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Short communication

Is behavioral sensitization to 3,4-methylenedioxymethamphetamine (MDMA) mediated in part by cholinergic receptors?

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HIGHLIGHTS

- Blocking development of behavioral sensitization to MDMA by muscarinic ACh-receptor antagonist.
- Nicotinic ACh-receptor antagonist did not block sensitization to MDMA.
- Expression of sensitization to MDMA despite pretreatment with muscarinic antagonist.

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ABSTRACT

Behavioral sensitization to the repeated administration of a psychostimulant presumably plays a key role in the pathogenesis of addiction and schizophrenia. Among other psychostimulants, 3,4-methylenedioxymethamphetamine (MDMA) is known to produce behavioral sensitization, too, but its mechanism of action is still not fully understood. Along with the strong release of catecholamines and serotonin, MDMA exerts actions at additional transmitter systems, including acetylcholine (ACh). To identify the cholinergic involvement in the development and expression of MDMA-induced sensitization, rats were treated daily with MDMA (5.0 mg/kg), MDMA plus the muscarinic antagonist atropine (4.28 mg/kg), or MDMA plus the nicotinic antagonist mecamylamine (1.0 mg/kg) for 13 consecutive days. The results show that atropine co-treatment was able to block the development of behavioral sensitization to MDMA, measured as horizontal activity and rearing, whereas mecamylamine did not. Pharmacological challenge with MDMA alone increased the locomotion in all substance pretreated groups with the MDMA plus atropine group showing the lowest values. The second challenge with MDMA plus atropine showed a decrease in locomotor behavior in the MDMA- and an increase in the MDMA plus atropine pretreated groups, resulting in similar levels of activity for both groups. A control experiment revealed no change in horizontal activity and rearing when only the cholinergic antagonists (atropine; mecamylamine) were administered. This is the first study that shows a substantial role of muscarinic receptors for the development of behavioral sensitization to MDMA.

The recreational drug 3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy") has a wide range of action, which is demonstrated by its diverse effects on several transmitter systems. MDMA enters serotonergic neurons via the serotonin transporter and interacts with the vesicular monoamine transporter-2. This interaction represents the putative mechanism of the serotonin (5-HT) release, the main pharmacological effect of MDMA. Additionally, MDMA elevates dopamine (DA) and norepinephrine (NE) levels, although in smaller amounts. The inhibiting effect of MDMA on monoamine oxidases and its weak direct binding affinities at distinct 5-HT and NE receptors contribute to the general increase of extracellular monoamines (for review see Ref. [1]). However, MDMA's complete mechanism of action is still not fully understood. Furthermore, the dose-related release of acetylcholine (ACh) after MDMA-administration substantially widens its pharmacological range. This effect was found in the prefrontal cortex, striatum and in the dorsal hippocampus in vivo [2–4]. Indirect striatal cholinergic activation such as this has been observed for other psychostimulants such as amphetamine [5]. Acquas et al. [2] suggested that the increased ACh-release is a result of stimulation of striatal histaminergic H_1-receptors by MDMA. In addition to the indirect cholinergic agonism, MDMA also shows direct ligand binding affinities at muscarinic receptors [6]. Considering these findings, it is most likely that ACh contributes to the behavioral effects of MDMA, and given that MDMA directly binds to muscarinic receptors one could expect a weak muscarinic dominance.

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In order to determine the different roles of nicotinic and muscarinic receptors within the behavioral pharmacology of MDMA, we performed experiments using the muscarinic receptor antagonist atropine and the nicotinic receptor antagonist mecamylamine. MDMA is known to produce acute behavioral hyperactivity and even locomotor sensitization during subchronic treatment [7–9]. In our experiment we monitored both effects, i.e., the acute MDMA-induced locomotor activity and the sensitizing effects of MDMA on horizontal activity and rearings in animals repeatedly treated with MDMA alone or with MDMA plus either atropine or mecamylamine, respectively.

For the main experiment, 32 male Sprague-Dawley rats (Charles River, Sulzfeld, Germany) weighing 215–300 g at the beginning and for the control experiment 24 male Sprague-Dawley rats weighing 220–270 g were housed in groups of 5–6 animals under constant climatic conditions in Makrolon cages (M IV) and a 12:12 h light/dark-cycle with light on at 8:00 a.m. Rats received 12 g of standard rat chow (Ssniff Spezialdiaeten, Soest, Germany) per rat daily and had access to water ad libitum. Behavioral experiments were performed in the light phase and were in accordance with the German Animal Protection Law.

All substances were calculated as free base and diluted in phosphate-buffered physiological saline (PBS, Biochrom KG Berlin, Germany). MDMA 5.0 mg/ml [(RS)-MDMA-hydrochloride generously provided by Prof. Kovar, Pharmaceutical Chemistry, University of Tuebingen, Germany], atropine 4.28 mg/ml (atropine-sulfate, Sigma–Aldrich, Steinheim, Germany) and mecamylamine 1.0 mg/ml (mecamylamine-hydrochloride, Research Biochemicals International, Natick, USA) were injected subcutaneously as 1.0 ml/kg body weight.

All animals were tested in an open field apparatus (Motility Test 302000, TSE, Bad Homburg, Germany, 47 cm × 47 cm × 44 cm) according to the a priori designed treatment schedule. The horizontal activity and rearings were measured by photocell beams placed 3 and 13 cm above the floor. The registered behavioral data were processed using the software MOTI V 4.14 (TSE, Bad Homburg, Germany).

For the main experiment, the rats were randomly divided into four groups (control, "M", "M-A" and "M-M") of $n = 8$ animals each. The control group animals received only PBS, the M group was treated with MDMA, the M-A group with MDMA plus atropine and the M-M group with MDMA plus mecamylamine. In the control experiment, which was performed to assess behavioral effects of the cholinergic antagonists alone, the control group also received PBS, while the atr group and mec group (each group $n = 8$) were treated with atropine or mecamylamine, respectively.

Both, the main and the control experiment were designed as follows: First, a sensitization phase over 13 consecutive days was performed. Every day, the animals were gently placed into the open field for 10 min of habituation. The rats were then shortly taken out, treated with the respective drug solution and placed back into the open field for further 25 min. Locomotor behavior was registered on days 1 and 13.

Following evaluation of the sensitization results, a "challenges" phase was conducted 14 days after the last day of the sensitization phase. In challenge 1 (day 27) all groups were treated with MDMA and in challenge 2 (day 29) groups M, M-A, atr and control were challenged with MDMA plus atropine and the locomotor activity was registered. The time schedule of the open field observation and the drug doses were the same as in the sensitization phase.

For each experiment, a two-way analysis of variance (ANOVA) with repeated measures was used for comparisons between day 1 and day 13 of the sensitization phase, between day 13 and challenge 1, and between challenge 1 and challenge 2, followed by a post hoc Tukey test for multiple comparisons. Statistical analysis was performed with OriginPro 8 (OriginLab Corporation, Northampton, USA) and all data are shown as means (±SEM).

Horizontal activity (Fig. 1A): During the sensitization phase of the main experiment, significant differences due to treatment, time and treatment × time interaction were revealed ($F(3,28) = 39.20$, $p < 0.001$; $F(1,28) = 19.18$, $p < 0.001$; $F(3,28) = 3.87$, $p = 0.02$). Post hoc tests showed that on day 1, locomotion of the M, M-A and M-M groups were elevated as compared to the control group. Repetitive treatment led to a further increase of the MDMA-induced hyperlocomotion but co-treatment with the anticholinergic substances determined the outcome: On day 13, the M and M-M groups showed increased locomotion while locomotion of the M-A group was not further elevated as compared to day 1. In challenge 1, following treatment with MDMA alone, significant effects on treatment and time were observed ($F(1,28) = 41.18$, $p < 0.001$; $F(1,28) = 42.49$, $p < 0.001$). Horizontal activity was increased in the M, M-M as well as the M-A group. Still, the locomotion of the M group was higher than that of the M-A group. In challenge 2, there were significant treatment, time and treatment × time interaction effects ($F(1,14) = 6.26$, $p = 0.025$; $F(1,14) = 8.65$, $p = 0.011$; $F(1,14) = 5.13$, $p = 0.04$). The administration of MDMA plus atropine resulted in a decrease of locomotion in the M group while the M-A group showed the same level of locomotion as in challenge 1, as indicated by post hoc test.

Rearings (Fig. 1B): Significant differences for treatment, time and treatment × time interaction were found ($F(3,28) = 10.56$, $p < 0.001$; $F(12,336) = 148.25$, $p < 0.001$; $F(3,28) = 10.68$, $p < 0.001$). Post hoc test indicated that all four groups showed the lowest level of rearings on the first experimental day, while on day 13, rearings in the M and M-M groups strongly increased. In the M-A and control group, rearings increased as well but resulted in a significantly lower level than in the M and M-M groups After treatment with MDMA alone (challenge 1) there was an effect on treatment ($F(1,28) = 19.89$, $p < 0.001$) and for challenge 2, a significant effect on treatment × time interaction was observed ($F(1,14) = 9.85$, $p = 0.007$).

In the control experiment, treatment with either atropine, mecamylamine or PBS alone did not affect the *horizontal activity* during the 13 consecutive days. For challenge 1, injection of MDMA had a significant effect on treatment ($F(1,21) = 12.06$, $p = 0.002$). The challenge 2 revealed an effect on time ($F(1,14) = 16.21$, $p = 0.001$; Fig. 1C). Regarding the *rearings*, repeated administration of the antagonists had a significant effect on time during the sensitization phase ($F(1,21) = 6.37$, $p = 0.02$). In challenge 1, no significant effects were observed, while in challenge 2 significant differences for time were found ($F(1,14) = 38.14$, $p < 0.001$; Fig. 1D).

We were able to show that the acute treatment with MDMA enhanced the horizontal activity. This result is in line with former findings [7,10], which support the hypothesis that MDMA causes hyperactivity which is generally discussed as an effect of presynaptic 5-HT- and DA-release [1,11]. In accordance with previous findings of our laboratory [9], acutely enhanced behavioral activity did not include rearings. Failure to alter vertical explorative activity or even an inhibition of explorative activity by MDMA has recently been described for vertical as well as social exploration [12]. Neither the selective muscarinic (atropine) nor the nicotinic (mecamylamine) ACh-antagonist affected the acute MDMA-induced hyperlocomotion directly. When administered alone, neither atropine nor mecamylamine had any influence on the basal horizontal activity. Therefore, the general MDMA-induced acute hyperlocomotion does not seem to be mediated by a cholinergic mechanism.

Subchronic treatment with MDMA resulted in a significant increase of both, horizontal activity and rearing. This phenomenon is well known as development of behavioral sensitization [13] and has already been observed for different doses of MDMA in several