Long-term neuronal damage and recovery after a single dose of MDMA: expression and distribution of serotonin transporter in the rat brain

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"Ecstasy", 3,4-methylenedioxymethamphetamine (MDMA), an amphetamine analogue is one of the most widely used recreational drugs. In spite of the fact that neurotoxic effects of MDMA has been found in several species from rodents to non-human primates, and results increasingly point to damage also in human MDMA users, data about the sensitivity of different brain areas and the recovery after neuronal damage are scarce. Serotonin transporter (5-HTT) mRNA in the raphe nuclei also has not been examined. Humans with genetic predisposition for the slow metabolism of MDMA, the so-called "poor metabolizers" of debrisoquin are at higher risk. Five- 9% of the Caucasian population is considered to carry this phenotype. These studies were carried out in Dark Agouti rats, a special strain that show decreased microsomal CYP2D1 isoenzyme activity, and thus may serve as a model of vulnerable human users. These works were designed to characterize MDMA-induced damage and recovery of the serotonergic system including sleep and morphological changes within 180 days. In our experiments we investigated the 5-HTT mRNA expression in the brainstem and medullary raphe nuclei, 5-HTT immunoreactive (IR) fibre densities in several brain areas, and 16 functional measures of sleep in response to a single dose of +/- MDMA (15mg\kg). Furthermore, behavioural experiments were performed 21 days after MDMA treatment. We found similar changes in 5-HTT mRNA expression in the examined raphe nuclei, namely transient increases 7 days after MDMA treatment followed by transient decreases at 21 days. Significant (20–40%), widespread reductions in 5-HTT-IR fibre density were detected in most brain areas at 7 and 21 days after MDMA administration. All cortical, but only some brainstem areas were damaged. Parallel to the neuronal damage we observed significant reductions in rapid eye movement (REM) sleep latency, increased fragmentation of sleep and increases in delta power spectra in non-REM sleep. At 180 days almost all functional changes in sleep were normalized together with 5-HTT mRNA expression in the examined raphe nuclei and the recovery of 5-HTT-IR fibre density in most brain areas. Our results also suggest that the acute MDMA administration abolished aggressive behaviour but MDMA pretreatment and the consequent depletion of serotonergic terminals did not affect aggression. Our findings concerning the changes detected in 5-HTT mRNA expression and fibre density indicate lasting impairment of the serotonergic system and suggest that a single use of MDMA may be associated with long-lasting cognitive, learning, memory and mood deficits and sleep disturbances particularly when a constellation of genetic vulnerability and certain environmental factors are present. Our data provide further evidence for the connection between altered serotonergic functions and sleep disturbance.

Keywords: MDMA, serotonin transporter, raphe nucleus, REM latency, aggression

The illegal recreational drug „ecstasy" contains the active ingredient (±)3,4-methylendioxymethamphetamine (MDMA), which is a ring-substituted amphetamine derivative with psychostimulant properties (Gudelsky and Nash, 1996; Shulgin, 1986) Ecstasy has become second in popularity to cannabis and exhibits mild hallucinogenic and rewarding properties as well as engenders feelings of connectedness and openness (Green et al., 2003). The unique psychopharmacological profile of this drug most likely derives from the property of MDMA to promote acutely the release mainly of serotonin (5-HT), but it also boost dopamine, norepinephrine and acethylcholine in multiple brain regions (Battaglia et al., 1991; Battaglia et al., 1987; Colado et al., 1999; Fitzgerald and Reid, 1990; Johnson et al., 1986).

In the rat central nervous system MDMA has characteristic and well-documented biphasic effects upon the serotonergic systems. First, shortly after administration, there is an acute and rapid release of 5-HT (Green et al., 2003; Mechan et al., 2002; Shankaran and Gudelsky, 1998). Second, in the brain of rodents and non-human primates, MDMA causes a long-term reduction in 5-HT and 5-HIAA (5-hydroxyindole acetic acid) concentration and serotonin transporter (5-HTT) density. The first phase lasts less than 24 h followed immediately by the second phase which lasts approximately 12 months (or possibly longer) (Schmidt, 1987; Stone et al., 1987). It has been shown that several months after MDMA treatment there is a considerable recovery of 5-HT axons (Battaglia et al., 1988; De Souza and Battaglia, 1989; Scanzello et al., 1993).

5-HTT is target of MDMA and also a marker of the established damage (Battaglia et al., 1988; Steele et al., 1987). It is known that 5-HTT plays a crucial role in the regulation of brain 5-HT neurotransmission, which has been linked to mood (Meltzer, 1989; Meltzer, 1990), anxiety (Nutt et al., 1990), sleep (Wauquier and Dugovic, 1990), circadian rhythm (Wesemann and Weiner, 1990), impulsiveness, aggression (Berman et al., 1997; Morgan, 1998), cognition, memory (Altman and Normile, 1988), appetite (Curzon, 1990), motor activity (Jacobs and Fornal, 1997), body temperature (Schwartz et al., 1995) and endocrine function (Jorgensen, 2007). Brain neuroimaging studies have suggested that human MDMA users may have long-lasting changes in brain function consistent with 5-HT toxicity e.g decreased 5-HTT density (Daumann et al., 2004; McCann et al., 1998; McCann et al., 2005; Reneman et al., 2001; Semple et al., 1999). The functional consequences of MDMA-induced 5-HT neurotoxicity are not clear, there have been reports of MDMA users who developed various abnormalities including psychiatric, vegetative, neuroendocrine and cognitive disorders following MDMA exposure (Daumann et al., 2004; McCann et al., 1998; McCann et al., 2005; Morgan, 2000; Parrott, 2002; Parrott et al., 2002; Reneman et al., 2001; Semple et al., 1999). Therefore, humans who sustain 5-HT injury as a result of their MDMA use may be at risk for developing several functional problems.

In humans, MDMA is metabolised principally by cytochrome P450 (CYP) 2D6 or debrisoquine hydroxylase (Tucker et al., 1994). Humans with a genetic predisposition for the slow metabolism of MDMA, the so-called "poor metabolizers" of debrisoquin are at higher risk (de la Torre et al., 2005). 5-9% of the Caucasian population is considered to carry this phenotype (Gonzalez and Meyer, 1991). In rats, MDMA is metabolised by an analogous isoenzyme, CYP2D1 (Balogh et al., 2004; Kirilly et al., 2006; O'Shea et al., 1998; Quate et al., 2004). Our studies were carried out in Dark Agouti rat that possesses decreased microsomal CYP2D1 isoenzyme activity, and thus this rat strain is a useful experimental tool to model a genetically-defined human sub-population in which clinical complications may be more likely to occur.

OBJECTIVES

The recreational drug ecstasy, 3,4-methylenedioxymethamphetamine (MDMA), has been found to selectively damage brain serotonin neurons in experimental animals and probably in human MDMA users, but detailed morphometric analyses and parallel functional measures during damage and recovery are missing. We specifically emphasized the long-term morphological effects of a single dose of MDMA from the brainstem up to the cerebral cortex. We hypothesized that long-lasting damage of the serotonergic system would be manifested in changes of several sleep parameters. Furthermore, we investigated the effects of MDMA on aggressive behaviour.

The main objectives in our studies were:

1. How does 5-HTT mRNA expression change in the different raphe nuclei during 6 months after MDMA treatment?
2. Can differences be observed in the changes concerning dorsal and median raphe nuclei?
3. How does 5-HTT-immunoreactive (IR) fibre density change in different brain areas?
4. Do the examined brain areas show differences in sensitivity after MDMA treatment?

5. How much regeneration can be observed 6 months after MDMA treatment?
6. How do sleep parameters change after MDMA treatment?
7. What sleep parameters change similarly and the opposite way compared to what has previously been observed during depression?
8. How does MDMA treatment alter aggressive behaviour?

MATERIALS AND METHODS

Animals and drugs

All animal experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and National Institutes of Health "Principles of laboratory animal care" (NIH Publications No. 85-23, revised 1985). In addition, specific national law was adhered to about animal studies (Hungarian Governmental Regulation December 31, 1998) and permission was obtained from local ethical committees. Six to seven-week old male Dark Agouti rats (Harlan, Olac Ltd, Shaw's Farm, Blackthorn, Bicester, Oxon, UK) were used in the experiments. The animals (4 per cage) were kept under controlled environmental conditions (temperature at 21±1 °C, and a 12 hour light-dark cycle starting at 10.00 a.m), with standard rat chow and water freely available. The rats received single intraperitoneal injection of MDMA (15 mg/kg) or saline (1 ml/kg) acutely, 3, 7, 21 or 180 days before experiments. In the case of resident– intruder test one group received MDMA pretreatment 21 days prior to the acute drug administration, which occured twenty minutes prior to the experiment.

Morphological studies

In situ hybridization histochemistry

The animals were decapitated 3, 7, 21 or 180 days after MDMA treatment. Coronal serial sections (12 μm) were cut througout the brainstem. In situ hybridization procedure was used to determine the 5-HTT mRNA expression in the brainstem [dorsal (DR) and median (MR) raphe nucleus] and medullary [nucleus raphe magnus (RMg), pallidus (RPa) and obscurus (ROb)] raphe nuclei. DNA template was generated by PCR from transporter specific cDNA using T7 RNA promoter and a downstream gene-specific sequence (anti-sense) (Hansson et al., 1998; Hoffman et al., 1998). Complementary RNA probes were transcribed from gel-purified DNA template using [^{35}S]-UTP and T7 polymerase to generate antisense probe. Sections were hybridized with [^{35}S]-labeled riboprobe then apposed to film (Imaging Plate) for 6-13 days before developing, depending on the hybridization signal. Quantitative analysis of 5-HTT mRNA expression was carried out on coronal sections of the brains. The mean grey values over the dorsal or median raphe nuclei were measured on the film autoradiography, and also over a similar size of the surrounding area that did not contain serotonergic cells. Differences measured in grey densities were used for the evaluation and statistical analysis. For quantification of hybridization signal we used NIH ImageJ software. To support these data and in case of RMg, RPa and ROb the sections were coated with nuclear track emulsion (NTB-3). After a 4 week exposure at 4 °C, slides were developed, fixed and counterstained with a Giemsa stain. Quantification of the silver grains associated with individual cells was accomplished with light- and dark-field microscopy. The threshold was adjusted to background signal level, the subdivision of interest was outlined and the mean grey density was automatically calculated with respect to the surface area of the outlined section. The mean grey density values reflect the mRNA expression in the cell. In the RPa and ROb the number of cells and number of silver grains overlying cell bodies was counted manually with light-field microscopy.

Immunohistochemistry

The animals were deeply anesthetized with nembutal, thoracotomized, and perfused transcardially of Zamboni fixative solution 7, 21 and 180 days after the single-dose of MDMA treatments. Brains were removed and postfixed overnight at 4 °C in the fixed solution. After fixation, brains were stored in 20% glucose solution one day before sectioning. Free-floating 40 μm-thick coronal sections were cut using a freezing microtome, then the sections were stored in a cryoprotectant solution at -20 °C until the immunohistochemical procedures. The presence of 5-HTT was detected using immunohistochemistry. We used a 1:3000 dilution of rabbit polyclonal anti-5-HTT antibody (Oncogene). Then the sections were processed with a peroxidase/DAB kit (En Vision TM, DAKO). Four to six representative, non-overlapping photographs were taken in each anatomical region: cerebral cortex layer I-II (somatosensory, somatomotor and parietal cortex), hippocampus (dentate

gyrus, CA1, CA2, CA3), hypothalamus (posteroventral preoptic areas, lateral hypothalamic area, posterior hypothalamic nucleus, paraventricular nucleus, suprachiasmatic nucleus, tuberomamillary nucleus), periaqueductal central grey (ventrolateral part, dorsomedial part, peripeduncular tegmental nucleus, medial pontine reticular formation), substantia nigra, limbic cortical areas (cingulate and piriform cortex), striatum (caudate-putamen, globus pallidus, dorsomedial striatum and ventrolateral striatum), bed nucleus of stria terminalis, amygdala (central and medial), mediodorsal thalamic nucleus, arcuate nucleus, anterior hypothalamic area, medial preoptic area, lateral septal nucleus. Quantitative analysis of serotonergic fibre density was performed using analySIS image software. The positively-stained fibres were distinguished from the background by means of density thresholding and the percentage area in each image occupied by fibres was recorded. These percentages were averaged across the six sections per brain region for each animal.

Resident – intruder test

We performed resident-intruder test to study the aggressive behavior (Holmes et al., 2002). To establish the resident status of the rats, these animals were housed singly for the final two weeks prior to the test. The behaviour of the animals was recorded on videotape for 15 minutes immediately after the introduction of the smaller male animal into the home cage of the resident rat (Kantor et al., 2000). ***Biting, boxing, kicking*** were scored and classified as impulsive aggressive behaviours, and the incidence of each parameter, as well as their sum were calculated. The latency of each behaviour was also recorded. An additional parameter, namely the latency of first impulsive aggressive behaviour (biting, boxing, and kicking) was also recorded. Duration and latency of ***wrestling*** was also scored and calculated. Duration of social behaviour and grooming was also analysed separately, scored and calculated. The ***motor activity*** and ***exploratory behaviour*** of each resident animal was observed and scored.

Vigilance studies

To investigate the effect of MDMA in the regulation of the sleep-wake cycle, EEG, EMG and motor activity was recorded during the subsequent 24 hours. The vigilance states were scored visually for 4 sec periods for first 2 hours after light onset. The polygraphic recordings were classified by sleep analysis software for the 24 h recordings (SleepSign for Animal; Kissei Comtec America Inc.). The sleep variables were divided into four groups: ***rapid eye movement (REM)*** and ***non-rapid eye movement (NREM)*** sleep indexes, ***wake parameters*** before and at around activity (dark) onset and ***sleep continuity***. The REM sleep parameters included REM latency (Mendelson, 1996), duration of REM in the first hour, time of REM maximum and the sum of REM in the light period. NREM sleep indeces included NREM latency (Deboer et al., 2003; Huber et al., 1998). NREM sleep in the first and second hour, light slow wave sleep (SWS-1) and deep slow wave sleep (SWS-2) latencies, sum of SWS-1 and SWS-2 in the light period. The wake parameters around activity onset were the activity onset and the passive wake before activity onset. The sleep continuity was defined as the sum of the number of awakenings (either active or passive wake) that disconnected any sleep periods. Quantitative analysis of NREM sleep, namely ***EEG power spectra*** of SWS-1 and SWS-2 were also performed (Kantor et al., 2004).

Statistical methods

For statistical analysis, STATISTICA 7.0 (Statsoft Inc., Tulsa, OK) and GraphPad Prism (GraphPad Software Inc., San Diego, CA, USA) softwares were used. Tests for homogenity of variances in the case of the resident-intruder test indicated that variances for duration of wrestling, social behaviour and grooming were not homogenous and thus nonparametric tests were performed for these parameters. Thus results for numbers and latencies of events, motor activity and exploratory behaviour and duration of all behaviours were analysed using Kruskal-Wallis nonparametric tests. Where statistical differences were detected, further comparisons were performed by Mann-Whitney u-tests. For the analysis of pretreatment-treatment interactions two-way analysis of variance (ANOVA) was used for all parameters.

Data from the 5-HTT immunohistochemistry, density of 5-HTT mRNA expressions were analyzed using two-way (treatment and time) analysis of variance and Newman-Keuls test for post-hoc comparisons. The REM, NREM, SWS-1 and SWS-2 latencies, the sum of REM, SWS-1 and SWS-2 in the light period, passive wake before activity onset and the fragmentation of sleep were analyzed using the same method. REM and NREM in the first and second hour were evaluated also by multivariate analysis of variance (MANOVA) for repeated measures. In case of time of REM maximum and activity onset Mann-

Whitney U test was performed. Log-transformed values were used for the statistical analysis of the EEG power spectra data.

RESULTS

Changes of 5-HTT mRNA expression in the brainstem (DR and MR) and medullary (RMg, RPa and ROb) raphe nuclei during a long period of time when the brain serotonergic system was damaged or partially recovered

Generally, a transient increase followed by a transient decrease and a complete recovery was found during the 180-day period in the ascending raphe nuclei. In the dorsal raphe nucleus a significant increase was observed 7 days after drug administration (+24%, p=0.008). In the MR 5-HTT mRNA expression significantly decreased 21 days (-27%, p=0.016) after drug exposure. To obtain more sensitive measures for the effects of MDMA, 5-HTT mRNA expressions of individual cells were counted 7 and 21 days after drug or saline treatment. We noted significantly elevated 5-HTT mRNA expressions in both nuclei 7 days (+53%, p=0.033 and +43%, p=0.049 for DR and MR, respectively) and significantly reduced 5-HTT mRNA expressions 21 days after MDMA treatment (-22%, p=0.025 and -18%, p=0.016, for DR and MR, respectively). In the descending raphe nuclei (RMg, ROb and RPa) 7 days after a single dose MDMA injection elevated 5-HTT mRNA expressions were measured. The average increases of 5-HTT per cell were 24% (p=0.101) in the RMg, 35% (p=0.003) in the RPa and 10% (p=0.012) in the ROb. The values of mean grey density were different in the three different subregions of the RMg (rostral, middle and caudal). Significantly higher values were found only in the middle portion of the nucleus (46%, p=0.019). MDMA did not affect levels of 5-HTT mRNA in the examined raphe nuclei compared to the control group 180 days after drug exposure.

Changes in the 5-HTT-IR fibre density in the brain areas relevant to sleep and aggression during a long period of time when the brain serotonergic system was damaged or partially recovered

Significant (20–40%), widespread reductions in 5-HTT-IR fibre density were detected in most investigated brain areas at 7 and 21 days after MDMA administration. All regions of the cerebral cortex and hippocampus, several nuclei of the hypothalamus and some cell groups of the brainstem were affected (Table 1). Recovery of serotonergic neurons in most areas examined was observed in animals treated with a single dose of MDMA 180 days previously. Among the exceptions hippocampus should be noted because we found a significant reduction in almost all hippocampal areas 180 days after MDMA treatment.

Effects of MDMA on sleep

Parallel to the neuronal damage significant decreases were detected in REM latency 7 and 21 days after MDMA administration. Changes in REM latency were - 55%, - 53%, and -29%, 7, 21 and 180 days after MDMA, respectively. Similarly, an increase in the amount of REM (+245%) was found in the first hour of the passive phase 21 days after MDMA. None of the other parameters characterizing REM sleep were altered. Among the nine parameters of NREM sleep, only three were altered by MDMA treatment and even these alterations failed to follow the time-course of serotonergic damage. Delta power measured either in SWS-1 or in SWS-2 was increased by MDMA both 7 and 21 days after treatment. Changes in the lower frequencies (1 and 2 Hz) were even more consistent. Interestingly, at day 7, the increase in delta power was more pronounced in SWS-1; the increase shifted mainly to SWS-2 at 21 days. In addition, SWS-1 in the passive phase was decreased at 21 days. Sleep fragmentation, an inverse measure of sleep continuity, was increased by 7 days after MDMA administration. Interestingly, this disturbance was normalized by day 21, despite of the continuous presence of serotonergic lesions in all brain areas. At 180 days almost all functional changes in sleep were normalized together with 5-HTT mRNA expression in the examined raphe nuclei and the recovery of 5-HTT-IR fibre density in most brain areas.

Acute and long-term effects of MDMA on aggression

Acute MDMA treatment decreased aggressive-type behaviour as measured by the resident-intruder test. In drug naïve rats, acute MDMA treatment caused massive decreases in all measures of aggressive behaviour, namely, incidence of biting (H=12.749, p=0.009), boxing (H=19.829, p<0.001), kicking (H=18.912, p=0.317), and both the sum (H=21.602, p<0.001) and latency of the three measures combined (H=15.832, p<0.001). The duration of wrestling (H=21.237, p=0.001) was also reduced and subsequently its latency was increased (H=20.350, p=0.001).

Furthermore, a marked reduction was also observed in other social behaviours (H=24.340, p=0.001) and grooming (H=24.473, p<0.001) Increases in locomotor activity (H=20.541, p=0.002) and exploratory behaviour (H=20.957, p=0.002) were attenuated in saline-pretreated animals after acute exposure to MDMA. In the MDMA-pretreated rats, all acute effects of MDMA were preserved.

Significant long term decreases in 5-HTT-IR fibre density were found in many brain areas including those involved in the regulation of aggression 21 days after MDMA administration, although at this time-point aggressive-type behaviour was unaltered. In rats exposed to MDMA 21 days prior to the experiment there was a significant increase in kicking (H=18.912, p=0.010) and a small decrease in grooming and social behaviour (H=24.473, p=0.008 and H=24.340, p=0.006, for grooming and social behaviour, respectively). Locomotor activity and exploratory behaviour was also unchanged in rats pretreated with MDMA.

CONCLUSIONS

Parallel measurements of 5-HTT mRNA expression in five raphe nuclei, 5-HTT-IR fibre density in several brain areas, and more than a dozen functional measures of sleep at three time-points within 180 days after MDMA administration allowed us to compare sleep disturbance with morphometric data and functions of raphe nuclei during a long period of time when the brain serotonergic system was damaged or partially recovered. Furthermore, we performed resident-intruder test to study the aggressive behaviour in drug-naïve rats and also in rats previously exposed to MDMA 21 days earlier.

Morphological effects of MDMA

In the present study, 5-HTT mRNA expression was measured in the two largest group of ascending projecting serotonergic neurons, the DR and the MR. A transient increase followed by a transient decrease and a complete recovery was found during the 180-day period in dorsal and median raphe nuclei. The effects of MDMA on 5-HTT mRNA expression in DR and MR did not differ in time or extent.

In the examined three descending raphe nuclei, RMg, Rob and RPa, we observed up-regulation of 5-HTT mRNA expressions at 7 days and also a complete recovery at 180 days. The up-regulation of 5-HTT mRNA expressions 7 days after MDMA treatment in all studied raphe nuclei may be explained by a compensatory phenomenon following decreased axonal transport or a possible distal down-regulation of the expression of 5-HTT (Kovacs et al., 2007).

Previous studies found that several amphetamine derivatives are more toxic to "fine" 5-HT fibres derived from the DR than "beaded" 5-HT fibres derived from the MR (Mamounas and Molliver, 1988; O'Hearn et al., 1988). However, later neuroanatomical and biochemical studies suggest that DR-selectivity of these amphetamines may not be exclusive (Haring et al., 1992; Hensler et al., 1994; Mamounas and Molliver, 1988; McQuade and Sharp, 1995). In our study the effects of MDMA on 5-HTT mRNA expression in the DR and MR did not differ in time or extent. Our immunohistochemical data also support the conclusion that axons of median raphe origin are also affected by MDMA. The origin of serotonergic projections to the suprachiasmatic nucleus is almost exclusively the MR, and MR fibres project heavily throughout the entire hippocampus. The suprachiasmatic nucleus and the three CA areas of the hippocampus were strongly affected by MDMA in our experiment. These results do not support the view that only "fine" axons derived from the DR are damaged by the drug.

Our results indicate that different brain areas are damaged to differing degrees after MDMA administration (Lew et al., 1996; Sabol et al., 1996). This observation supports previous data that the rate and degree of recovery is region-dependent and the hippocampus is more resistant to recovery compared to other brain regions (Scanzello et al., 1993). The explanation for this might be the specific hierarchy of hippocampal neurons and/or alteration of synthesis or metabolism of neurotrophic factors (Martinez-Turrillas et al., 2006).000 Among the consequences of these findings may be long-term alterations and disturbances in cognitive and memory functions in previous ecstasy users.

In addition, 5-HTT-IR fibre density was also measured in a number of brain regions. Decreases were detected in several brain areas, from the brainstem up to the cerebral cortex at 7 and 21 days. Evidence for partial recovery was found in most affected brain regions by 180 days. Our earlier results show diminished TPH-fibre density in several brain regions 3 days, 1 and 3 weeks after the administration of MDMA (Adori et al., 2006; Ando et al., 2006).

Effects of MDMA on sleep

In the literature we were first to characterize the chronic sleep effects of MDMA. We measured 16

Table 1. 5-HTT-IR fibre densities 7, 21 and 180 days after MDMA (15 mg/kg) treatment in brain areas of Dark Agouti rats

BRAIN AREAS	7 days	21 days	180 days
	Changes (%)		
Cerebral cortex			
Somatosensory cortex	-31 *	-33*	-15
Somatomotor cortex	-36 *	-32 *	-14
Hippocampus			
CA1	-26 *	-37 *	-36 *
CA2	-35 *	-41 *	-32 *
CA3	-33 *	-46 *	-31 *
Dentate gyrus	-24	-35	-19
Hypothalamus			
Posteroventral preoptic areas	-11	-17	-2
Lateral hypothalamic area	-12	-19	-8
Posterior hypothalamic nucleus	-22 *	-38 *	-30 *
Paraventricular nucleus	-2	-6	-3
Suprachiasmatic nucleus	-30 *	-35 *	-17
Tuberomamillary nucleus	-19 *	-35 *	-23 *
Brainstem			
Peripeduncular tegmental nucleus	-7	-12	-7
Medial pontine reticular formation	-3	-14	-5
Periaqueductal grey			
Ventrolateral part	-15	-30 *	-15
Dorsomedial part	-17	-38 *	-16

* significant changes, $p < 0.05$

sleep parameters in 3 different time-points. The most important results are reduced REM latency, increased sleep fragmentation and the changes in the EEG delta power density.

Our results support the view that initiation of REM sleep and delta power NREM sleep are modulated by the serotonergic system (Adrien, 2002; Bhatti et al., 1998; Portas et al., 1996). MDMA administration is followed by depletions in 5-HTT-IR fibre density in some, but not all, brain areas involved in the regulation of REM sleep examined in this study. Significant damage was found in the suprachiasmatic and tuberomamillary nucleus, parallel with reduced REM latency 7 and 21 days after MDMA treatment. In contrast, we could not find significant changes in 5-HTT-IR fibre density around the "effector" neurons located in the medial pontine reticular formation (Sinton and McCarley, 2004) and the peripeduncular tegmental nucleus (Adrien, 2002) in the brainstem, thus, it is unlikely that the observed decrease in REM latency could be a consequence of changes in the serotonergic system of these areas.

The importance of our findings is further supported by the fact that disturbances of sleep are typical for most depressed patients and belong to the core symptoms of depression according to DSM-IV (Adrien et al., 1991; Akiskal et al., 1982; Riemann et al., 2001). More than 90% of depressed patients

complain about disturbances in sleep quality (Mendelson et al., 1977; Riemann et al., 2001). Difficulties in falling asleep, frequent nocturnal awakenings and early morning awakening are the most frequent complaints, and reduced REM latency and increased sleep fragmentation are the most characteristic alterations described in depression (Riemann et al., 2001). Altered sleep functions were found after a single dose of MDMA. Some of these (e.g. reductions in REM latency and sleep fragmentation) were consistent with those described in depression. Effects on other (e.g., NREM) parameters failed to resemble disturbances described in depression. We may conclude that all sleep disturbances described in depression can not be explained by this type of serotonergic damage or the changes in the serotonergic system in depression are only partially similar to the effects of MDMA, and thus point to the involvement of other alterations or the involvement of additional neurotransmitter systems in the pathophysiology of depression.

Acute and long-term effects of MDMA on aggression

Acute MDMA administration abolished aggressive behaviour in the resident-intruder test as expected (Miczek and Haney, 1994; Navarro and Maldonado, 1999). Unexpectedly, MDMA pretreatment and the consequent partial depletion of serotonergic terminals did not increase aggression.

The data from the present study support previous observations that acute MDMA treatment decreases aggressive-type behaviour in rats, as measured by the resident-intruder test (Miczek and Haney, 1994; Navarro and Maldonado, 1999; Verheyden et al., 2002), as well as in human abusers (Curran et al., 2004). However, in the present study there were some differences in the effects of MDMA upon separate parameters of aggression, for example in the frequency of biting, boxing and kicking. Closer examination of the data reveals that this is mainly due to differences in the manifestation of attack between individuals, with some animals preferring to kick, while others showing a greater tendency to box or bite.

The lack of evidence for increased aggression in this test may be explained in many ways. First, the level of aggression exhibited by rats in this test is normally relatively high. It could be difficult to detect further small increases in an already high aggressive state, which is described as an essential factor in this test paradigm (Navarro and Maldonado, 1999). Furthermore, chronic social isolation, which is necessary to confer resident status, has been reported to decrease serotonin levels in forebrain areas in the long-term (Dalley et al., 2002; Fulford and Marsden, 1998; Robbins et al., 1996), and indeed this could be one explanation for the increased baseline aggression in the resident-intruder test. A further decrease in 5-HT content as a result of MDMA pre-treatment may not elicit any further increase in aggression.

Our results draw attention to the long-term dangers of ecstasy use particularly when a constellation of genetic vulnerability and certain environmental factors are present.

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Egyszeri MDMA kezelés tartós neuronkárosító hatása és az azt követő regeneráció hosszú-távú vizsgálata: a szerotonin transzporter expressziója és eloszlása patkány agyban

A rekreációs drogok körébe tartozó 3,4-metiléndioxi-metamfetamin (MDMA), közismert nevén az „ecstasy" használata a hazai és európai fiatalok körében egyre nő. Kutatásunk témája az MDMA neuronkárosító hatásának részletes leírása. Annak ellenére, hogy az MDMA neuronkárosító hatását a rágcsálóktól a majmokig számos fajban kimutatták, és egyre több adat szól amellett, hogy ennek veszélye a drogot fogyasztó személyek között is fennáll, kevés adatot találunk a különböző agyterületek eltérő érzékenységéről, szinte alig rendelkezünk információval a neuronkárosodást követő regenerációról, és semmit sem tudunk a raphe magokban található szerotonerg sejtek válaszáról. Az MDMA lebontásáért a debrizoquin-4-hidroxiláz vagy CYP2D6 enzim felelős. Az európai népesség 5-9%-ában ennek az enzimnek genetikailag meghatározottan csökkent a működése, melynek következtében ebben a populációban feltehetőleg nagyobb az MDMA toxicitása. Kutatásaink során Dark Agouti patkánytörzset alkalmaztuk, mely mintegy modellje a genetikai okok miatt lassan metabolizáló humán fenotípusnak. Vizsgálatainkban a humán hatások szempontjából releváns egyszeri dózis +/- MDMA (15 mg/kg) kezelést követően párhuzamosan vizsgáltuk a 5-HTT mRNS expresszióját az agytörzsi és nyúltvelői raphe magokban, a 5-HTT tartalmú rostok sűrűségét számos agyterületen és 16 alvásparamétert a kezelést követő fél éves időszak alatt. Mivel a vizsgált morfológiai paraméterekben 21 napnál igen markáns változást találtunk, ugyanakkor a regenerációnak még semmi jelét sem láttuk, ebben az időpontban magatartás vizsgálatot is végeztünk. Kísérleteink alapján az agytörzsi, valamint a nyúltvelői raphe magokban hasonló változásokat találtunk a 5-HTT mRNS expressziójában, nevezetesen a 7 nappal a beadást követően mért átmeneti növekedést egy átmeneti csökkenés követi 21 nappal az MDMA kezelés után. A 5-HTT fehérje mennyisége a legtöbb általunk vizsgált agyterületen jelentős (20-40%) csökkenést mutatott. A kérgi területek mind, az agytörzsi területek közül azonban csak némelyik sérült. Ezzel párhuzamosan a vizsgált alvásparaméterek közül szignifikánsan csökkent a REM (paradox alvás) latencia, fokozódott az alvástöredezettség, míg a non-REM alvás idején az ún. delta EEG teljesítmény-sűrűség fokozódott. Hat hónappal a kezelés után az 5-HTT mRNS expressziója az összes vizsgált raphe magban, valamint az 5-HTT rostdenzitás szinte az összes általunk mért agyterületen normalizálódott, párhuzamosan az alvásparaméterekkel. A magatartás vizsgálatban kapott eredményeink szerint az MDMA akut hatása, hogy csökkenti az agressziót. Ugyanakkor a később kialakult neuronkárosodás nem okozott jelentős változást az agresszió mértékében. Mivel az 5-HTT mRNS expressziójában és a rostdenzitásban talált változások egyaránt a szerotonerg rendszer tartós károsodására utalnak, a genetikailag vagy az egyéb körülmények miatt lassan metabolizáló személyek már egyszeri fogyasztás után is komoly veszélynek vannak kitéve, amely számos funkció – pl. a tanulás, memória, hangulat, alvás-ébrenlét ciklus – zavarához, egyensúlyának felbomlásához vezethet. Eredményeink alátámasztják az összefüggést a szerotonerg rendszer változása és az alvásproblémák között.

Kulcsszavak: MDMA, szerotonin transzporter, raphe magok, REM latencia, agresszió