFC is subject to acute plasticity during relapse to nicotine seeking, and that SHPS-1 is an interesting candidate for future investigations of the molecular mechanisms that regulate relapse to nicotine seeking.

From the literature and the research described in this thesis it follows that relapse to different drugs of abuse, such as nicotine, heroin and cocaine, is partially regulated by overlapping neural substrates. For example, the activity of and plasticity in the glutamatergic projections from the mPFC to the nucleus accumbens have a central role in regulating relapse to these drugs. However, it can also be concluded from this thesis that relapse is regulated by molecular mechanisms that are specific for different drugs of abuse. This indicates that, with the knowledge of the molecular mechanisms that regulate relapse to drug seeking, it should be possible to develop general, as well as drug-specific, addiction therapies.