Chapter 7

GENERAL DISCUSSION
SUMMARY AND CONCLUSIONS

The studies presented in this thesis were aimed to investigate the role of individual variation in the consequences of MDMA treatment. Chapter 2 addressed the question to what extent vulnerability to MDMA induced 5-HT depletion would depend on baseline, trait like differences in monoaminergic neurotransmission. The study used SAL and LAL mice, known to differ in their monoamine functioning. Both lines did not differ in dopamine and serotonin depletion three days after MDMA treatment, however SAL and LAL mice were found to be differential vulnerable for the long-term MDMA-induced serotonergic depletion. Chapter 3 investigated whether gender is an important factor causing variability. It was shown that male rats are more vulnerable for both the acute and the long-term consequences of MDMA treatment than females. Males showed a stronger acute hyperthermic response and had a higher lethality rate than females. Furthermore, males showed a long-lasting increase in body temperature. No gender difference in long-term serotonin depletion was observed. Male rats were found to have a long-term decrease in brain 5-HIAA tissue concentrations, however interpretation with regard to serotonergic neurotoxicity remains difficult at present. Chapter 4 addressed the question whether individuals differ in the behavioral consequences of MDMA. Aggressive behavior was used as a strongly serotonin-mediated behavior. On average, MDMA treatment did not result in long-term changes in aggressive behavior. However, when the initial individual differences in aggressive behavior and the underlying mechanisms of serotonergic homeostasis were taken into account, it was found that only rats that initially expressed low levels of aggression showed an increase in aggressive behavior three weeks after MDMA treatment. This individual
difference in behavioral response was not reflected in the MDMA-induced long-term serotonergic neurotoxicity as measured by quantification of the number 5-HT fibers in the prefrontal cortex and hippocampus. MDMA is generally considered to exert its action through the serotonin transporter (SERT). Genetic polymorphisms of the SERT gene in primates are known to be related to a wide variety of affective diseases and aggression. Therefore, Chapter 5, we used a SERT knockout rat model to test the involvement of the serotonin transporter in the long-term effects of MDMA. MDMA treatment resulted in serotonergic depletion in both SERT⁻/⁻ and SERT⁺/⁺ rats, however the reduction was stronger in SERT⁺/⁺ rats. This indicates that the MDMA-induced long-term serotonin depletion is not completely dependent on its action through SERT. Furthermore, the MDMA-induced hyperthermic response was partially mediated by serotonin release, suggesting that also in rats that do not express SERT, serotonin is released after MDMA treatment. The long-term consequences of MDMA on serotonin are known to depend on a complex interaction between environmental temperature and core body temperature. Chapter 6 explored in detail the role of temperature on the long-term consequences of MDMA use. Centrally applied MDMA itself did not induce 5-HT depletion in conditions where peripherally applied MDMA would induce a lasting reduction in 5-HT tissue concentrations. The difference between centrally and peripherally administered MDMA is the absence of hyperthermia after central administration. Under conditions of hyperthermia induced by high ambient temperature, MDMA administered directly into the brain can induce long-term serotonin depletion. Surprisingly, MDMA metabolites were detected in the brain after central MDMA administration. This supports the theory that metabolites of MDMA play a role in the serotonin depleting process.
Regarding the main aim of this thesis on individual vulnerability to the long-term consequences of MDMA use, the experiments show the importance of baseline differences in monoaminergic neurotransmitter systems, coping style, gender and the serotonin transporter as intrinsic factors and temperature as extrinsic factor.

DOES MDMA HAVE NEURODEGENERATIVE POTENCY?

Numerous preclinical reports have been published regarding the long-term adverse effects of MDMA on the serotonergic system. These studies generally demonstrate that MDMA administration results in persistent reductions of markers of the serotonergic system (see general introduction section) in various brain areas. Despite the abundance of evidence showing that MDMA causes serotonergic depletion, it remains uncertain whether MDMA causes serotonergic neuron degeneration. In fact, there is a paucity of studies that examined the effects of MDMA on established markers of neuronal damage. Neurotoxicity in its strict sense is characterized by cell death and glial cell hypertrophy. It seems unlikely that MDMA induces death of serotonergic cells, since evidence has shown that despite profound loss of serotonin in forebrain projection areas (O'Hearn et al., 1988), MDMA administration has no effect on serotonergic cell bodies in the dorsal raphe nuclei, neither in number nor in morphology. Intra-raphe infusion of MDMA did not result in a long-term decrease of serotonergic markers (Paris & Cunningham, 1992).

Nevertheless, several studies indicate that administration of MDMA can cause neuronal damage as indicated by sensitive silver staining methods (i.e.
Fink-Heimer method) and Fluoro-Jade B (Commins et al., 1987; Schmued, 2003). However, these methods do not discriminate between different neurotransmitters. Therefore it remains uncertain whether the damaged cells identified by the two staining methods are serotonergic. Another approach used to demonstrate neuronal damage is by measuring glial activity. As a response to structural neuronal damage, astrocytic reactivity is known to be enhanced. This enhanced reactivity can be demonstrated by the expression of glial fibrillary acidic protein (GFAP). GFAP can thus function as a marker for neuronal degeneration (O'Callaghan & Sriram, 2005). Studies investigating the expression of glial fibrillary acidic protein (GFAP) after MDMA administration sometimes do, but also do not show an increase in GFAP expression (Aguirre et al., 1999; Pubill et al., 2003; Wang et al., 2004).

All together, the majority of the data indicates that MDMA in doses that cause significant serotonin depletion does not induce clear signs of serotonergic neuronal damage. Hence, it seems that although brain 5-HT levels are strongly depleted after MDMA, serotonin axons and nerve terminals remain intact. This would suggest that MDMA-induced deficits in serotonergic systems are due to adaptive changes in gene expression or protein function, and these changes reflect a state of metabolic exhaustion, rather than neurotoxic damage. Indeed, some studies indicate that the initial serotonin depletion can be (partially) restored over time (Hatzidimitriou et al., 1999; Stone et al., 1987c). Hence, in view of the long term reduction in serotonin consistently found in various brain areas, it can safely be concluded that MDMA has lasting effects on serotonergic signalling. Whether MDMA actually induces long-term structural damage or otherwise irreversible changes in the 5-HT neuron, as opposed to a reversible down
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regulation of the mechanisms involved in serotonergic neuron homeostasis remains to be investigated.

BEHAVIORAL CHANGES AFTER MDMA INDUCED 5-HT DEPLETION

Regardless of the question whether MDMA has neurotoxic potency, MDMA treatment results in long-term changes in several serotonergic markers. Remarkably, studies that have investigated the long-term behavioral changes after MDMA treatment fail to show long-lasting effects. The lack of long-term changes in behavior after MDMA treatment might be explained by the plasticity and redundancy of the serotonergic system. In most studies MDMA-induced changes (for example depletion in serotonin tissue concentrations) in the serotonergic system have been investigated in baseline conditions. It is important to notice that these measurements do not necessarily reflect the potency of a neuron to release serotonin. Serotonin release and the binding of extracellular serotonin to serotonin receptors are important in mediating behavioral output and are only distantly related to serotonin tissue concentrations. It can be hypothesized that only when the capacity of the neuron to release serotonin is under pressure, behavioral changes will occur.

In this respect, female rats (chapter 3) that seem to have increased serotonin turnover (that is thought to reflect increased, compensatory 5-HT release) might be more vulnerable to behavioral changes in situations of pressure on the serotonergic system, for example under conditions of chronic stress.
Individual variation in general may be another reason for the absence of clear long-term behavioral effects of MDMA treatment. The importance of individual variability was demonstrated in chapter 4, where only low aggressive male rats showed an increase in aggression after MDMA. This result is consistent with observations in humans indicating that only specific subpopulations might show long term behavioral effects of MDMA.

**HOW DO THE CURRENT RESULTS TRANSLATE TO HUMANS?**

Examining the effects of MDMA in animals enables researchers to overcome some of the limitations inherent in human studies. For example, acute neurochemical changes and long term serotonergic impairments after MDMA administration are well reported in animals, but to obtain corresponding data in humans is very problematic due to invasive nature of these analyses and ethical complications. Also, research in laboratory animals provides the opportunity to control the amount and quality of the drug, the environment of MDMA administration and the history of drug use in contrast to research in humans. However, animal studies present their own limitations making the translation of data from animals to humans sometimes difficult.

Acutely, MDMA has been shown to induce similar physiological and behavioral effects in rats and humans, which include cardiovascular, body temperature and locomotor responses. Comparing humans and rats for their thermoregulatory abilities, humans seem to be better at coping with overheating. In rats the MDMA-induced hyperthermia depends on the ambient temperature, whereas in humans this does not seem to be the case.
(Freedman et al., 2005). On the other hand, in rats there is a strong relationship between the serotonin depletion and the height of the hyperthermic response. In humans it is not known whether this relationship exists. In view of the importance of body temperature in the long term serotonin depletion explored in chapter 6, it seems important that further studies should address this issue in humans as well.

An argument that is often used in the discussion of the translation of animal experimental data to humans concerns the difference in dose-response relationships. Indeed, the doses of MDMA administered in animal experiments are usually much higher than those used by humans. One cannot make direct comparisons between results obtained in animals and human studies especially since small mammals tend to eliminated drugs at a faster rate than large animals. To account for differences in dosage, interspecies scaling has been used. With the formula \( \text{Dose}_{\text{human}} = \text{Dose}_{\text{animal}} \times (\frac{\text{BW}_{\text{human}}}{\text{BW}_{\text{animal}}})^{0.7} \) dosages can be translated between species (McCann & Ricaurte, 2000). However, the validity of interspecies dose scaling in relation to MDMA has been questioned by Vollenweider (Vollenweider et al., 2001), who argued that allometric interspecies scaling methods may not apply to MDMA neurotoxicity. The argument is based on the strong differences in MDMA pharmacokinetics between rats and humans (de la Torre & Farre, 2004) and consequently the synthesis of potential neurotoxic MDMA metabolites.

At present, the molecular mechanisms underlying the MDMA-induced serotonergic depleting effects are not completely known. Importantly, in both rats and humans MDMA seems to induce mainly long-term effects on the serotonergic system. In view of the importance of the serotonergic system in emotional processes and mood, a close collaboration between
human and animal research is required to be able to reveal the process of MDMA-induced serotonergic depletion and its long-term consequences.

CONSEQUENCES OF MDMA: WILL THE FUTURE BRING THE PROBLEMS?

MDMA has been shown to induce long-lasting, possibly permanent depletion of serotonin. The present thesis has demonstrated that severity of the MDMA-induced serotonin depletion and the behavioral changes can vary between individuals. The observed individual differences seem to be related to variation in intrinsic factors and environmental conditions. Extensive evidence has implicated a deficit in serotonergic neurotransmission in the development of major depression. It can be suggested that individual variation in changes in the serotonergic system (e.g. individual variation in MDMA-induced serotonin depletion) at a certain point in life might lead to an increased vulnerability in serotonin-related mood disorders like depression for some but not all individuals.

McEwen has introduced the concept of allostasis and allostatic load (McEwen, 2003) that seems to be relevant in the present context. In this concept, the physiological adaptation of an individual to challenges of daily life is referred to as allostasis. Allostatic load is defined as the wear and tear (daily experiences, lifestyle and major life stressors) throughout life that interacts with the individual’s genetic constitution and predisposing early life experiences and is therefore also referred to as the cost of allostasis.

The MDMA-induced long-lasting serotonin depletion can be hypothesized to have long-term physiological costs. To compensate for the severe loss of serotonin, the serotonergic system might adapt to the situation by increasing
for example post-synaptic receptor sensitivity, structural regeneration, enhancing neurotransmitter synthesis, and decreased neurotransmitter release.

Adaptations like this might be considered as allostatic load and increase the vulnerability for serotonin-related mood disorders like depression.

Regarding the possible influence of individual variability, it can be hypothesized that individuals might differ in their capacity to adapt to the MDMA-induced serotonergic depletion, implicating that MDMA abuse might result in an increased allostatic load for some but not for all individuals.

Another factor that contributes to an increase in allostatic load of the serotonergic system is aging. Serotonin receptors are known to be denser at birth than in the mature brain. Several post mortem human studies have reported a reduction in the number of cortical 5-HT₁A, 5-HT₁B/D, and 5-HT₂A binding sites with age in frontal lobe, occipital lobe, and hippocampus (Arranz et al., 1993; Gross-Isseroff et al., 1990; Marcusson et al., 1984a; Marcusson et al., 1984b; Shih & Young, 1978). This line of reasoning implies that a history of ecstasy use might lead to an increased vulnerability for serotonin related disorders during aging in particular. The urgency to investigate the possible negative consequences of MDMA (ab)use during aging in both clinical and preclinical models is indicated by the fact that the first people using MDMA during the mid 1980’s are around 50 years of age at present, increases the necessity to investigate the long-term consequences of MDMA abuse during aging in preclinical models.
REFERENCE LIST