

Changes in serotonin, dopamine and noradrenaline levels in striatum and nucleus accumbens after repeated administration of the abused drug MDMA in rats

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Abstract

The selective neurotoxic action of the abused drug 3,4-methylenedioxymethamphetamine (MDMA) on the serotonergic axons ascending from the dorsal raphe nucleus (DRN) is well known. The present study examined the long-term effects of subchronic MDMA treatment on rat brain tissue contents of catecholaminergic neurotransmitters. Two and four weeks after cessation of repeated MDMA treatment (ten consecutive days, 20 mg/kg/day), the tissue neurotransmitter concentrations were measured by means of electrochemical detected HPLC in several forebrain areas and DRN. We found reduced serotonin levels in the whole forebrain at both instants of time. In nucleus accumbens (NAC), the noradrenaline levels were also decreased, whereas dopamine levels were increased 4 weeks after treatment. It is concluded that MDMA causes changes of monoamine transmitter levels outlasting cessation of drug intake for at least 4 weeks. Decreased noradrenaline and/or serotonin may subsequently cause the augmentation of dopamine in NAC, a structure crucially involved in motivation circuits. With exception of transmitter alterations in the NAC, the post drug effects are opposite to the acute effects of MDMA and may underlie the psychiatric changes after MDMA intake in humans.

Keywords: 3,4-methylenedioxymethamphetamine; Neurotoxicity; Sensitization; Serotonin; Dopamine; Noradrenaline; Nucleus accumbens

The synthetic drug 3,4-methylenedioxymethamphetamine (MDMA) is widely known among young people since the 1980's and the street consumption is still growing. Its hallucinogenic and mood altering properties are probably the reason for the widespread abuse of this drug. Parallel to the popularity, concern about the long-term neurotoxic effects of MDMA has increased. In animal studies, MDMA produces long-lasting decreases in biochemical markers for serotonergic function, i.e. serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA), loss of activity of the synthesis enzyme tryptophanhydroxylase and a persistent reduction of [³H]-paroxetine labeled serotonin uptake sites. Immunohistochemical studies showed a loss of the fine serotonergic axons throughout the forebrain, ascending from the dorsal raphe nucleus (reviewed in Ref. [5]).

In humans, repeated MDMA usage has been correlated

with the decrease of 5-HIAA in liquor [14]. Moreover, several case reports indicate the development of cognitive deficits, panic disorder and psychotic episodes often occurring as 'flash-backs' after excessive MDMA abuse [11]. Corresponding to this, behavioural and neurochemical sensitization to MDMA could be shown in rats [7], a phenomenon of progressive enhancement of behavioural effects of a repeatedly administered drug accompanied with increased dopaminergic neurotransmission in basal ganglia. This phenomenon is considered an important factor in the development of psychostimulant induced psychosis and drug addiction [15,16].

It is well known that the serotonergic system is mainly involved in the pathogenesis of depression and may also play an important role in schizophrenia by an interaction with the dopaminergic system [12]. Thus, a direct connection between the serotonergic depletion of the forebrain and the psychiatric side effects after excessive use of MDMA may exist. Apart from the crucial role of the catecholamines dopamine (DA) and noradrenaline (NA) in treatment of

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schizoaffective disorders, the role of NA in mediating the actions of amphetaminergic psychostimulants has been underestimated by far. Recently, Rothman and colleagues could demonstrate that amphetamines like amphetamine, methamphetamine and MDMA release NA more potently than DA [17]. Therefore we have examined the effects of repeated MDMA treatment on the tissue levels of 5-HT and 5-HIAA, but also of DA and NA in rat brain. To obtain data about long term alterations of neurotransmitter levels, we compared the results from two different instants of time, i.e. 2 and 4 weeks after cessation of repeated MDMA treatment. As forebrain areas relevant for our measurements we chose the medial prefrontal cortex (PFC), striatum (CPu) and nucleus accumbens (NAC), which are known as the main targets of both dopaminergic and serotonergic projections from the midbrain.

Since retrograde degeneration may be a very important topic in the emergence of neurodegenerative disorders, transmitter levels within the origin of the vulnerable serotonergic axons, the dorsal raphe nucleus (DRN), were also examined by means of electrochemical detected HPLC.

For the experiment, 32 male Sprague–Dawley rats with an average body weight of 240 g (Charles-River, Germany) were used. The animals were group-housed under constant conditions in a 12/12 h light/dark cycle (lights on at 07:00 h). Water was available *ad libitum*, food was delivered once daily at 12 g/animal. MDMA hydrochloride was synthesized from Piperonal, according to Braun et al [1], and was dissolved in phosphate buffered saline (PBS, Sigma Deisenhofen, Germany) to a concentration of 20 mg/ml for experimental use. For ten consecutive days, the animals were injected subcutaneously into the neck with either vehicle ($n = 12$) or MDMA-HCl ($n = 20$) at 1.0 ml/kg body weight. In contrast to the common method of treatment (20 mg/kg MDMA, twice daily at four consecutive days) used to induce serotonergic neurotoxicity, we decided to inject the animals once daily for ten consecutive days to facilitate daily “micro-withdrawals” which seem to be necessary for induction of behavioural sensitization.

For neurochemical analysis, animals were sacrificed 14 or 30 days after treatment. The brains were removed and cut into 1.5 mm thick frontal sections. The preparation of the brain areas followed immediately according to the method of Heffner et al. [6], obtaining tissue samples from PFC, NAC, the anterior, posteromedial and posterolateral part of the dorsal striatum (a-, pm-, pl-CPu) and, additionally, the DRN.

For the HPLC analysis the tissue was homogenized in eluent (sodium acetate 6.983 g; citric acid 7.355 g; disodium EDTA 0.048 g; sodium octansulfonate 0.105 g; methanol 7.0% [V/V] in 1000 ml aqua dest) containing the internal standard 3,4-dihydroxybenzylamine (Sigma Deisenhofen, Germany). The separation and quantification of the neurotransmitters followed on a reversed phase column (60 × 3.1 mm ProntoSil RP18, Bischoff, Germany) with an electrochemical detector (Esa Coulochem, Bischoff, Germany)

with data input from both a guard cell ($U = 450$ mV) and an analytical cell ($U = 20/320$ mV *rsp.*). The flow rate of the HPLC pump was 0.8 ml/min.

For statistical analysis, the two control groups from both instants of time were pooled. Data were analyzed using completely randomized one-way analysis of variance (ANOVA) with post-hoc Newman–Keuls corrected *t*-tests. Data are given as means ± SEM and a *P*-value of <0.05 was accepted as significant.

In MDMA treated rats a massive and consistent depletion of 5-HT and 5-HIAA was seen in PFC, NAC and all compartments of CPu to a minimum of 44% of the control values (Fig. 1) 2 and 4 weeks after cessation of repetitive MDMA treatment. In DRN, our study did not show any significant changes of 5-HT or 5-HIAA levels. This finding is in good accordance with the well reported signs of MDMA induced serotonergic neurotoxicity: degeneration of fine serotonergic terminals in the whole forebrain while sparing the cell bodies in DRN (reviewed in Ref. [5]). With longer time span between MDMA treatment and preparation, the tissue levels of 5-HT and 5-HIAA rose slightly in the preferentially serotonergic innervated regions of the basal ganglia, i.e. the NAC and pm-CPu (Fig. 1).

Since no changes in 5-HT contents have been found in the dorsal raphe and since there was some recovery of 5-HT concentrations after 4 weeks, it may be concluded that no retrograde degeneration of 5-HT neurons and even recovery of damaged terminals take place within our time frame of 1 month after cessation of repeated MDMA treatment. However, the time available for recovery may have been too short, thus, recovery was only slight and not in all areas significant. Previous studies have already reported that the damaged brain serotonin neurons are able to recover from axonal damage. Nevertheless, the sprouting and reinnervation pattern of the fibres is highly abnormal, showing denervated distal target regions and hyperinnervated proximal target regions [4]. Our study indicates that mainly dense serotonergic innervation areas of the forebrain are involved in this recovery process.

MDMA is an amphetamine analogue which is capable of releasing both DA and 5-HT [18]. Interestingly, the neurotoxic actions of MDMA affect only the fine serotonergic axons ascending from the DRN, the dopaminergic pathways are spared from damage. Consistent with this current opinion are our results: The DA, DOPAC and HVA concentrations were not decreased in the forebrain except a transient deficiency in DA levels in a-CPu 2 weeks after MDMA treatment. Surprisingly, in NAC the DA tissue levels were elevated 4 weeks after treatment (Fig. 2). Since nucleus accumbens is discussed as one crucial structure in the mediation of reward [9], long-term elevation of DA function in NAC may be responsible for increasing the rewarding actions of other psychostimulants like Cocaine [13].

Generally, 5-HT is reported to have an inhibitory influence on the activity of DA neurons (for review see Ref. [8]). This is consistent with our findings in NAC: The heightened

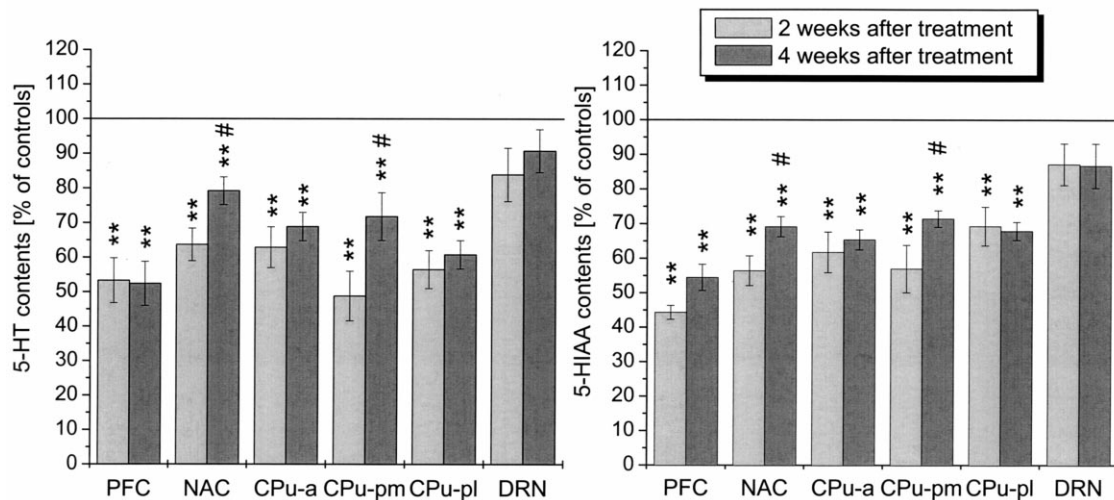


Fig. 1. Changes in tissue levels of 5-HT and 5-HIAA in analyzed brain regions. Shown are tissue contents of MDMA groups against controls (100%-line) as means $\pm$ SEM. Post-hoc Newman-Keuls t -tests: (* $P < 0.05$, ** $P < 0.01$ vs controls; # $P < 0.05$, ## $P < 0.01$ vs "2 weeks after treatment"). ANOVA-Statistics: PFC: 5-HT; $F[2, 27] = 19.2$, $P < 0.0001$, 5-HIAA; $F[2, 27] = 59.1$, $P < 0.0001$. NAC: 5-HT; $F[2, 27] = 21.4$, $P < 0.0001$, 5-HIAA; $F[2, 27] = 38.9$, $P < 0.0001$. Cpu-a: 5-HT; $F[2, 27] = 10.2$, $P = 0.0005$, 5-HIAA; $F[2, 27] = 27.5$, $P < 0.0001$. Cpu-pm: 5-HT; $F[2, 27] = 12.9$, $P = 0.0001$, 5-HIAA; $F[2, 27] = 22.9$, $P < 0.0001$. Cpu-pl: 5-HT; $F[2, 27] = 10.7$, $P = 0.0004$, 5-HIAA; $F[2, 27] = 12.55$, $P = 0.0001$.

DA levels might result from a disinhibition of the mesocorticolimbic dopaminergic pathway due to a massive 5-HT depletion in the forebrain. Moreover a recent study with knockout mice showed that serotonin-(1B) receptor knockout results in a regiospecific disinhibition of basal and cocaine evoked extracellular DA levels in NAC [19]. In contrast, electrical stimulation of the DRN leads to an enhancement of accumbal DA release and reduces dorsostriatal DA release concomitantly [2]. Consequently, decreased accumbal and increased striatal DA levels should be measured in a 5-HT depleted brain. However, we could show opposite results after neurotoxic pretreatment with

MDMA, i.e. augmentation of DA levels in NAC in comparison to a transient decrease in a-CPu. Therefore, the changes in DA are not fully explainable by an altered serotonergic neurotransmission.

To explain the prominent elevations of DA in NAC, a closer view to the experimental design may be useful. With repeated administration of MDMA for ten consecutive days, we could show development of behavioural sensitization [10], confirming the findings of Kalivas et al [7]. Kalivas and colleagues have also demonstrated that both neurotoxic and non-neurotoxic MDMA treatments lead to behavioural sensitization and increased accumbal DA.

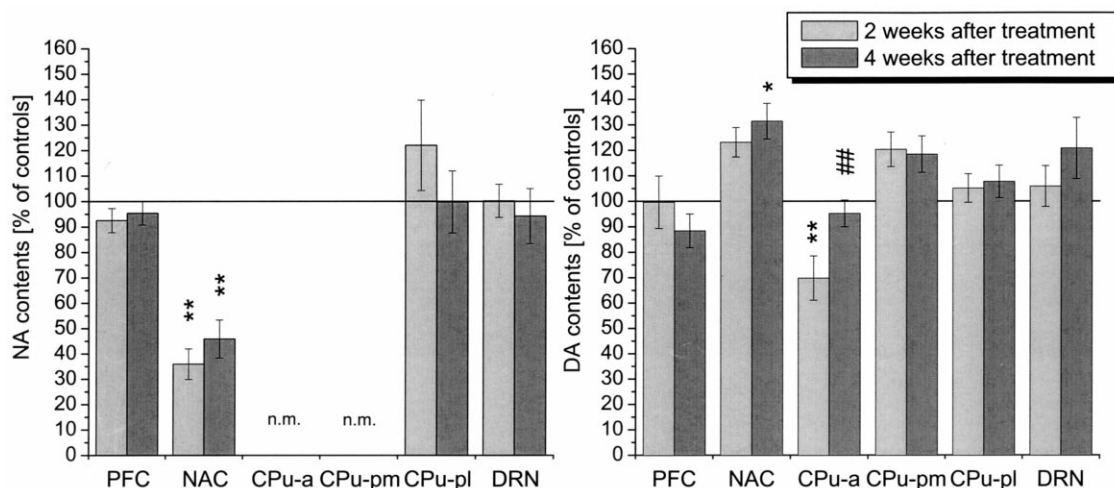


Fig. 2. Changes in tissue levels of NA and DA in analyzed brain regions. Shown are tissue contents of MDMA groups against controls (100%-line) as means $\pm$ SEM. Post-hoc Newman-Keuls t -tests: (* $P < 0.05$, ** $P < 0.01$ vs controls; # $P < 0.05$, ## $P < 0.01$ vs "2 weeks after treatment"; n.m. = not measured). ANOVA-Statistics: NAC: NA; $F[2, 27] = 8.8$, $P < 0.0011$, DA; $F[2, 27] = 4.1$, $P = 0.0279$. a-CPu: DA; $F[2, 27] = 6.68$, $P = 0.0044$.

Therefore, serotonin depletion might not be necessary for the induction of behavioural sensitization; elevated accumbal DA must rather be the element which contributes to augmented drug-induced behaviour after repeated MDMA treatment. However, depletion of 5-HT brain levels by drugs like p-chlorophenylalanine or 5,7-dihydroxytryptamine, which do not release neuronal dopamine or produce sensitization, clearly augments behavioural responses to cocaine and amphetamine [13].

NA was decreased in the NAC 2 and 4 weeks after cessation of MDMA-treatment (Fig. 2). Surprisingly, a regioselective decrease of NA concentrations occurs in NAC. The reason for this effect could be the involvement of two different subpopulations of noradrenergic neurones. In fact, there is evidence that the primary source of noradrenergic afferents to NAC shell is the A2 region of nucleus tractus solitarius [3].

It is well known that NA is regulating release of other neurotransmitters via presynaptic α_2 -adrenoceptors [20], and novel antidepressant agents indicate an important role of NA in the treatment of endogenous depression. According to the altered noradrenergic neurotransmission in NAC, it can be discussed that decreased NA at α_2 -adrenoceptors located on dopaminergic axons disinhibits dopaminergic terminals and subsequently causes the heightened DA levels.

In summary, we could not show any signs for retrograde degeneration of the serotonergic neurons, rather the capability of the serotonergic system to recover from damage became evident. In addition, we could show changes in dopaminergic and noradrenergic systems after subchronic intermittent treatment with high doses of MDMA. These changes, although transient in nature, may chronically affect the equilibrium of the forebrain neurotransmission to such an extent that latent psychiatric disorders could break through and manifest themselves in psychotic episodes or a heightened vulnerability for addictive drugs.

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