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Repeated-testing of place preference expression for evaluation of anti-craving-drug effects

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Summary. In addiction research, the conditioned place preference (CPP) paradigm is a widely used animal model of conditioned reward. Usually, CPP development is studied, while only few studies examine CPP expression. In the present study, the suitability of a schedule allowing repeated testing of CPP expression was evaluated. Two groups of rats were either conditioned with cocaine or morphine then the repeated-testing-schedule was applied. This schedule consisted of four repeated applications of a sequence of drug- (i.e. cocaine or morphine), saline- and anti-craving-drug- (i.e. acamprosate, naloxone, their joint administration or saline as internal control) tests. Methodologically, the repeated-testing-schedule produced stable CPP expression in both groups over 12 subsequent tests. In conclusion, it is suggested as a useful method to study effects of anti-craving-drugs on CPP expression, thereby reducing the overall number of experimental animals. The evaluation of the anti-craving-drug effects revealed that neither acamprosate and naloxone given separately nor their combined administration significantly reduced cocaine- or morphine-CPP expression. Thus, we suggest that these anti-craving-drugs are unlikely to be effective for relapse prevention in cocaine- or morphine-addicts.

Keywords: Conditioned place preference (CPP) expression – Conditioned reward – Repeated testing – Morphine – Cocaine – Naloxone – Acamprosate

Introduction

Acamprosate (calcium acetylhomotaurinate), a drug showing functional NMDA-glutamate receptor antagonistic properties (Spanagel and Zieglgänsberger, 1997), has been reported to be effective against alcohol-craving. In rodents, acamprosate suppressed the alcohol deprivation effect and reduced ethanol-intake as well as some signs of physical alcohol-withdrawal (Spanagel et al., 1996; for review see Spanagel and Zieglgänsberger, 1997). In weaned alcoholic patients, acamprosate was effective in reducing the probability of relapses. Animal experiments further revealed that some morphine-induced behaviours are affected by acamprosate, i.e. acamprosate reduced the intensity of physical morphine-dependence in mice (Sepulveda et al., 2002) and inhibited the motivational component of morphine-withdrawal (Kratzer and Schmidt, 1998). Additionally, acamprosate suppressed the expression (Spanagel et al., 1998) but not the development (Kratzer et al., 2003) of morphine-sensitization, indicating that expression and development of sensitization are different processes. A potential effect of acamprosate on development of cocaine-conditioned reward was recently reported (McGeehan and Olive, 2003). As yet, acamprosate has not yet been tested on expression of cocaine- or morphine-conditioned reward.

Another compound with possible anti-craving-drug properties is naloxone, a μ-opiate-receptor antagonist that was shown to facilitate extinction of conditioned ethanol-reward in mice (Cunningham et al., 1998). The very similar μ-opiate-receptor antagonist naltrexone has also been shown to reduce ethanol-intake and facilitate abstinence in patients (for review see Spanagel and Zieglgänsberger, 1997). Animal experiments report that naloxone infusion into the ventral tegmental area or the periaqueductal gray blocked the development of morphine-conditioned place preference (CPP) (Olmstead and Franklin, 1997). However, morphine-CPP expression was increased in both mice and rats after naloxone treatment (Noble et al., 1993; Neisewander et al., 1990). Additionally, cocaine-induced reinstatement of cocaine-seeking was not affected by naltrexone pretreatment in rats (Comer et al., 1993). The effectiveness of naloxone on expression of cocaine-conditioned reward has not yet been examined. Interestingly, a joint administration of acamprosate and naloxone in alcoholic patients produced superior effects than both

drugs given separately, resulting in a lower probability for relapse (Kiefer et al., 2003). However, neither animal nor human studies examined potential additive or synergistic effects of acamprosate plus naloxone in reduction of morphine- or cocaine-conditioned reward.

In the present study a CPP paradigm was used as an animal model of context-conditioned drug reward. The study aimed to establish a testing-design (the repeated-testing-schedule) that allows the repeated testing of anti-craving-drug effects on CPP expression in the same rats. Therefore, two groups of rats were either conditioned with cocaine or morphine, then expression of drug-CPP was repeatedly tested under the influence of different anti-craving-drugs (i.e. acamprosate, naloxone or their joint administration), each anti-craving-drug test being preceded by a drug- and a saline-test.

Material and methods

Animals

All animal experiments were conducted in accordance with the NIH guide for care and use of laboratory animals and comply with the national laws on animal experiments. Both the morphine- and the cocaine-group consisted of 12 male Sprague-Dawley rats (Charles River, Sulzfeld, Germany) each, weighing about 230–270 g at the first day of the experiment. All rats were housed in groups of 6 rats and water was supplied *ad libitum*. Food was restricted to 12 g of standard rat chow per rat and day. All rats were maintained in a temperature-controlled room (21–22°C) under a 12 hour light-dark-cycle (light-onset 8 a.m.) and the experiments were carried out during the light-phase.

CPP-apparatus

The experimental setup consisted of six CPP boxes (TSE Systems, Germany), each with three different coloured and textured chambers and has been described in detail in our previous study (Herzig and Schmidt, 2004). In each CPP box, one main chamber (about 31 cm × 25 cm) had grey walls and a rough-textured floor, while the other had striped black and white walls and a smooth floor. The smaller, middle chamber (11 cm × 25 cm) had white walls and also a smooth floor. All chambers were equipped with photo sensors to detect the location of the rat and to measure the locomotion. 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. The walls separating the chambers (during conditioning), could be replaced by chamber-walls with open doors (during pretest and tests), to allow the rats to pass into the other chambers. The middle chamber was illuminated during all tests as previous experience (unpublished results) showed that light reduced the time spent in the illuminated chamber. Locomotion and the times spent in each chamber were automatically determined according to the number of light-beam breaks. Thus, our CPP setup allows the analysis of treatment-effects on both CPP and locomotion (the latter might be important to recognize potential side-effects of a treatment).

Drugs

All drugs were dissolved in physiological saline (0.9% NaCl, Fresenius Kabi GmbH, Bad Homburg, Germany) and injected intraperitoneally (i.p.) in a volume of 1 ml/kg. Both morphine (morphine-sulphate, lot 20737, Th. Geyer, Renningen, Germany) and cocaine (cocaine-hydrochloride, lot L447362 931, Merck, Germany) were used at doses of 10 mg/kg during conditioning and applied 10 min prior to the start of the experiment. During the intervening drug-tests lower doses of cocaine and morphine (5 mg/kg) were used in order to avoid strong locomotor effects (which might in turn affect CPP expression, see discussion in Cunningham et al., 1998). Acamprosate (Merck, Germany) was used at a dose of 200 mg/kg (injected 30 min before start) that proved most effective in preventing the motivational aspect of naloxone-induced morphine-withdrawal (Kratzer and Schmidt, 1998). A dose of 2 mg/kg naloxone (naloxone hydrochloride, lot 16H1461, Sigma, Germany) was used (injected 10 min prior to the test), since it showed behavioural effectiveness in other studies (Mucha et al., 1982; Bardo and Neisewander, 1986).

CPP-experiment

An unbiased CPP procedure was used. The applied treatment-schedule for the present CPP-experiment is indicated in Table 1.

The pretests and the conditioning-phase were carried out in the same way as during our previous study (Herzig and Schmidt, 2004). First, three pretests (days 1–3) were performed to determine a stable baseline of the unconditioned place preference for each rat. During each pretest, the rats were placed for 20 min into the middle chamber without prior injection and with free access to all chambers. After completing the pretests, the drug-associated (“rewarded”) chamber was randomly assigned to the cocaine- and the morphine-group in a counterbalanced manner. In order to induce a strong CPP for each drug, ten days of conditioning (days 4–13) followed when rats were placed into one of the main chambers for 30 min without access to the other chambers. On even days during conditioning, the cocaine- and the morphine-group received an injection of 10 mg/kg of cocaine or morphine, respectively, before being placed into the drug-associated chamber. On odd days, all rats received a saline injection before being placed into the saline-associated chamber. After conditioning, one day (day 14) without testing (rats remained in their home-cage without treatment) followed to overcome the regular schedule of drug- and saline-application that was used during conditioning. All subsequent tests (days 15–26) were carried out with for 20 min with free access to all chambers to enable comparison with the pretest data. The tests started on day 15 with a test in the drugged-state (i.e. after administration of the respective conditioning-drug), then a test (day 16) in the undrugged-state (i.e. after saline administration) was performed and an anti-craving-drug test (day 17) after administration of one of four testing-drugs (i.e. saline as an internal control, acamprosate, naloxone and combination of acamprosate plus naloxone) followed on the subsequent day. Thereafter, this testing-scheme (i.e. drug-test, saline-test and anti-craving-drug-test) was repeated for three times that finally all rats underwent four drug- and four saline-tests and also four tests after administration of one of the three anti-craving-drugs or saline. The anti-craving-drugs and the internal saline control were applied according to a Latin square design in a way that one fourth of the rats of each group started (day 17) the tests with one of the four treatments (i.e. acamprosate, naloxone, their joint administration or internal saline control). According to the Latin square design, the remaining anti-craving-drug treatments followed on the following anti-craving-drug tests (days 20, 23, 26) that finally each rat received each of the three anti-craving-drugs and the internal saline control just once. The Latin square design was used to account for possible extinction-related effects on drug-CPP expression.

Data analysis

Statistical analysis was performed by using the program GB-Stat 7.0 (Dynamic Microsystems Inc.). In all statistical tests, significance levels were set to $p < 0.05$ and $p < 0.01$. Locomotion during the tests was calculated according to the number of light-beam breaks in all three chambers.

Table 1. Repeated-testing-schedule. The conditioned place preference experiment was divided into three phases: The pretest-phase consisted of three pretests, followed by a conditioning-phase of ten days when cocaine or morphine (both 10 mg/kg i.p.) was alternately applied with saline to the cocaine- or morphine-group, respectively. After one day (day 14) without testing (rats remained untreated in their home-cage), the testing-phase followed (days 15–26) with four anti-craving-drug tests (=$\mathbf{X_{1-4}}$, i.e. saline as internal control, acamprosate, naloxone or a combination of acamprosate plus naloxone; applied according to a Latin Square design; for details see materials and methods section) and four intervening drug- (=$drug_{1-4}$; i.e. treatment with the respective conditioning drug, using 5 mg/kg i.p. of each drug) and saline- (=$saline_{1-4}$) tests

Day	1	2	3	4–13	14	15	16	17	18	19	20	21	22	23	24	25	26
Test	pretest 1	pretest 2	pretest 3	conditioning	no test	test 1	test 2	test 3	test 4	test 5	test 6	test 7	test 8	test 9	test 10	test 11	test 12
Treatment	–	–	–	drug or saline	–	$drug_1$	$saline_1$	$\mathbf{X_1}$	$drug_2$	$saline_2$	$\mathbf{X_2}$	$drug_3$	$saline_3$	$\mathbf{X_3}$	$drug_4$	$saline_4$	$\mathbf{X_4}$
	pretest-phase			conditioning-phase		testing-phase											

The CPP values were calculated by subtracting the time spent in the saline-associated chamber from the time spent in the drug-associated chamber for each rat. The locomotion and CPP data of the cocaine- and the morphine-conditioned groups were analysed by repeated measures ANOVA followed by multiple post-hoc tests (Fisher's LSD). In each group the values of the three pretests were averaged for statistical analysis. To control for expression of CPP, the unconditioned place preference during the average pretest (av pre) served as a reference. To further account for the effects of the anti-craving-drugs (i.e. saline, acamprosate, naloxone and combination of acamprosate plus naloxone) on CPP expression the average CPP during the intervening saline-tests (av sal = $saline_{1-4}$) was used as reference. For analysis of locomotion data, the average saline (av sal = $saline_{1-4}$) locomotion served as reference. Rats that did not show stable CPP expression during the four intervening saline-tests were excluded from statistical analysis. The criterion for stable CPP expression for each rat was reached if the average CPP expression during the intervening saline-tests minus the average pretest-preference was >100 s. The 100 s-criterion has already been used in a previous study (Herzig and Schmidt, 2004) where it proved effective in selecting only the rats that showed stable cocaine- or morphine-CPP expression.

Results

Four rats in the cocaine- and two rats in the morphine-group missed the criterion for stable CPP expression and were consequently excluded from further analysis. Additionally, the time spent in the middle chamber was not significantly altered (e.g. increased or decreased) by repeated testing in both the cocaine- and the morphine-conditioned group. Repeated measures ANOVA revealed no effect of repeated testing on the time spent in the middle chamber in the cocaine-group during the intervening cocaine- ($F_{3,21} = 0.700$; $p = 0.5628$) or saline-tests ($F_{3,21} = 1.140$; $p = 0.356$) and also in the morphine-group during the intervening morphine- ($F_{3,27} = 1.029$; $p = 0.3953$) or saline-tests ($F_{3,27} = 2.360$; $p = 0.0937$). As no effect of repeated testing on the time spent in the middle chamber was observed in both groups, these data are not presented.

A stable cocaine-CPP expression was found during all tests in the cocaine-group (Fig. 1a). All treatments with anti-craving-drugs had no effect on cocaine-CPP expression. Repeated measures ANOVA that was used to account for significant CPP expression showed a significant effect of treatment ($F_{6,42} = 4.597$; $p = 0.011$). Multiple post-hoc tests (Fisher's LSD) comparing CPP expression during all tests with the CPP during the average pretest detected significant cocaine-CPP expression ($p < 0.01$) during all tests. Multiple post-hoc tests (Fisher's LSD) with the average saline CPP as reference did not show significant effects of any treatment ($p > 0.05$ for each test) on cocaine-CPP expression. Separate analysis (repeated measures ANOVA) of the intervening cocaine- and saline-tests (data not shown) revealed no alteration of CPP-expression by repeated testing in the cocaine-group

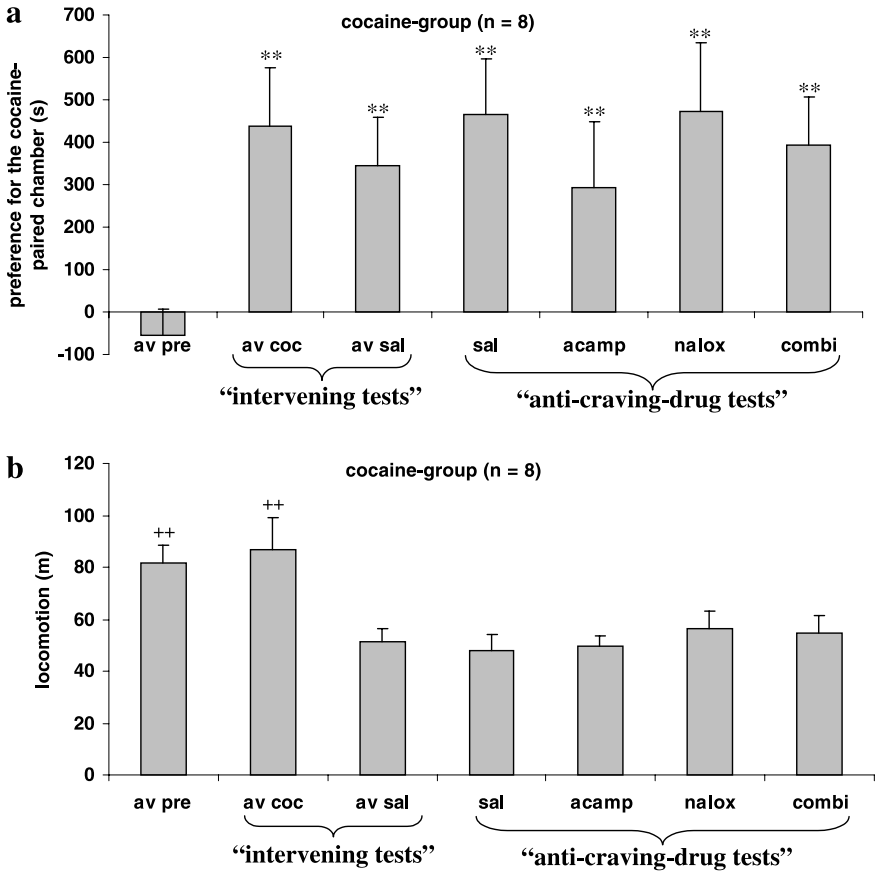

Fig. 1. Influence of treatment on cocaine-CPP expression. (**a**) Cocaine-CPP expression (=time spent in cocaine- minus time spent in saline-associated chamber) and (**b**) locomotion (in all three chambers) in the cocaine-conditioned group ($n = 8$). The average of three pretests is indicated by "av pre", while "av coc" and "av sal" indicate the average of four intervening cocaine- (i.e. $cocaine_{1-4}$) or saline- (i.e. $saline_{1-4}$) tests, respectively. Furthermore, the anti-craving-drug tests after saline ("sal"), acamprosate ("acamp"), naloxone ("nalox") and combination of acamprosate plus naloxone ("combi") are presented. For the detailed testing-order see Table 1 and materials and methods section. Repeated measures ANOVA followed by Fisher's LSD post-hoc comparison showed significant CPP expression during all tests ($^{**}p < 0.01$, average pretest-CPP as reference), but no effect of any anti-craving-drug treatment on CPP expression (all $p > 0.05$, average saline-CPP as reference). Locomotion was significantly increased ($^{++}p < 0.01$, average saline locomotion as reference) during the average pretest and the average cocaine-test

($cocaine_{1-4}$-tests: $F_{3,21} = 0.242$; $p = 0.8658$; $saline_{1-4}$-tests: $F_{3,21} = 0.062$; $p = 0.9794$).

Analysis of the locomotion data in the cocaine-group (Fig. 1b) showed a significant effect of treatment ($F_{6,42} = 5.850$; $p = 0.0002$) and multiple post-hoc tests (Fisher's LSD) revealed significantly ($p < 0.01$) increased locomotion (as compared to average saline locomotion) during the average pretest and during the average cocaine-test.

In the morphine-group (Fig. 2a), also stable morphine-CPP expression was found during all tests. Parallel to the results in the cocaine-group, all treatments with anti-craving-drugs had no effect on morphine-CPP expression. Repeated measures ANOVA of the average pretest and all tests showed a significant effect of repeated treatments ($F_{6,54} = 9.293$; $p < 0.0001$). Multiple post-hoc tests (Fisher's LSD) comparing CPP expression during all tests with the CPP during the average pretest detected significant morphine-CPP expression ($p < 0.01$) during all tests. Multiple post-hoc tests (Fisher's LSD) with the average saline CPP as reference revealed significantly increased CPP expression during the average morphine-test ($p < 0.05$) but no significant effects of any other tests ($p > 0.05$ for each test). Separate analysis (repeated measures ANOVA) of the intervening morphine- and saline-tests (data not shown) revealed no extinction-induced decrease in CPP-expression by repeated testing in the morphine-group ($morphine_{1-4}$-tests: $F_{3,27} = 0.585$; $p = 0.63$; $saline_{1-4}$-tests: $F_{3,27} = 2.047$; $p = 0.131$).

Analysis of the locomotion data in the morphine-group (Fig. 2b) showed a significant effect ($F_{6,54} = 6.483$; $p < 0.0001$) and multiple post-hoc tests (Fisher's LSD) revealed significantly ($p < 0.01$) increased locomotion (as compared to average saline locomotion) during the average pretest.

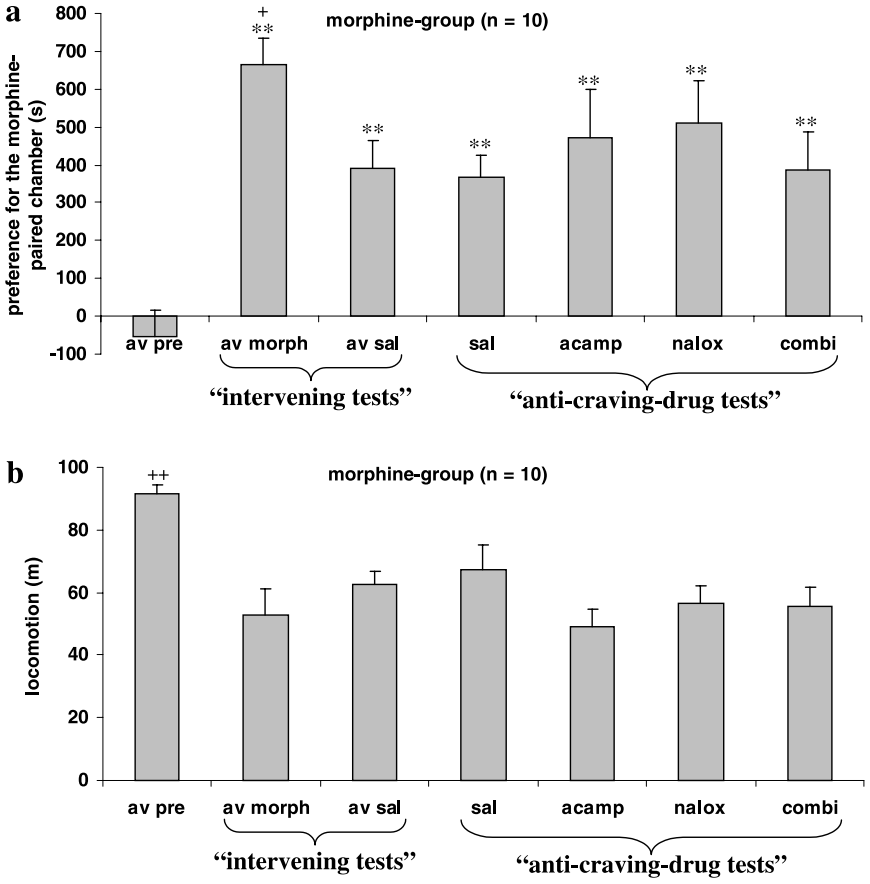

Fig. 2. Influence of treatment on morphine-CPP expression. (**a**) Morphine-CPP expression (=time spent in morphine- minus time spent in saline-associated chamber) and (**b**) locomotion (in all three chambers) in the morphine-conditioned group (n = 10). The average of three pretests is indicated by "av pre", while "av morph" and "av sal" indicate the average of four intervening morphine- (i.e. morphine$_{1-4}$) or saline- (i.e. saline$_{1-4}$) tests, respectively. Furthermore, the anti-craving-drug tests after saline ("sal"), acamprosate ("acamp"), naloxone ("nalox") and combination of acamprosate plus naloxone ("combi") are presented. For the detailed testing-order see Table 1 and materials and methods section. Repeated measures ANOVA followed by Fisher's LSD post-hoc comparison showed significant CPP expression during all tests ($^{**}p < 0.01$, average pretest-CPP as reference). No effect of any anti-craving-drug treatment on CPP expression was observed (all $p > 0.05$, average saline-CPP as reference), but CPP expression was significantly increased during the average morphine test ($^{+}p < 0.05$). Locomotion was significantly increased ($^{++}p < 0.01$, average saline locomotion as reference) during the average pretest

Discussion

The exclusion of four rats in the cocaine- and two rats in the morphine-group produced sub-groups of rats showing stable CPP-expression for at least 12 repeated tests. The stable CPP expression enabled testing of different anti-craving-drugs in the same rats. The reasons for the exclusion of some rats were either random fluctuations in preference behaviour during the intervening drug- and saline-tests (ranging from CPP to conditioned place aversion) or a fast extinction of CPP expression as shown by other rats. In our opinion, both cases indicate that these rats had not acquired stable CPP. However, a good animal model of conditioned drug-reward should produce a stable behaviour similar to the difficult-to-extinguish conditioned drug effects observed in drug addicts. In weaned cocaine addicts for example, drug-associated cues can induce craving even after years of abstinence finally leading to relapse (Childress et al., 1999). We assume that only anti-craving-drugs that show effectiveness under such strict conditions (i.e. that they are effective against robust drug-CPP expression) might have some effectiveness in later clinical trials. However, rats selected for their robust CPP expression might also be particularly resistant to anti-craving-drug treatment, resulting in an underestimation of the acamprosate- or naloxone-effect in the present study. Thus, the use of subgroups of rats has to be kept in mind by comparing the present results with results of other studies that included all rats (incl. the non-responders) into the analysis.

Suitability of the repeated-testing-schedule

In the present study, each rat in both groups was repeatedly tested (i.e. 12 repeated tests, see Table 1) for CPP expression after different treatments. The advantage of repeated testing is that different anti-craving-drugs can be compared for their effectiveness in reducing drug-CPP expression. Additionally, a similar testing-schedule might also be applied to test different doses of the same anti-craving-drug within the same rats. Another advantage is that a more favourable statistical comparison can be used (within-group comparison instead of between-group comparison). Most importantly, repeated testing can contribute to the reduction of the overall number of required experimental animals.

The strongest argument against repeated testing of the same rats in a CPP paradigm is that drug-CPP expression usually declines continuously due to extinction processes. Mueller and Stewart (2000) for example showed that significant extinction of cocaine-CPP expression can be observed after eight subsequent tests under saline. This is relevant, since 12 subsequent tests have been carried out in the present study. However, Mueller and Stewart (2000) included all rats (presumably incl. rats that show fast extinction of CPP expression) into their analysis which might explain the observed reduction of CPP expression after eight tests. Additionally, another important study that already applied repeated tests of ethanol-CPP expression showed a remarkable degree of resistance to extinction despite a decrease in CPP expression after repeated saline-tests in mice (Cunningham et al., 1998). This might indicate that one part of the mice show fast extinction of CPP expression (contributing to the decrease) while the other part of the mice show stable CPP expression (contributing to the resistance to extinction). In the present study, the use of a 100 s selection-criterion (that has already been applied in Herzig and Schmidt, 2004) excluded rats that show fast extinction. Furthermore, the application of intervening drug-tests might have also contributed to the observed lack of CPP extinction, as drug-tests were reported to maintain CPP expression on a high level (Mueller and Stewart, 2000; Mueller et al., 2002). In conclusion, the selection of rats showing stable CPP expression in combination with the present testing-schedule (i.e. intervening drug-tests) produced stable CPP expression during 12 repeated tests and largely excluded extinction-induced effects.

Besides extinction, another argument against repeated testing of CPP expression is the use of the intervening drug- and saline-tests. The intervening drug-tests have been applied to maintain high CPP expression while the intervening saline-tests functioned as control-values indicating the stability of CPP expression. Furthermore, possible acute withdrawal effects were minimized by this testing-design, since always one saline-test separated the drug-tests from the anti-craving-drug tests. However, one could argue that a test of CPP expression in the drugged-state (when rats have access to all chambers) is a ''new-conditioning'', since the entire CPP-box (instead of the previously associated rewarded chamber) becomes conditioned to the drug. This ''new-conditioning''-effect should therefore result in a decrease of CPP expression by repeated tests. Additionally, intervening saline-tests should also produce a decrease of CPP expression due to extinction. However, in both the cocaine- and the morphine-group no statistically significant decrease of CPP expression was observed during the intervening drug- and saline-tests, indicating that four intervening drug-tests were not sufficient to alter the stable CPP expression and supporting the usefulness of the present testing-schedule. A possible explanation for the lack of the ''new-conditioning''-effect might be the fact that each of the four intervening drug-tests lasted only for 20 minutes (with half of the conditioning-dose of the respective conditioning drug), while the five initial drug-conditionings lasted for 30 minutes each. However, in extent of 12 repeated tests the ''new-conditioning''-effect might become more important and might probably limit the maximum number of repeated tests that still yield stable drug-CPP expression. Additionally, due to the intervening drug- and saline-tests, the present repeated-testing schedule may also not be useful to evaluate effects that require repeated treatment with the same drug.

A remaining concern of the present testing-schedule is that the anti-craving-drug (i.e. acamprosate, naloxone, or the combination of acamprosate and naloxone) that is tested first might influence (e.g. due to induction of tolerance or sensitization) the behavioural outcome for the drug tested thereafter. Such effects cannot be completely excluded by the present testing-schedule, but the applied Latin square design with the internal saline-control tests (i.e. one of the four treatments during the anti-craving-drug tests was saline) minimizes this effects. Furthermore, since there were always a drug- and a saline-test between two anti-craving-drug tests, only anti-craving-drugs with a very long half-life can still be present in a behaviourally relevant amount to directly affect subsequent tests. In summary, we conclude that the applied

repeated-testing-schedule (Table 1) produced stable drug-CPP expression during all tests and may therefore be used to evaluate the effects of different anti-craving-drugs (or different doses of the same anti-craving-drug) on CPP expression. Thereby, the overall number of experimental animals used for future CPP studies might be reduced.

Influence of acamprosate on CPP expression

In the present study, acamprosate showed no effect on cocaine- or morphine-CPP expression. Regarding morphine, this fits to our previous results (Herzig and Schmidt, 2005), suggesting that the outcome of the repeated-testing schedule is comparable to the results of an ordinary testing procedure. The observed ineffectiveness of acamprosate cannot be explained by possible alterations in locomotion, as acamprosate did not show any effect on locomotion in both groups. However, the SEM of the cocaine-CPP data during the acamprosate test is relatively large. Consequently, it cannot be excluded that by using a larger number of animals, the acamprosate-effect in the cocaine-group might have been significant. Thus, we suggest further studies to clarify the ineffectiveness of acamprosate on cocaine-CPP expression. Additionally, a recent study demonstrated that acamprosate inhibited development of cocaine- but not morphine-CPP (McGeehan and Olive, 2003). However, we assume that development of CPP is not the appropriate measurement for evaluation of anti-craving-drug effects, as these drugs should be effective after the behaviour has developed (not during development). Based on the present results, we therefore conclude that acamprosate is not likely to be effective in reducing conditioned reward and craving in humans addicted to cocaine or morphine. Regarding morphine, this conclusion fits to the suggested ineffectiveness of acamprosate in treatment of cue-induced relapse in opiate addicts (Spanagel et al., 1998). Additionally, heroin self-administration, heroin- and stress-induced relapse, development of morphine-sensitization (Spanagel et al., 1998; Kratzer et al., 2003) and the discriminative stimulus properties of morphine were not affected by acamprosate (Pascucci et al., 1999). However, acamprosate suppressed expression of morphine-sensitization (Spanagel et al., 1998) and inhibited the motivational component of morphine withdrawal (Kratzer and Schmidt, 1998). Furthermore, the intensity of physical morphine-dependence was also reduced by acamprosate in mice (Sepulveda et al., 2002). Thus, acamprosate only alters specific aspects of opiate-induced behaviours, leaving others unaffected. Overall, the observed ineffectiveness of acamprosate in cocaine- and morphine-conditioned reward does not support further clinical studies using acamprosate as anti-craving-drug in cocaine- or morphine-addicts. Nevertheless, this contrasts the already reported clinical effectiveness of acamprosate in ethanol-induced behaviours (for review see Spanagel and Zieglgänsberger, 1997). Thus, we suggest different mechanisms underlying expression of cocaine- and morphine-conditioned reward on the one side and ethanol-conditioned reward on the other side. Additionally, the discussed NMDA-antagonistic properties of acamprosate (see Spanagel and Zieglgänsberger, 1997) are obviously rather weak, because a strong NMDA-antagonistic action of acamprosate should have produced a reduction of morphine-CPP expression similar to the reduction observed for several other NMDA-antagonists (Popik and Danysz, 1997; Tzschentke and Schmidt, 1997; Kotlinska and Biala, 1999; Popik and Kolasiewicz, 1999; Papp et al., 2002; Popik et al., 2003).

Influence of naloxone on CPP expression

The present results show that just like acamprosate, naloxone did not significantly affect cocaine- or morphine-CPP expression. Regarding morphine-CPP expression, the ineffectiveness of naloxone in the present study reproduced our previous results (Herzig and Schmidt, 2005) further emphasizing the suitability of the repeated-testing schedule. The ineffectiveness of naloxone cannot be explained by an altered locomotion, as naloxone-treatment did not affect locomotion in both groups. However, these results fit to the observation that cocaine-induced reinstatement of cocaine-seeking was not affected by pre-treatment with the similar μ-opiate-receptor antagonist naltrexone (Comer et al., 1993). Regarding opiates, morphine- (Stewart, 1983) and heroin-induced (Shaham and Stewart, 1996) relapse to heroin self-administration were reduced by naltrexone. On the other hand, heroin-seeking was not affected by naloxone pretreatment (Alderson et al., 2000) while even an increase of morphine-CPP expression was demonstrated in mice and rats after naloxone treatment (Noble et al., 1993; Neisewander et al., 1990). The lack of increased morphine-CPP expression in the present study might therefore be explained by the use of different procedural designs (e.g. strain of rats, duration of conditioning and testing, etc.). Overall, the present results do not suggest that naloxone might be effective as an anti-craving-drug in cocaine- or morphine-addicts.

Influence of acamprosate-naloxone-combination on CPP expression

In line with the results for the single anti-craving-drugs, combined administration of acamprosate and naloxone also failed to affect locomotion and cocaine- or morphine-CPP expression. Another preclinical study also failed to detect any additive or synergistic effects of a combined treatment in reducing ethanol consumption (Stromberg et al., 2001). However, this contrasts recent preclinical and clinical studies suggesting that the combination-treatment has a superior effect in relapse prevention of alcoholism than both drugs given separately (Kim et al., 2004; Kiefer et al., 2003). Nevertheless, it is in line with our suggestion (see above) of different mechanisms underlying expression of cocaine- or morphine- vs. ethanol-conditioned reward.

In summary, neither acamprosate and naloxone given separately nor their combined administration significantly reduced cocaine- or morphine-CPP expression. Thus, we suggest that these anti-craving-drugs may not be effective in cocaine- or morphine-addicts. Methodologically, the applied repeated-testing-schedule proved to be suitable for testing the effectiveness of different anti-craving-drugs on drug-CPP expression and may therefore help to reduce the number of experimental animals.

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