The conditioned place preference (CPP) paradigm has frequently been used as a method of assessing the reinforcing properties of drugs of abuse and to study the neural mechanisms underlying conditioned reinforcement. The majority of drugs abused by humans, including psychostimulants, opiates, ethanol, and benzodiazepines, will also produce a CPP (see, Carr et al., 1989, for review). During recent years, it has been reported that both the development and expression of a CPP are dependent on glutamatergic transmission. Specifically, it has been proposed that there are different roles for two of the main receptor subtypes for glutamate: the N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptors (Cervo and Samanin, 1995; Tzschentke and Schmidt, 1997).

Transmission via the NMDA receptor subtype has been argued to be necessary for both the induction and the expression of a CPP because the NMDA channel blocker MK-801 prevented the induction of a cocaine (Cervo and Samanin, 1995), methamphetamine (Kim and Jang, 1997), and morphine CPP in rats (Tzschentke and Schmidt, 1995, 1997; Kim et al., 1996). This effect has been replicated with the competitive NMDA receptor antagonist CGP37849, preventing the induction of a morphine CPP, again in rats (Tzschentke and Schmidt, 1995). However, Hoffman (1994) reported that MK-801 failed to affect the induction of an amphetamine (AMPH)-induced CPP. Studies investigating the effect of NMDA receptor antagonists on the expression of a CPP reveal conflicting results. MK-801 failed to prevent the expression of a cocaine-induced CPP (Cervo and Samanin, 1995) but did prevent the expression of a morphine-induced CPP (Tzschentke and Schmidt, 1997). Furthermore, Bespalov (1996) reported that the competitive antagonist (±)-3-(2-carboxy-piperazine-4-yl)-propyl-1-phosphonic acid prevented the expression of an AMPH-induced CPP.

The underlying involvement of AMPA receptors in CPP appears to be much clearer. First, the AMPA receptor antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX) prevented the expression of a CPP induced by cocaine (Cervo and Samanin, 1995; Kaddis et al., 1995), AMPH, and morphine (Layer et al., 1993). Second, the noncompetitive AMPA receptor antagonist GYKI 52466 has been reported to prevent the expres-
sion of both a morphine and an AMPH-induced CPP (Tzschtentke and Schmidt, 1997). Although DNQX infused into the nucleus accumbens was shown to be ineffective against the induction of a morphine CPP (Layer et al., 1993) and intracerebroventricular DNQX was ineffective against the induction of a cocaine-induced CPP (Cervo and Samanin, 1995), intra-accumbens DNQX did attenuate the expression of an AMPH (Layer et al., 1993) and a cocaine-induced CPP (Kaddis et al., 1995). Therefore, the current literature suggests that AMPA receptors mediate the expression of drug-induced CPPs but that AMPA receptor antagonists show a more inconsistent profile when tested on the induction.

One consistent problem with the studies investigating the role of AMPA receptors is that they have all involved the use of AMPA receptor antagonists that have relatively low selectivity at the AMPA receptor. DNQX also possesses antagonistic properties at the strychnine-insensitive glycine site of the NMDA receptor (Kessler et al., 1989; Pellegrini-Giampietro et al., 1989; Sheardown et al., 1990; Goldstein and Litwin, 1993; Yoneda et al., 1993; Birch et al., 1988), and there is evidence that GYKI 52466 may also influence NMDA receptor-mediated transmission (Steppuhn and Turski, 1993), although it does not act at NMDA receptors (Honore`, 1991; Ouardouz and Durand, 1991). Because NMDA receptor antagonists can prevent the expression of a CPP (Bespalov, 1996; Tzschtentke and Schmidt, 1997), caution must be exercised in interpreting the results from the less selective AMPA receptor antagonists.

The purpose of the studies reported here was to assess the role of glutamatergic transmission via AMPA receptors in an AMPH-induced CPP in mice using the selective competitive AMPA receptor antagonist 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzofuran-2(1H)-quinoline (NBQX) (Sheardown et al., 1990). For comparison, we also investigated the effect of the less selective AMPA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), which has a similar pharmacological profile to DNQX (Birch et al., 1988; Harris and Miller, 1989; Kessler et al., 1989; Pellegrini-Giampietro et al., 1989), although its selectivity for AMPA receptors compared with NMDA receptors is slightly higher than that for DNQX (Sheardown et al., 1990). Subsequently, we explored the involvement of the glycine site of the NMDA receptor by assessing the effect of the strychnine-insensitive glycine-site antagonist L-701,324. The results of these studies allowed us to make conclusions concerning the reliability of the previous studies investigating AMPA receptor involvement in a drug-induced CPP.

**Materials and Methods**

**Animals**

Male C57Bl/6J × SV129 mice, bred at the University of Sussex, were used in all experiments. Animals weighed 21 to 29 g at the start of experiments and were housed in groups of two or three under a 12:12-h light/dark cycle (lights on at 7:00 AM) with ad libitum access to food and water. Holding room temperature was maintained at 20–21°C, and humidity was 40 to 60%. Experiments were performed between 8:30 AM and 3:00 PM. All experiments were carried out under U.K. legislation on animal experimentation.

**Apparatus**

All experiments were performed in a three-compartment place conditioning apparatus. The two outer compartments (200 × 200 × 200 mm) were separated by a central compartment (200 × 50 × 200 mm) and differed in visual and tactile cues. One outer compartment had white walls with a perforated metal floor, whereas the other had black and white walls (each wall was split along the diagonal, with the top half painted black and the bottom half painted white) with a smooth clear Perspex floor. The central compartment allowed movement from one compartment to the other and could also be blocked from the outer compartments with clear doors, to restrict animals to a particular compartment during the conditioning phase. The movement and location of animals during the preconditioning and test phases were recorded using a video camera (Sony SPT-M106CE) connected to a video cassette recorder (Panasonic AG-5700) to allow subsequent analysis of preference and activity.

**Procedure**

Four separate experiments were performed, for which the basic protocol was identical. In all experiments, we used an AMPH-induced (0.25 mg/kg) CPP as the paradigm to assess the role of glutamatergic mechanisms in conditioned reinforcement. In experiments 1 and 2, we investigated the effect of NBQX on the induction and the expression of a CPP. In experiments 3 and 4, we investigated the effect of CNQX and 7-chloro-4-hydroxy-3-(2-phenoxy)phenyl-2(1H)-quinolone (L-701,324) on the expression of a CPP. The design of these experiments is shown in Table 1.

**CPP Procedure**

The CPP procedure took place over 10 days and consisted of the following three phases.

**Preconditioning Phase (Day 1).** Animals received vehicle (t = 0 min), followed by saline (t = 0). Consequently, they were placed in the CPP apparatus and allowed to explore freely for 10 min. The behavior of the animals was recorded for subsequent analysis. These data provided the baseline preferences for the two compartments. Initial preferences for the two compartments did not differ significantly (mean ± S.E.M. time, 199 ± 3.7 s in compartment A, 201 ± 4.1 s in compartment B during a 10-min test, calculated from all experiments).

**Conditioning Phase (Days 2–9).** On every second day, in experiment 1, animals received NBQX (vehicle, 3, 10, or 30 mg/kg) 20 min before AMPH or NBQX (vehicle, 30 mg/kg) 20 min before saline, immediately before confinement to one compartment of the CPP apparatus. On every second day, in experiments 2, 3, and 4, animals received vehicle 20 min before AMPH and confinement to one compartment. In all four experiments, on alternate days, all animals received vehicle 20 min before saline and confinement to the other compartment. Confinement to compartments lasted for 20 min. As-
signment of drug-paired compartments was performed randomly and was fully counterbalanced across groups.

Test Phase (Day 10). Animals received NBQX vehicle (experiment 1), NBQX vehicle or 10 mg/kg (experiment 2), CNQX vehicle, 1, 3, or 10 mg/kg (experiment 3) 20 min before saline, or L-701,324 vehicle, 1, 3, or 10 mg/kg (test 1) or L-701,324 vehicle, 0.03, 0.1, or 0.3 mg/kg (test 2) 25 min before saline (experiment 4). After the saline injection, animals were allowed to freely explore the apparatus for 10 min. The behavior of the animals was recorded for subsequent analysis to determine the extent of place conditioning and activity levels.

Drugs

d-AMPH sulfate was obtained from Sigma Chemical (Poole, Dorset, UK). NBQX and CNQX were donated by Schering AG (Berlin, Germany). L-701,324 was donated by Merck Sharp and Dohme (Essex, UK). AMPH was dissolved in 0.9% saline. NBQX was dissolved in 1 M sodium hydroxide, and 0.1 M hydrochloric acid was added to take the pH back toward neutral. The solution was made up to its final volume with distilled water. This resulted in drug solutions of pH 9 to 10. NBQX vehicle was pH matched to drug by adding 0.1 M sodium hydroxide to distilled water. CNQX and L-701,324 were suspended in 0.3% Tween-80 in distilled water, which was also used as the vehicle. All drugs were administered i.p. at an injection volume of 10 ml/kg. All doses refer to drug base.

Data Analysis

The time spent in each compartment and activity within the compartments were scored blind. An animal was considered to be in a compartment only when its entire body was within that compartment. Activity was measured as line crossings of two perpendicular lines crossing the midpoint and extending to the walls of the compartment. For analysis of place conditioning, the time spent in the drug-paired compartment minus the time spent in the vehicle-paired compartment was used as the dependent variable. For analysis of activity levels, total line crossings in both compartments were used as the dependent variable. Data for place conditioning were initially analyzed using a two-way mixed-factor ANOVA (except for test 1 in experiment 4), with test session (preconditioning and test phase) and treatment as independent variables. After a significant interaction, a one-way between-subject ANOVA was performed on activity levels, total line crossings in both compartments were used as the dependent variable. Data for place conditioning were initially analyzed using a two-way mixed-factor ANOVA (except for test 1 in experiment 4), with test session (preconditioning and test phase) and treatment as independent variables. After a significant interaction, a one-way between-subject ANOVA was performed on the test session data only. If this produced a significant effect of treatment, post hoc analysis was performed using the Duncan’s test. Due to the sedative effect of the higher dose range of L-701,324 (experiment 4, test 1), the variance between groups was heterogeneous, and the data were not suitable for parametric analysis. For this reason, a comparison of the preference during the test phase and the preconditioning phase for each group was made using Wilcoxon’s signed rank test to deduce whether conditioning had occurred. Data for activity levels were analyzed using a one-way ANOVA on activity levels during the test phase, followed by Duncan’s test if a significant effect of treatment was found. All statistical analysis was performed using the SPSS statistical package for Windows (Version 6.1, SPSS Inc., Chicago, IL).

Results

CPP. The results from experiment 1, showing the effect of NBQX on the induction of place conditioning, are shown in Fig. 1. Analysis revealed a significant interaction between treatment and test session ($F_{5,53} = 4.90, p < .01$). The one-way ANOVA on the preference for the AMPH-paired compartment during the test phase revealed a significant effect of treatment ($F_{5,53} = 12.1, p < .001$), indicating that significant place conditioning had taken place. Post hoc comparisons showed a significant effect of AMPH compared with saline, indicating place conditioning ($p < .05$). NBQX, at doses of 3 and 10 mg/kg, failed to affect the induction of place conditioning, whereas a dose of 30 mg/kg significantly attenuated the preference ($p < .05$). NBQX, at a dose of 30 mg/kg, also produced a significant place aversion, compared with saline controls ($p < .05$).

The results from experiment 2, showing the effect of NBQX on the expression of AMPH-induced place conditioning, are shown in Fig. 2. The two-way ANOVA on treatment by test session revealed a significant interaction ($F_{3,31} = 3.88, p = .018$) and a subsequent one-way ANOVA on test-phase data revealed a significant effect of treatment ($F_{3,31} = 3.53, p = .026$). Post hoc analysis showed that groups conditioned to AMPH displayed a significant place preference compared with saline-conditioned groups ($p < .05$). NBQX at 10 mg/kg had no effect on this place conditioning.

The effect of CNQX on the expression of an AMPH-induced CPP (experiment 3) is shown in Fig. 3. A two-way ANOVA on conditioning...
The effect of treatment during test phase revealed a significant effect of treatment (Duncan's test). CNQX vehicle during test phase. †

Fig. 3. The effect of CNQX on the expression of an AMPH-induced CPP. The data shown represent mean seconds spent in the drug-paired [AMPH 0.25 mg/kg (Amph CPP) or saline (saline CPP)] compartment minus seconds spent in the saline-paired compartment (±S.E.M.) during the 10-min test sessions (preconditioning or test phase). All groups received CNQX (veh, 1, 3, or 10 mg/kg) 20 min before saline, immediately before test session (n = 8–10/group). *p < .05 compared with saline CPP plus CNQX vehicle during test phase. †p < .05 compared with Amph CPP plus CNQX vehicle during test phase (Duncan’s test).

treatment by test session revealed a significant interaction (F\(_{5,52} = 3.35, p = .011\)), and a subsequent one-way ANOVA on test-phase data revealed a significant effect of treatment (F\(_{5,52} = 3.25, p = .013\)). Post hoc analysis again showed significant place conditioning, with AMPH treatment resulting in more time spent in the drug compartment than saline treatment (p < .05). CNQX, at doses ranging from 1 to 10 mg/kg, blocked the expression of this CPP (p < .05).

Figure 4 shows the results of experiment 4, in which the effect of the higher dose range of L-701,324 (1–10 mg/kg) on the expression of an AMPH-induced CPP was assessed. The extreme variation observed in groups treated with L-701,324 was due to a large spread in the preference scores, which appeared to occur as a result of the locomotor suppressant effects of the compound. Wilcoxon’s signed rank test revealed that only the group conditioned to AMPH and tested with L-701,324 vehicle displayed significant place conditioning (z = −2.80, p < .01). Groups conditioned to AMPH and tested with L-701,324 (1–10 mg/kg) failed to exhibit place conditioning.

The effects of the lower dose range of L-701,324 on the expression of an AMPH-induced CPP are shown in Fig. 5. Two-way ANOVA revealed a significant treatment by test-session interaction (F\(_{5,54} = 5.19, p < .01\)), and subsequent ANOVA on the test-session data revealed a significant effect of treatment (F\(_{5,54} = 4.49, p < .01\)). Post hoc analysis revealed that AMPH produced a significant CPP (p < .05), which was prevented by L-701,324 at doses of 0.1 and 0.3 mg/kg. A dose of 0.03 mg/kg L-701,324 failed to prevent the expression of the AMPH CPP.

Activity during CPP Test. Figure 6 shows the effects of the various drug treatments on activity levels during the test phase. Figure 6A shows the activity levels during the test assessing the effect of NBQX on the expression of the CPP (experiment 2). One-way ANOVA revealed a significant effect of treatment (F\(_{3,31} = 3.31, p < .05\)), and post hoc analysis indicated that the groups receiving AMPH-NBQX and saline-NBQX displayed a significant reduction in activity compared with the group receiving AMPH-vehicle (p < .05). In contrast, CNQX (experiment 3) had no effect on activity levels during the test phase (F\(_{5,52} = 0.69, p > .05\)), as shown in Fig. 6B. The effects of L-701,324 are shown in Fig. 6, C and D. At the higher dose range (Fig. 6C), one-way ANOVA revealed a significant effect of group (F\(_{5,54} = 7.78, p < .001\)). Subsequent post hoc analysis revealed that L-701,324 significantly reduced activity at all three doses (1–10 mg/kg) compared with groups that received L-701,324 vehicle. At the lower dose range (Fig. 6D), one-way ANOVA revealed no effect of L-701,324 (0.03–0.3 mg/kg) on activity during the test-phase (F\(_{5,54} = 1.16, p > .05\)).

Discussion

The studies reported here show that the selective, competitive AMPA receptor antagonist NBQX does not prevent the
induction of an AMPH-induced CPP, except at a dose that also produces a significant conditioned place aversion (CPA). NBQX also had no effect on the expression of an AMPH-induced CPP at a dose high enough to cause a significant reduction in activity. Conversely, both the less selective AMPA receptor antagonist CNQX and the glycine-site antagonist L-701,324 prevented the expression of an AMPH-induced CPP at doses that did not affect activity levels. A summary of the relative affinities of the tested compounds for the AMPA receptor and glycine site of the NMDA receptor is shown in Table 2.

The fact that NBQX affected the induction of a CPP only at a dose that also produced a significant conditioned place aversion (CPA). NBQX also had no effect on the expression of an AMPH-induced CPP at a dose high enough to cause a significant reduction in activity. Conversely, both the less selective AMPA receptor antagonist CNQX and the glycine-site antagonist L-701,324 prevented the expression of an AMPH-induced CPP at doses that did not affect activity levels. A summary of the relative affinities of the tested compounds for the AMPA receptor and glycine site of the NMDA receptor is shown in Table 2.

TABLE 2
Affinities of NBQX, CNQX, and L-701,324 for AMPA receptors and the glycine site of the NMDA receptor

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC_{50} (nm)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AMPA</td>
<td>Glycine</td>
</tr>
<tr>
<td>NBQX</td>
<td>150^a</td>
<td>&gt;100,000^b</td>
</tr>
<tr>
<td>CNQX</td>
<td>300^a</td>
<td>14,000^b</td>
</tr>
<tr>
<td>L-701,324</td>
<td>&gt;100,000^a</td>
<td>2^c</td>
</tr>
</tbody>
</table>

*IC_{50} values for displacement of [3H]AMPA, [3H]glycine, or [3H]-689,560 binding in rat brain membranes. L-689,560 is a highly selective antagonist at the strychnine-insensitive glycine site of the NMDA receptor (Leeson et al., 1992).*
activity levels argues against the possibility of a species difference, as the effect of CNQX in mice was consistent with effects of DNQX in rats. Second, it suggests that the route of drug administration was not a decisive factor in explaining differences in published results. The previous reports on AMPA receptor antagonists preventing the expression of CPP various routes of administration used, including intra-accumbens, intracerebroventricular, and systemic. The present study confirms that the less selective AMPA receptor antagonists prevent the expression of drug-induced CPPs when given systemically. This appears to suggest that the reason for the different results found with NBQX and the other AMPA receptor antagonists (DNQX, CNQX, and GYKI 52466) is that the latter substances influence NMDA receptor-mediated transmission. In particular, the affinity of CNQX and DNQX for the glycine site of the NMDA receptor may be important for their activity in CPP experiments. We therefore predict that a glycine-site antagonist would also prevent the expression of an AMPH-induced CPP.

The results of experiment 4 provide evidence in support of this explanation. The selective glycine site antagonist L-701,324 prevented the expression of an AMPH-induced CPP, at doses that did not affect activity levels. The results of test 1 were complicated by the sedative effects of the higher dose range of L-701,324 (1–10 mg/kg), although analysis revealed that these doses prevented the expression of the CPP. Test 2, however, revealed a dose-dependent effect of L-701,324 on the expression of the CPP, with the two higher doses (0.1 and 0.3 mg/kg i.p.) preventing the CPP, whereas a dose of 0.03 mg/kg reduced the CPP but not significantly. All three of these lower doses did not affect activity levels. Recently, a similar effect of L-701,324 has been reported by Danysz et al. (1998), whereby an attenuation in the expression of a morphine-induced CPP in rats was observed.

The results of the present study suggest that previous findings with the less selective AMPA receptor antagonists may be misleading. We report here that the effects of an antagonist such as CNQX differ from those found with the more selective antagonist NBQX. Furthermore, we report that this difference can be explained by the action of CNQX at the modulatory strychnine-insensitive glycine site of the NMDA receptor, as an antagonist at this site shows a similar behavioral profile to CNQX. The implications of this study are not necessarily restricted to the AMPH-CPP paradigm. Further work is necessary to see whether this finding extends to other drugs of abuse that produce a CPP, such as morphine and cocaine, although the report by Danysz et al. (1998) suggests that this may be the case. Perhaps more importantly, care must be taken in interpreting previous in vivo studies using the class of less selective AMPA receptor antagonists in other paradigms.

It has been suggested that AMPA receptors play an important role in mediating AMPH-enhanced responding for conditioned reinforcement (Burns et al., 1994) and responding for a drug-paired stimulus (Hitchcott and Phillips, 1997). Both of these studies involved the use of CNQX as a method of investigating AMPA receptor involvement in the behavioral response. Similarly, many reports of drug-induced behavioral sensitization have also used CNQX and DNQX as tools for studying AMPA receptor involvement in the behavioral effects of drugs of abuse (Karler et al., 1991, 1994; Pierce et al., 1996). Our present findings suggest it may be necessary to reexamine the conclusions of these studies if more selective antagonists give rise to different effects.

In summary, the selective competitive AMPA receptor antagonist NBQX did not affect either the induction or the expression of an AMPH-induced CPP. In contrast, both the less selective AMPA receptor antagonist CNQX and the selective glycine-site antagonist L-701,324 prevented the expression of an AMPH-induced CPP. These results suggest that the induction and the expression of an AMPH-induced CPP in mice do not depend on glutamatergic transmission via AMPA receptors but that the expression of the response is dependent on modulation of NMDA receptor-mediated transmission via the glycine site. This importance of the glycine site of the NMDA receptor in mediating drug-induced conditioned reinforcement suggests that compounds acting at this site may have therapeutic potential in the treatment of drug craving. The present study also raises doubts concerning the conclusions drawn from previous studies using the less selective AMPA receptor antagonists, such as DNQX and CNQX. Further work is necessary to deduce whether the conclusions drawn from these studies are indeed misleading.

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References


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AMPA Receptors and Conditioned Place Preference


