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RESEARCH ARTICLE

Effects of MPEP on expression of food-, MDMA- or amphetamine-conditioned place preference in rats

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Abstract

Recent studies have revealed the effectiveness of 2-methyl-6-(phenylethynyl)pyridine (MPEP), a highly selective antagonist of metabotropic glutamate receptors subtype 5 (mGluR5), in conditioned drug reward. In a previous study we showed that MPEP blocks expression of context-conditioned morphine- but not cocaine reward in the rat. The present study now examines the effectiveness of MPEP in the expression of context-conditioned food, MDMA ('ecstasy') or amphetamine reward. Therefore, three groups of rats were conditioned either to food, MDMA or amphetamine in the conditioned place preference (CPP) paradigm. After conditioning, CPP expression and locomotion were determined simultaneously in the presence and absence of the respective reward (i.e. food or drug), or after application of 50 mg/kg MPEP (the dose that was most effective in reducing morphine CPP expression in our previous study). As a result, MPEP reduced locomotion in all groups. Furthermore, only expression of amphetamine CPP was inhibited by MPEP, while expression of food and MDMA CPP was not affected, suggesting that the MPEP-induced inhibition of amphetamine CPP expression was not causally linked to the reduction of locomotion. Overall, we conclude that MPEP reduces expression of context-conditioned amphetamine but not MDMA or food reward.

Introduction

Recent years of research have revealed the importance of glutamate in processes underlying drug addiction (for reviews see Tzschentke and Schmidt, 2003; Kalivas, 2004). Several studies using either the self-administration or the conditioned place preference (CPP) paradigm further suggest an involvement of metabotropic glutamate receptors subtype 5 (mGluR5) (Chiamulera *et al.*, 2001; Popik and Wrobel, 2002; McGeehan and Olive, 2003; Herzig and Schmidt, 2004). The first hint of an involvement of mGluR5 in addiction came from the study of Chiamulera *et al.* (2001), showing that mGluR5 knockout (k.o.) mice did not develop cocaine self-administration and that the selective mGluR5 antagonist 2-methyl-6-(phenylethynyl)pyridine (MPEP) blocked cocaine self-administration in wild-type mice. Using the CPP paradigm, it was shown that MPEP reduced expression of morphine CPP in mice (Popik and Wrobel, 2002) and in rats but had no effect on expression of cocaine CPP (Herzig and Schmidt, 2004). The CPP paradigm, a model of context-conditioned drug reward, is especially relevant for addiction research, because contextual stimuli can induce craving that might finally lead to relapse (Childress *et al.*, 1999).

Regarding other drugs of abuse, a reduction of both ethanol-seeking behaviour and the alcohol deprivation effect by MPEP has been reported (Marcon *et al.*, 2003; Bäckström *et al.*, 2004). Additionally, MPEP had no effect on the development of CPP to amphetamine, nicotine and ethanol (McGeehan and Olive, 2003). In our opinion CPP expression rather than CPP development could provide an opportunity to explore the involvement of conditioned reward in craving. We therefore suggest that potential anti-craving drugs should be tested on CPP expression (not development), as these compounds should be effective long after development of the conditioned behaviour (see Herzig and Schmidt, 2005a). As yet, no data exist about the effect of MPEP on amphetamine or MDMA ('ecstasy') CPP expression. Furthermore, a previous study at our laboratory indicated that only isolated but not group-housed rats develop MDMA CPP (Meyer *et al.*, 2002), but it remains to be determined if a subgroup within the group-housed rats also develops MDMA CPP.

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Regarding natural reward, Chiamulera *et al.* (2001) report that lever-pressing for food is not affected by MPEP. The effects of MPEP on conditioned food reward have not yet been examined. Nevertheless, an effect of MPEP on conditioned food reward would question its selectivity to conditioned drug reward and thereby its potential usefulness in addiction therapy. In the present study a CPP paradigm was used in rats to examine the effects of MPEP on the expression of context-conditioned natural (food) or drug (MDMA or amphetamine) reward. Despite the report of a reduction of cocaine CPP development by low doses (i.e. 5–20 mg/kg) of MPEP (McGeehan and Olive, 2003), the only studies that examined expression of drug CPP in this respect failed to show the effects of low-dose MPEP (Popik and Wrobel, 2002; Herzig and Schmidt, 2004). Thus, an intraperitoneal (i.p.) dose of 50 mg/kg MPEP was used in the present study because it proved to be the most effective in blocking morphine CPP expression and neither induced CPP nor conditioned place aversion in our previous study. Furthermore, according to Anderson *et al.* (2002), higher doses of MPEP may elicit off-target, non-mGluR5-mediated effects.

Materials and methods

Animals

All animal experiments were conducted in accordance with the principles of animal care and the national laws on animal experiments. For this study 54 male Sprague–Dawley rats were used (F_1 generation of Charles River rats, Sulzfeld, Germany) approximately 7 weeks old, weighing 182–260 g at the start of the experiment. All rats were naive and housed in groups of six animals. Water was supplied *ad libitum* while food was restricted to 12 g of standard rat chow per rat per day. The rats were maintained under a 12-hour light–dark cycle with lights on at 8 a.m. and the experiments were carried out during the light-phase. All rats were allowed to habituate to the colony room for 3 weeks, and they were habituated to handling three times prior to the experiments.

Drugs

The doses for all drugs were based on their salt form and the injection volume was 1 ml/kg for each drug. Amphetamine (R, S-amphetamine-sulphate, lot 23097, Th. Geyer, Renningen, Germany) was dissolved in physiological (0.9 %) saline (Fresenius Kabi GmbH, Bad Homburg, Germany) and injected i.p. 10 minutes prior to the start of the experiment at doses of 4 mg/kg (during conditioning) or 2 mg/kg (during the amphetamine test). MDMA (R,S-3,4-methylenedioxy-methamphetamine; supplied by the Pharmaceutical Institute, Department of Pharmaceutical Chemistry/Analysis, University of Tübingen, Germany) was dissolved in phosphate-buffered saline (PBS) solution and injected subcutaneously (s.c.) 10 minutes prior to the experiment at a dose of 5 mg/kg (during conditioning) or 2.5 mg/kg (during the MDMA test). The selective mGluR5 antagonist MPEP was supplied by Novartis Pharma AG (Basel, Switzerland) and a dose of 50 mg/kg, dissolved in physiological saline, was injected i.p. 30 minutes prior to the start of the MPEP50-test.

CPP apparatus

The applied CPP apparatus was the same as described in our recent study (Herzig and Schmidt, 2005b) and consisted of six boxes (TSE Systems, Germany), each with three different coloured and textured chambers. One of the main chambers (both about 31 × 25 cm) had grey walls and a rough-textured plastic floor, while the other had striped black-and-white walls and a smooth plastic floor. The smaller, middle chamber (11 × 25 cm) had white walls and a smooth metal floor. All chambers were equipped with photo sensors to detect the location of the rat to analyse the locomotion and the time spent in each chamber. Three CPP boxes were placed with the grey-coloured chambers facing the centre of the room and the other three boxes with the grey-coloured chambers facing the room wall. Each lid of each chamber contained a bulb for illumination and the middle chamber was always illuminated to reduce the time spent inside. The walls separating the chambers (during conditioning) were replaced by walls with open doors (during the pre-tests and the tests), allowing the rats to enter the other chambers.

CPP experiment

An unbiased CPP procedure was used and the rats were assigned randomly to three groups: amphetamine ($n = 12$), MDMA ($n = 24$) and food ($n = 18$). More rats were used in the food group, as we expected a weaker CPP after food conditioning. Based on previous results we further expected that only a fraction of group-housed rats would develop MDMA CPP (Meyer *et al.*, 2002); therefore, the number of rats in the MDMA group was also increased. For all groups, the experiment consisted of three phases: pre-test (days 1–3), conditioning (days 4–13) and four tests (days 15–18). One day without testing (day 14, rats remaining untreated in their home-cage) was performed because food or drug was administered on alternating days during conditioning. Each rat was always tested in the same CPP box and after the end of each run the CPP boxes were cleaned with water and wiped dry. The initial pre-test phase consisted of three pre-tests, and for each pre-test the rats were placed into the middle chamber without prior injection and tested for 20 minutes with free access to all chambers. The subsequent conditioning phase consisted of five alternating pairings of the unconditioned stimulus (i.e. food, MDMA or amphetamine) or vehicle [i.e. 1 ml/kg saline i.p. or PBS-solution subcutaneously (s.c.)] with the respective chamber context. Each conditioning session lasted for 30 minutes and rats were confined to one of the main chambers. Within each group, the assignment of the food- or drug-associated chamber was counterbalanced between the differentially coloured and orientated chambers. For food conditioning, rats received a saline i.p. injection and two standard pellets (offered on the chamber floor) that had been macerated in water for 30 minutes and that were readily accepted and consumed by all rats. After each food conditioning session (at the normal feeding time) the food group received the usual amount of food minus the amount of the food reward to maintain their usual daily food amount. For drug conditioning, 4 mg/kg amphetamine or 5 mg/kg MDMA were used. Four subsequent tests were carried out during the testing phase, each test lasting for 20 minutes with free access to all chambers, and for the start the rats were

placed into the middle chamber. In order to account for possible extinction-induced effects, half the rats in each group received 50 mg/kg MPEP before the first test and the respective vehicle before the second test, while the other half received the opposite treatment order. To determine whether potential effects induced by MPEP are of a temporary or permanent nature, a third test consisting of challenge with the unconditioned stimulus (i.e. one macerated food pellet placed into the centre of the middle chamber or an injection of 2 mg/kg amphetamine or 2.5 mg/kg MDMA, respectively) was carried out to produce a full CPP expression in each group. In order to avoid strong locomotor side effects during the challenge, which might alter CPP expression (see discussion in Cunningham *et al.*, 1998), lower doses of the unconditioned stimuli were administered. The fourth test again consisted of a vehicle test.

Data analysis

For statistical analysis the program GB-Stat version7.0 (Dynamic Microsystems Inc.) was used and significance levels were set at $^{*}p < 0.05$ and $^{**}p < 0.01$. CPP was calculated as the time difference between the food- or drug-associated and the vehicle-associated chambers and locomotion was calculated as the distance travelled in all three chambers. Repeated-measures analysis of variance (ANOVA) (with the factor tests) followed by Fisher's least-squares difference (LSD) *post-hoc* comparison were carried out for the CPP and locomotion data of each group. The three pre-tests (= av pre-test) were averaged before statistical analysis to serve as a reference for determination of significant CPP expression or to indicate a lack (i.e. inhibition) of CPP expression. Also the data of the two vehicle tests were averaged (= av vehicle) and served as a reference to indicate reduction or potentiation of CPP expression. For the locomotion data, the av pre-test was used as a reference. As we have observed previously that not all rats respond to drug-conditioning in the CPP paradigm (Herzig and Schmidt, 2004), a criterion was used to select only rats that respond to the respective reward. The minimum criterion for the inclusion of each rat into the statistical analysis was: {CPP (av vehicle)} – {CPP (av pre-test)} > 0 seconds. Overall, five rats in the food group, eight rats in the MDMA group and five rats in the amphetamine group were excluded.

Results

Conditioned place preference (CPP)

In all groups, the two vehicle tests were averaged (= av vehicle) for reasons of clarity, as Student's *t*-test comparisons did not show significant differences (each $p > 0.05$) between the first and second vehicle tests (food group: 204.9 ± 55.1 seconds (first vehicle test) and 160.5 ± 82.9 seconds (second vehicle test); MDMA group: 306.3 ± 89.1 seconds and 253.9 ± 64.7 seconds; amphetamine group: 442.5 ± 82.4 seconds and 274.0 ± 118.1 seconds).

Analysis of the CPP data in the food group ($n = 13$) by repeated-measures ANOVA (factor tests) revealed a significant effect ($F_{4,50}$; $p = 0.0088$; Fig. 1a). Fisher's LSD *post-hoc* test (av pre-test CPP as reference) showed significant CPP expression during the av vehicle test ($p < 0.05$) and during the MPEP50 test ($p < 0.01$), but not during the food test ($p > 0.05$). However, CPP expression during the MPEP50 and the food tests was not significantly different from the av vehicle test ($p > 0.05$). Thus, MPEP did not affect expression of food CPP, but the food challenge inhibited food CPP expression. Analysis of the locomotion data in the food group also showed a significant effect ($F_{55,30}$; $p < 0.0001$; Fig. 1b). Fisher's LSD *post-hoc* test (av pre-test locomotion as reference) showed significantly decreased locomotion during the MPEP50 test ($p < 0.01$) and during the food test ($p < 0.05$). Thus, MPEP and food intake reduced the locomotion in the food group.

In the MDMA group ($n = 16$), a significant effect of tests on CPP expression was found ($F_{5,34}$; $p = 0.0031$; Fig. 2a). *Post-hoc* tests (Fisher's LSD) revealed significant ($p < 0.01$) CPP expression after all tests (i.e. av vehicle, MPEP50 and MDMA tests) compared to the av pre-test CPP. Furthermore, CPP expression during the MPEP50 and the MDMA tests was not significantly different from the av vehicle test ($p > 0.05$). Thus, the MPEP and MDMA tests did not affect expression of MDMA CPP. Analysis of the locomotion data in the MDMA group showed a significant effect ($F_{46,15}$; $p < 0.0001$; Fig. 2b). *Post-hoc* comparison revealed that locomotion during the av vehicle and the MPEP50 tests was significantly reduced (both $p < 0.01$). Thus, locomotion in the MDMA-group was reduced during the MPEP and the vehicle test.

In the amphetamine-group ($n = 7$) the effect of the tests on CPP expression just missed significance ($F_{2,93}$; $p = 0.0614$; Fig. 3a). However, *post-hoc* comparisons showed significant CPP expression during the av vehicle and the amphetamine test (both $p < 0.05$), but not during the MPEP50 test ($p > 0.05$). Furthermore, CPP expression during the MPEP50 and the amphetamine test was not significantly different from the av vehicle test ($p > 0.05$). Thus, MPEP inhibited the expression of amphetamine CPP. Analysis of the locomotion data in the amphetamine group revealed a significant effect of the tests ($F_{45,56}$; $p < 0.0001$, Fig. 3b). *Post-hoc* comparison showed significantly decreased locomotion during the MPEP50 test ($p < 0.01$) and significantly increased locomotion during the amphetamine test ($p < 0.01$), but no alteration during the av vehicle test ($p > 0.05$). Thus, amphetamine (2 mg/kg) increased and MPEP (50 mg/kg) reduced the locomotion in the amphetamine group.

Discussion

In order to avoid the inclusion of non-responding rats, all rats that did not show an increase in CPP expression between the av pre-test and the av vehicle were excluded from statistical analysis. Thus, it has to be kept in mind that only a subpopulation of responders was analysed and that CPP expression is probably more stable than reported from other studies that also included the non-responders. Overall, the main finding of the present study is the inhibition of amphetamine CPP expression by 50 mg/kg (i.p.) of the mGluR5 antagonist MPEP while food and MDMA CPP expression remained unaffected. Moreover, MPEP reduced locomotion in all groups.

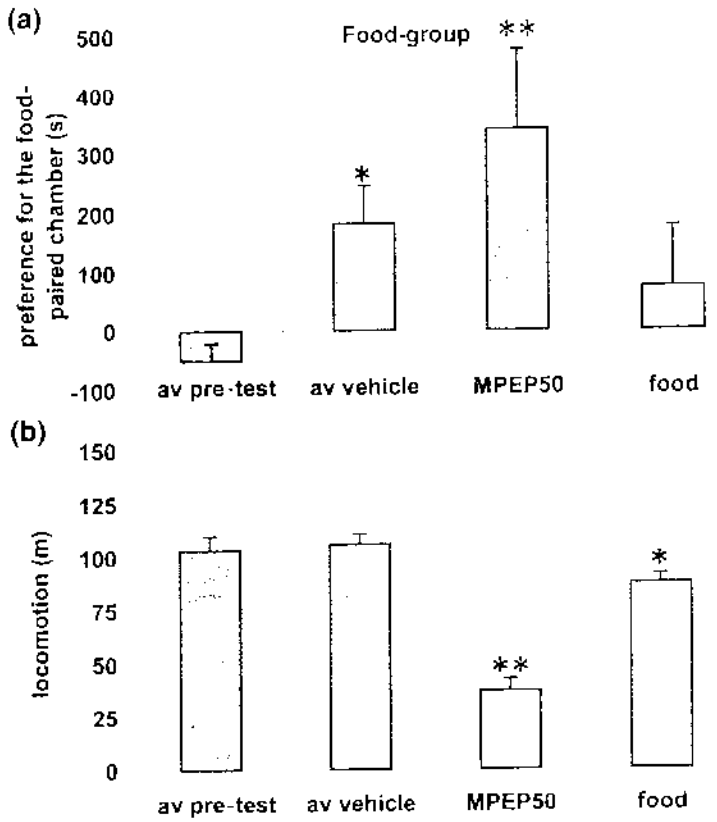

Figure 1. *Food group. (a) Conditioned place preference (CPP = time difference between food- and vehicle-associated chamber) and (b) locomotion (= distance travelled in all three chambers) for the food group during three pre-tests (their average is indicated by 'av pre-test') and all tests (i.e. two vehicle tests (average indicated by 'av vehicl'), a test after 50 mg/kg MPEP ('MPEP50') and the food test ('food') consisting of a single macerated food pellet placed inside the middle chamber). Each pre-test or test lasted for 20 minutes using three-chambered CPP boxes. The respective SEM is shown. *$p < 0.05$) and **$p < 0.01$ indicate significant difference from av pre-test.*

Locomotion

The observed reduction of locomotion by 50 mg/kg MPEP is in accordance with a previous report that showed an inhibition of spontaneous locomotion by high doses of MPEP (30 and 100 mg/kg, p.o.) in rats (Spooren *et al.*, 2000). However, it contrasts a recent study that reported an increased locomotion by MPEP (5 and 20 mg/kg, i.p.) in mice (McGeehan *et al.*, 2004). A possible explanation for these contradictory results might be a biphasic effect of MPEP on locomotion, i.e. increasing locomotion at low doses and decreasing locomotion at high doses. Also the use of different rodent species or procedural issues might play a role, because MPEP was always injected 30 minutes prior to measurement of locomotion in the present study, while it was applied immediately prior to the experiment in the McGeehan *et al.* (2004) study. Therefore, another possible explanation might be a time-dependent change in the effects of MPEP. At least, it may be concluded that MPEP modulates spontaneous locomotion.

It is obvious that the CPP paradigm depends to a large extent on locomotor activity. Thus, one might argue that the MPEP-induced inhibition of amphetamine CPP expression is caused by MPEPs modulatory effects on locomotion. However, food and MDMA CPP expression was not inhibited by MPEP, despite a similar decrease in locomotion. Furthermore, in our previous study (Herzig and Schmidt, 2004), the same dose of MPEP reduced morphine but not cocaine CPP expression, while locomotion was reduced in both groups. Thus, the general decrease in locomotion induced by MPEP does not explain the specific effects on amphetamine and morphine but not on food, MDMA or cocaine CPP expression. Additionally, as pointed out by Cunningham *et al.* (1998), lower activity levels are associated typically with stronger (not weaker) preference. We therefore conclude that the decrease in locomotion produced by MPEP was not sufficient to alter CPP expression, probably because locomotion was never blocked completely by MPEP and the remaining locomotion was still enough to express the respective CPP behaviour.

Food CPP expression

The present results show clearly that food CPP expression was inhibited during the food test and that MPEP did not alter food CPP expression. The role of food consumption is highlighted further by the reduced locomotion during the food test. The inhibition of food CPP expression during the food test is in contrast to the unaltered CPP expression in both drug-conditioned groups during the drug test. However, both drugs were administered by the experimenter and therefore no consumption behaviour was involved, while food consumption during the food test obviously played a role in the food group. Overall, we conclude that food conditioned contextual

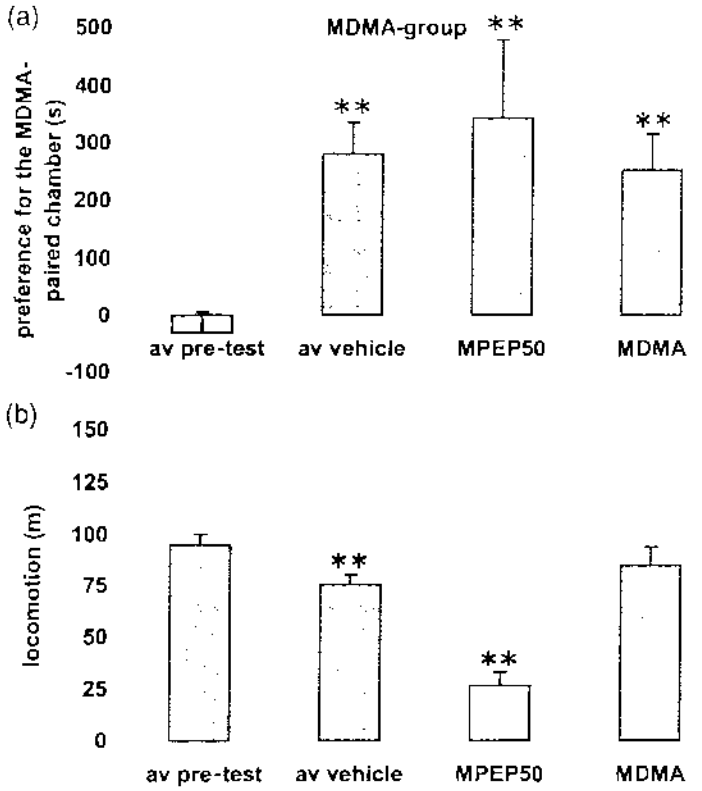

Figure 2. *MDMA group. (a) Conditioned place preference (CPP = time difference between MDMA- and vehicle-associated chamber) and (b) locomotion (= distance travelled in all three chambers) for the MDMA group during three pre-tests (their average is indicated by 'av pre-test') and all tests (i.e. two vehicle tests (average indicated by 'av vehicle'), a test after 50 mg/kg MPEP ('MPEP50') and the test with 2.5 mg/kg R,S-MDMA ('MDMA'). Each pre-test or test lasted for 20 minutes using three-chambered CPP boxes. The respective SEM is shown. **p < 0.01 indicates significant difference from av pre-test.*

effects are reduced by food consumption. Regarding the ineffectiveness of MPEP in reducing food CPP expression, the present findings complement previous results from other groups showing that MPEP did not affect food self administration (Chiamulera *et al.*, 2001; Paterson *et al.*, 2003), suggesting that MPEP does not alter the mechanisms regulating food reward in rodents. This is especially interesting, as we have shown previously that repeated MPEP leads to a decrease in the body weight of the rats (Herzig and Schmidt, 2004). Consequently, the MPEP-induced reduction in body weight cannot be explained by an alteration of the rewarding properties of food.

MDMA CPP expression

The present results show that a significant CPP expression was observed in 66.7% of the MDMA-conditioned rats and that MPEP did not affect MDMA CPP expression. Thus, the question of whether a subgroup of group-housed rats develops MDMA CPP can be answered positively. The fact that one-third of the group-housed rats did not develop MDMA CPP might explain the failure to detect MDMA CPP in our previous study (Meyer *et al.*, 2002) that used the same MDMA dose for conditioning, but included all rats (not only the responders) into the analysis. As isolated rats developed CPP in the latter study, we conclude that a higher proportion of isolated rats develop MDMA CPP in comparison to group-housed rats. A possible reason for such differences in individual MDMA susceptibility due to the housing conditions might be different social ranks within group-housed rats that lead to different levels of social stress for each individual. A behavioural differentiation due to social pressure within a group has, for example, been described by Grasmuck and Desor (2002). In consequence, the higher social stress in the lower-ranked individuals may have caused a similar change in their susceptibility to MDMA than reported previously for individuals that were maintained in isolation (Meyer *et al.*, 2002). Unfortunately, we did not find any reference that indicates the proportion of high- and low-ranked individuals within a cage of six male rats to prove our hypothesis.

So far no study has tested the effects of MPEP (or any other drug) on the expression of MDMA CPP. The observed failure of MPEP to affect MDMA CPP expression at least suggests different mechanisms involved in conditioned reward to MDMA, food or cocaine, on one hand, and amphetamine (see Discussion below) or morphine (Popik and Wrobel, 2002; Herzig and Schmidt, 2004) on the other hand. Overall, an involvement of glutamate in expression of contextual MDMA reward cannot be excluded completely, but at least mGluR5 do not seem to play a role.

Amphetamine CPP expression

The observation that MPEP inhibits amphetamine CPP expression is a new finding and the strong response during the amphetamine test indicates that the inhibiting effect of

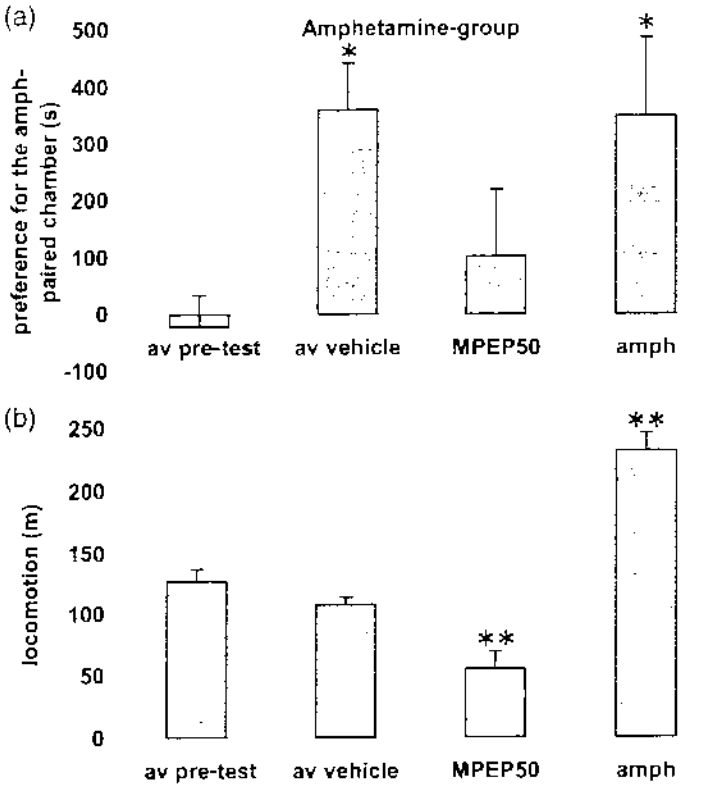

Figure 3. *Amphetamine group. (a) Conditioned place preference (CPP = time difference between amphetamine- and vehicle-associated chamber) and (b) locomotion (= distance travelled in all three chambers) for the amphetamine group during three pre-tests (their average is indicated by 'av pre-test') and all tests (i.e. two vehicle tests (average indicated by 'av vehicle'), a test after 50 mg/kg MPEP ('MPEP50') and the test with 2 mg/kg R,S-amphetamine ('amph'). Each pre-test or test lasted for 20 minutes using three-chambered CPP boxes. The respective SEM is shown. *$p < 0.05$ and **$p < 0.01$ indicate significant difference from av pre-test.*

MPEP on amphetamine CPP expression does not persist permanently. However, as several subsequent tests have been carried out, one might argue that extinction could have contributed to the MPEP-induced inhibition of amphetamine CPP expression. As the first test was either a vehicle or a MPEP test for half the rats in each group, such extinction effects were minimized. Moreover, no significant differences between the first and the second vehicle tests were observed in all groups, indicating that there was no strong extinction present. In line with these results, the work of Mueller and Stewart (2000) and our recent study (Herzig and Schmidt, 2005h) showed that extinction of cocaine or morphine CPP expression is small during the first few testing days. Overall, we conclude that extinction cannot account for the MPEP-induced inhibition of amphetamine CPP expression. Interestingly, amphetamine CPP development in mice was not affected by MPEP (McGeehan and Olive, 2003). However, this does not contradict the present results, as CPP development and expression are two different processes. Our previous observation, that cocaine CPP expression is not affected by MPEP (Herzig and Schmidt, 2004), renders the MPEP-induced inhibition amphetamine CPP expression especially interesting. Based on the similarities in the mode of action of both psychostimulants as dopamine-reuptake blockers, one would assume that MPEP modulates CPP expression to both drugs in a similar way. However, previous studies already documented differences between both drugs as, for example, lesions of the prelimbic medial prefrontal cortex blocked development of cocaine CPP but not amphetamine CPP (Tzschentke and Schmidt, 1998). Unfortunately, CPP expression has not been examined in this respect, but if the brain nuclei responsible for development of CPP differ between both drugs, it might be possible that such differences exist for CPP expression, raising the possibility that one drug (e.g. MPEP) causes different effects in amphetamine- or cocaine-conditioned rats.

Regarding the mechanisms underlying the inhibition of amphetamine CPP expression by MPEP, it remains unclear whether this effect is mainly mediated via mGluR5. Thus Anderson *et al.* (2002) suggested not to use MPEP doses in excess of 50 mg/kg in rats to avoid off-target, non-mGluR5-mediated effects, and some recent reports showed several unspecific effects of MPEP, i.e. that MPEP acts as a positive allosteric modulator for human mGluR4 (Mathiesen *et al.*, 2003) and as an inhibitor of human noradrenalin transporters (Heidbreder *et al.*, 2003). Additionally, non-competitive NMDA-receptor antagonistic properties of MPEP were also reported (O'Leary *et al.*, 2000). However, we cannot decide whether such off-target mechanisms may have contributed to the observed reduction of amphetamine CPP expression by MPEP, as no literature is available examining the role of mGluR4 or inhibitors of noradrenalin transporters in the expression of amphetamine CPP. In addition, possible non-competitive NMDA-receptor antagonistic properties of

MPEP can also not be excluded as a reason for the observed effects, especially as the relevant literature is confounding. On one hand, the non-competitive NMDA-receptor antagonist MK-801 blocked expression of amphetamine CPP and morphine CPP (Tzschentke and Schmidt, 1997) but not cocaine CPP expression (Cervo and Samanin, 1995), nicely paralleling the reported specific effects of MPEP (Herzig and Schmidt, 2004 and present results). On the other hand, memantine as another non-competitive NMDA-receptor antagonist blocked CPP expression to both morphine (Popik and Danysz, 1997) and cocaine (Kotlinska and Biala, 2000). Overall, it cannot be concluded finally whether the mGluR5-antagonistic activity accounts solely for the observed reduction of amphetamine CPP expression by MPEP. To reach a final conclusion, we suggest further studies in amphetamine-conditioned animals using a more selective mGluR5-antagonist, such as MTEP (see Cosford *et al.*, 2003).

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