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ORIGINAL INVESTIGATION

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Attenuation of cocaine-seeking behaviour by the AMPA/kainate receptor antagonist CNQX in rats

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Abstract *Rationale:* It has been suggested previously that conditioned effects on drug-seeking behaviour are in part mediated through glutamatergic neurotransmission. *Objectives:* To optimise a second-order schedule of IV cocaine reinforcement in Wistar rats and investigate the effects of the systemic AMPA/kainate receptor antagonist CNQX on cocaine-seeking behaviour under this schedule. *Methods:* Free-feeding Wistar rats were trained to respond for an IV cocaine infusion (0.25 mg/infusion) under a FI15 min(FR7:S) schedule, whereby the completion of FR7 responses led to the presentation of a conditioned stimulus (CS). After two 15-min fixed intervals, rats were allowed to respond for cocaine under an FR4(FR7:S) second-order schedule for another 120 min. After acquisition of stable responding, the cocaine unit dose was increased to 0.50 mg/infusion. The effects of CNQX (0, 0.75, 1.5, and 3 mg/kg IP) on cocaine seeking were then examined using a within-subjects design. *Results:* Increasing the cocaine unit dose increased responding during the first and second intervals, with a decrease in the latency to the first CS. CNQX decreased the number of cocaine responses in a dose-dependent manner during the first 15-min cocaine-free interval, but did not affect cocaine responding during either the second interval or the latter part of the session under the FR4(FR7:S) schedule. In the locomotor activity test, reductions in rearing were produced by higher CNQX doses than those that attenuated significantly responding during the first fixed interval. *Conclusions:* These results suggest that AMPA/kainate receptors are involved in mediation of cocaine-seeking behaviour controlled partly by cocaine-associated cues.

Keywords Cocaine · Self-administration · Drug-seeking · Second-order schedule · Glutamate receptors

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Introduction

Environmental stimuli present during drug taking may acquire powerful incentive-motivational properties through classical conditioning and elicit drug craving and relapse, sometimes following years of abstinence. Such conditioned responses have been demonstrated for many abused drugs (Carter and Tiffany 1999). For example, abstinent cocaine users report intense drug craving and arousal when exposed to environmental cues previously associated with cocaine use (Childress et al. 1993; Foltin and Haney 2000). These conditioned responses to drug-associated stimuli have been suggested to contribute to the chronic, relapsing nature of drug addiction (O'Brien et al. 1998).

Dopaminergic transmission has been shown to be one of the key neurobiological substrates mediating conditioned responses elicited by environmental cues both in humans and laboratory animals. Human brain imaging data have demonstrated activation of many dopamine-rich forebrain regions in cocaine craving (Grant et al. 1996; Childress et al. 1999; Kilts et al. 2001). In rats, exposure to stimuli associated with cocaine availability enhanced dopamine release in the nucleus accumbens and amygdala, and increased Fos immunoreactivity in the medial prefrontal cortex and amygdala (Ito et al. 2000; Weiss et al. 2000; Ciccocioppo et al. 2001). Moreover, dopamine antagonists attenuated cue-induced neural activation and cocaine-seeking (Weiss et al. 2001).

There is also evidence for the involvement of glutamate transmission in procession of drug-paired cues. Infusion of an AMPA receptor antagonist into the nucleus accumbens decreased responding for cocaine-associated cues under a second-order schedule of cocaine reinforcement, while an NMDA antagonist was without effect (Di Ciano and Everitt 2001). In contrast, extinguished cocaine-seeking behaviour could be reinstated by infusion of both NMDA and AMPA glutamate receptor agonists into the nucleus accumbens, with the AMPA agonist producing a more selective effect on cocaine-seeking (Cornish et al. 1999; Cornish and Kalivas 2000). The

nucleus accumbens glutamate transmission has also been suggested to mediate enhancement of behavioural sensitisation by environmental stimuli paired with repeated cocaine injections (Bell et al. 2000).

The importance of drug-associated environmental stimuli has been demonstrated in many animal models of cocaine-seeking and relapse, including second-order schedules of cocaine reinforcement (Markou et al. 1993; Everitt and Robbins 2000). These schedules are particularly useful for measuring drug-seeking behaviour, because under them responding is controlled at least partly by the contingent presentation of previously neutral stimuli that have acquired conditioned reinforcing properties through repeated pairing with the primary reinforcer. Moreover, responding for the primary drug reinforcer can be maintained for prolonged periods of time without the confounding pharmacological effects of the reinforcer.

Animal models employing second-order schedules may be valuable for developing systemically administered pharmacological therapies aiming at diminishing the motivational effects of stimuli associated with abused drugs, especially psychomotor stimulants, in human addicts. For example, systemic administration of a partial dopamine D_3 receptor agonist was shown to suppress cocaine-seeking under a second-order schedule in rats (Pilla et al. 1999). Although both neuroanatomical and pharmacological data suggest that glutamate transmission is involved in mediation of drug-associated stimuli, modulation of cue-controlled drug-seeking behaviour by systemically administered antagonists at AMPA or NMDA glutamate receptors has not been demonstrated. Consequently, our goal was to examine the effects of systemic injections of a water-soluble formulation of the AMPA receptor antagonist CNQX on cocaine responding under a second-order schedule in rats. It is important to show that glutamate antagonists can modulate drug-seeking behaviour without their well-known effects on motor functions (Jackson et al. 2000). Therefore, we tested the effects of CNQX also on spontaneous locomotor activity. The second-order schedule developed and optimised for the present experiments was based on procedures described previously (Whitelaw et al. 1996; Arroyo et al. 1998).

Materials and methods

Animals

Male Wistar rats (Harlan, The Netherlands) weighing 180–200 g upon arrival were housed in pairs in Macrolon IV cages in a temperature and humidity controlled room under a reversed 12-h light-dark cycle (lights off at 0600 hours). Water and pellet food (RM1, SDS, Witham, UK) were available ad libitum in the home cage except during food training (see below). All behavioural testing was carried out during the dark phase of the light-dark cycle between 0800 and 1500 hours, 5 days a week. All experimental procedures using animals were conducted under the National Animal Welfare Act and were approved by the Institutional Animal Care and Use Committee at the National Public Health Institute and the Chief Veterinarian of the County Administrative Board.

Apparatus

Both the initial food training and cocaine self-administration took place in operant chambers (Model ENV-112B; Med Associates, Georgia, Vt., USA), enclosed in ventilated sound-attenuating cubicles. The chambers were equipped with a food hopper in the middle of the front panel and two retractable levers on both sides of the food hopper. A white stimulus light was mounted above each lever. Auditory stimuli were delivered from a speaker positioned on the front panel. A food dispenser located behind the front panel delivered 45 mg Noyes pellets to the food hopper. Intravenous (IV) infusions were delivered at the volume of 0.1 ml by means of activating an infusion pump outside the sound-attenuating cubicle. The infusion pump was attached to a counterbalanced liquid swivel with Tygon tubing. From the swivel, Tygon tubing protected by a steel spring passed through a hole in the operant chamber and was connected to the catheter base at the midscapular region of the animal. Schedule contingencies and data collection were controlled by a computer using the MED-PC behavioural software (Med Associates).

Surgery

Rats were anaesthetised with an isoflurane-air mixture and implanted with a chronic jugular catheter, as described previously (Caine et al. 1993). Briefly, the catheter assembly made in-house consisted of a 13-cm length of silastic tubing (inside diameter 0.31 mm; outside diameter 0.64 mm), attached to a guide cannula that was bent at a right angle. The cannula was embedded into a dental cement base and anchored with surgical polypropylene mesh. The catheters were implanted with the proximal end reaching the heart through the right jugular vein, continuing subcutaneously over the shoulder, and exiting between the scapulae. After surgery, catheters were flushed once a day for the next 10 days with a 0.05 ml infusion of an antibiotic (Oriprim; Orion Pharma, Espoo, Finland) and a 0.1 ml infusion of 0.9% saline containing heparin (30 IU/ml) to prevent infection. Thereafter, the catheters were flushed daily with heparinised saline at the end of each self-administration session. Catheter patency was tested by infusing the short-acting anaesthetic methohexital (Brietal, Eli Lilly and Co., Indianapolis, Ind., USA) through the catheter whenever an animal not receiving drug treatment displayed self-administration outside baseline performance. Animals with patent catheters exhibited a rapid loss of muscle tone within 2 s of the methohexital infusion.

Cocaine self-administration procedure

Prior to implantation of IV catheters, rats were trained to lever press for food reinforcement. During training rats were restricted to 4 g of standard pellet food per day. Rats were trained under a fixed ratio 1 (FR1) schedule with a time-out (TO) duration of 1 s on both response levers. A lever press response resulted in the delivery of a 45 mg Noyes pellet and illumination of the white stimulus light above the lever for the 1-s TO period. Rats were trained during daily 60-min sessions until they earned 100 pellets during the session. Once rats had reached this criterion (two or three sessions), they were returned to ad libitum food and implanted with IV catheters.

One week after surgery, rats were allowed to respond for a 0.1 ml IV infusion of cocaine (0.25 mg/infusion, dissolved in 0.9% physiological saline) on an FR1 TO 20-s schedule during daily 2-h sessions. At the beginning of each session, the house light was turned off and the two response levers were extended. Responding on the active lever resulted in an infusion that was signalled by a 20-s illumination of the stimulus light and a 3.5-s tone. Responses on the inactive lever were recorded but had no programmed

consequences. Once animals had acquired reliable responding on FR1 TO 20-s schedule, a second-order schedule of reinforcement of the type FR*x*(FR*y*:S) was introduced, where *x* was the number of conditioned stimulus (CS) presentations (1-s stimulus light and tone) required for the delivery of a cocaine infusion and *y* was the number of lever presses required for a CS presentation. Therefore, rats were presented a 1-s stimulus light and tone after *y* responses, and a 20-s stimulus light and a 3.5-s tone after completion of *x*, i.e., during each cocaine infusion. The schedule requirements were gradually increased as follows: FR1(FR2:S), FR1(FR3:S), FR1(FR5:S), FR1(FR7:S), FR2(FR5:S), FR3(FR5:S), FR4(FR5:S), FR5(FR7:S). When rats had reached FR5(FR7:S) or self-administered fewer than ten infusions during the 2-h session, a second-order schedule consisting of two fixed intervals (FI) followed by a 2-h period of self-administration under FR4(FR7:S) was introduced. The length of the fixed intervals was gradually increased from 5 to 15 min. Thus, under the final reinforcement schedule, rats were allowed to respond for the first two cocaine infusions under the FI15 min(FR7:S) schedule, and then take an unlimited number of cocaine infusions under FR4(FR7:S) schedule for 2 h. The purpose of the latter schedule was to strengthen action-outcome associations and the importance of the CS in the maintenance of cocaine responding.

Experimental procedures

Experiment 1: cocaine dose-response function under a second-order schedule

To examine the effect of cocaine unit dose on responding, rats were allowed to establish stable baseline responding for cocaine (0.25 mg/infusion) under the two-component second-order schedule described above. A stable baseline was defined as three consecutive self-administration sessions with less than 20% variation in the number of responses during the first interval and less than 10% variation in the total number of infusions earned. After ten sessions, the cocaine unit dose was increased from 0.25 mg/infusion to 0.50 mg/infusion and responding was allowed to stabilise. The data from the last three sessions at each unit dose were used for data analysis.

Experiment 2: effect of CNQX on cocaine responding under a second-order schedule

The AMPA/kainate antagonist CNQX (6-cyano-7-nitroquinoxaline-2,3-dione disodium) was obtained from Tockris Cookson Ltd (Bristol, UK) and dissolved in distilled water. CNQX (0, 0.75, 1.5, 3 mg/kg) was administered intraperitoneally (IP, 1 ml/kg) 20 min before cocaine self-administration sessions at the 0.50 mg/infusion unit dose under the second-order schedule described above. The order of doses was counterbalanced with a Latin square design, and only one drug dose was tested weekly.

Experiment 3: effect of CNQX on spontaneous locomotor activity

Locomotor activity was measured in transparent Macrolon III cages (18×33×15 cm) that were placed inside photocell frames (Cage Rack Activity System; San Diego Instruments, Calif., USA). The frames were equipped with seven pairs of photocells (5 cm off the cage floor) for measuring horizontal activity and eight pairs of photocells (12 cm off the floor) for measuring vertical activity. The number of photocell interruptions was recorded by a computer at 5-min intervals for 60 min and was used as the measure of locomotor activity. During the first four sessions, rats were habituated to the test cages after vehicle injections. The effects of CNQX (0, 0.75, 1.5, 3 mg/kg, IP, 20 min before testing) on locomotor activity were examined in a within-subjects Latin square design with at least two vehicle injection days separating the different CNQX doses.

Statistical analysis

The following measures were taken from all cocaine self-administration sessions: (a) the number of responses on the active lever during the two fixed intervals, (b) the time to emit the first response, (c) the time to complete the first FR7 response unit, i.e. the latency to the first CS, (d) the number of responses on the inactive lever during the fixed intervals, and (e) the total number of cocaine infusions under FR4(FR7:S). For examining the effects of the two cocaine unit doses on cocaine responding, the means of the above measures across the last three sessions at each dose were calculated and compared with paired *t*-tests. The effects of CNQX on cocaine responding and locomotor activity were analysed with one-way repeated measures ANOVA. Following a significant main effect of dose, each individual drug dose was compared with the vehicle condition using a post hoc comparison with Bonferroni correction. The number of the inactive lever responses during the first fixed interval was square root transformed to preserve homogeneity of variance. All statistical analyses were performed with the SPSS statistical package (SPSS Inc., Chicago, Ill., USA).

Results

Experiment 1: cocaine dose-response function under a second-order schedule

Rats acquired stable responding under the final FI15 min(FR7:S) second-order schedule within 8–9 weeks, and they were then maintained for 2 weeks at the 0.25 mg/infusion unit dose and for 8 weeks at the 0.50 mg/infusion unit dose. Ultimately, the temporal limiting factor for individual rats was catheter patency.

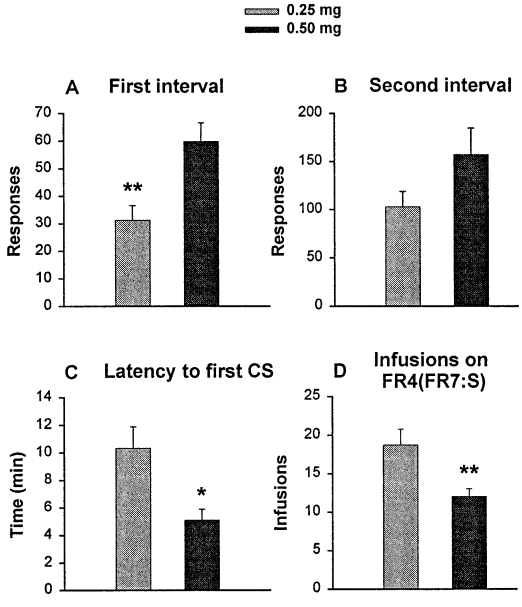

Fig. 1A–D Effects of increasing the unit dose of cocaine (from 0.25 mg to 0.50 mg/infusion) on responding under the second-order schedule of IV cocaine reinforcement (n=11). **A** Number of responses during the first interval, **B** number of responses during the second interval, **C** latency to the first CS presentation, and **D** number of cocaine infusions during the latter half of the session under FR4(FR7:S). Data points represent the mean±SEM. *P<0.05, **P<0.01

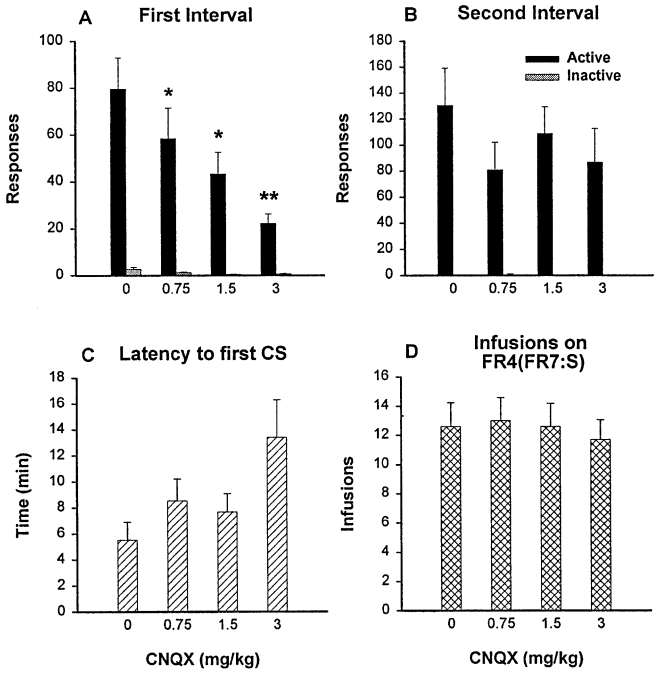

Fig. 2A–D Effects of CNQX on responding under the second-order schedule of IV cocaine reinforcement (0.50 mg/infusion) (n=10). **A** Number of active and inactive lever responses during the first interval, **B** number of active and inactive lever responses during the second interval, **C** latency to the first CS presentation, and **D** number of cocaine infusions during the latter half of the session under FR4(FR7:S). Data points represent the mean±SEM. *P<0.05, **P<0.01

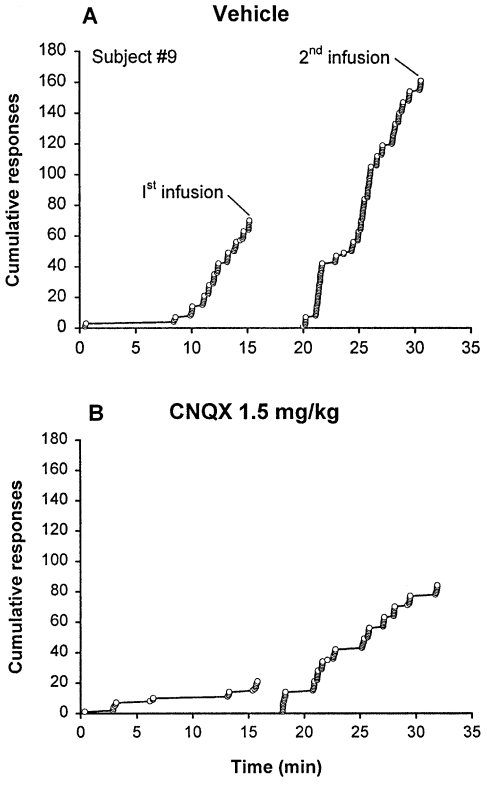

Fig. 3A, B Pattern of responding by a single rat during the first two intervals under the F115 min(FR7:S) second-order schedule of IV cocaine reinforcement (0.50 mg/infusion). The cumulative number of responses is plotted against the elapsed time from the start of the session. Each *open circle* indicates a response on the active lever. **A** Responding after the vehicle injection, and **B** after the 1.5 mg/kg injection of CNQX

From the 16 subjects that started the experiment, four lost their catheters during the final weeks, and did not complete either the dose response function or the CNQX experiment. Another two animals were excluded from the data analysis because they never emitted more than seven responses during the first fixed interval.

Figure 1 shows cocaine-seeking at the 0.25 and 0.50 mg/infusion unit doses. Doubling the unit dose increased the number of first interval responses by approximately 100%, while the number of cocaine infusions during the latter part of the session under FR4(FR7:S) decreased significantly. The increase in responding during the first interval was accompanied by a significant decrease in the latency to obtain the first CS. Responding during the second fixed interval after the first cocaine infusion was clearly higher than during the first interval, but it was less sensitive to the change in unit dose, as revealed by a non-significant (P=0.078) increase in responding at the 0.50 mg/infusion unit dose.

Experiment 2: effect of CNQX on cocaine responding under a second-order schedule

Comparison of the number of the first interval responses after a baseline vehicle injection with the vehicle injections included in the Latin square indicated that rats maintained stable responding during the 4 weeks of CNQX testing. The number of the first interval responses (mean±SEM) during the baseline was 71.7±8.5 and that during testing 79.5±13.4.

Figure 2 shows that systemic CNQX suppressed cocaine-seeking in a dose-dependent manner during the first fixed interval [F(3,27)=8.95, P<0.001], but failed to affect responding during the second interval, i.e. after the first cocaine infusion [F(3,27)=1.80, P=0.37], or the number of cocaine infusions during the latter part of the session under FR4(FR7:S) [F(3,27)=0.71, P=0.56]. A more detailed analysis of responding during the first interval revealed that CNQX did not change the latency to initiate responding (the time to the first response) [F(3,27)=0.98, P=0.42] (data not shown) or the number of the inactive lever responses [F(3,27)=2.73, P=0.063] that was very low, only 2.7±0.8 responses after the vehicle injection. However, CNQX increased the latency to the first CS [F(3,27)=4.20, P=0.015], particularly at the highest 3 mg/kg dose, although the post hoc test did not indicate it to be different from the vehicle condition.

Figure 3 presents an example of cocaine responding after vehicle and the 1.5 mg/kg CNQX dose. The rat showed accelerated responding during the first interval,

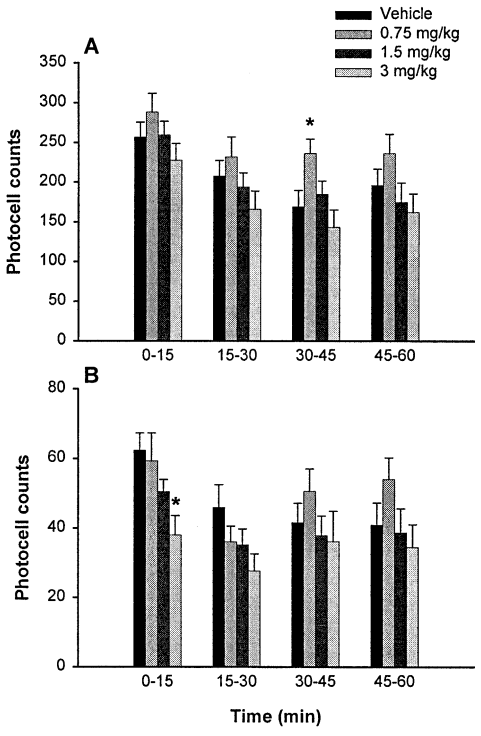

Fig. 4A, B Effects of CNQX on locomotor activity (n=16). The number of photocell counts (mean±SEM) is presented at 15-min intervals during the 120-min measuring period. **A** Total horizontal activity, **B** rearing. *P<0.05

with the typical short pauses after each CS, and the pronounced increase in responding after the first cocaine infusion. The 1.5 mg/kg CNQX did not slow the initiation of responding but tended to increase the intervals between CS, thus decreasing the total number of responses especially during the first interval.

Experiment 3: effect of CNQX on spontaneous locomotor activity

Figure 4 shows the effects of CNQX on spontaneous horizontal and vertical locomotor activity. In order to facilitate the comparison of CNQX effects on locomotion and cocaine seeking, the data are presented and analysed at 15-min intervals through the 60-min measuring period. CNQX had opposing effects on horizontal and vertical activity. ANOVAs revealed that CNQX increased horizontal activity during the third 15-min interval [F(3,45)=4.92 P=0.005]. In contrast, rearing was significantly suppressed during the first [F(3,45)=5.37, P=0.003] and the fourth interval [F(3,45)=3.15, P=0.034].

Discussion

Under the present second-order schedule of cocaine reinforcement, Wistar rats showed characteristic response patterns described previously. During the first cocaine-free fixed interval, responding accelerated towards the end of the interval, with short pauses after each burst of seven responses that lead to presentation of the cocaine-associated stimulus (CS). The delivery of the first cocaine infusion at the end of the first interval stimulated responding noticeably during the second interval. An increase in the cocaine unit dose increased responding during the first and second intervals, shortened the latency to the first CS presentation, but decreased the number of cocaine infusions during the latter half of the session under the FR4(FR7:S) schedule. Together, these findings suggest that responding under the present schedule, and especially during the first interval, was influenced by both the expected reinforcing value of the self-administered cocaine dose and the contingent presentation of the CS.

It is worth noting that the interpretation of the role of cocaine-associated stimuli in controlling behaviour is difficult. In addition to acting as a conditioned reinforcer, the CS may also have a role as a discriminative stimulus for responding. Moreover, the relationship between the CS and the primary reinforcer under second-order schedules is not fully understood. The effects of stimuli that come to control behaviour as a result of their association with the primary reinforcer are often transitory and diminish over time. In our work, rats were allowed to take an unlimited number of cocaine infusions under the FR4(FR7:S) schedule for 2 h during the daily sessions in order to strengthen the association of CS with cocaine infusions. The motivational significance of the CS could also be attenuated by development of stimulus-response habits under fixed interval schedules, making behaviour less sensitive to changes in reinforcer value (Everitt and Robbins 2000). Our cocaine dose-response data showed, however, that responding was significantly affected by the cocaine unit dose. Finally, omission of the CS has been demonstrated to decrease responding significantly under a second-order schedule of cocaine reinforcement in rats, suggesting that CS exerts at least partial control over responding under these schedules (Arroyo et al. 1998).

Systemic administration of the AMPA/kainate antagonist CNQX dose-dependently attenuated cocaine-seeking only during the first interval. It is not likely that this decrease by CNQX could be caused by any changes in the reinforcing effects of self-administered cocaine, because the first interval responding is affected by cocaine self-administered during the previous session. Rather, CNQX effects could be interpreted either as attenuation in the efficacy of cocaine-associated stimuli to control cocaine-seeking behaviour or as decreased motivation to respond for cocaine. Since motor impairment produced by the antagonist could also lead to decreases in responding, we examined CNQX effects on spontaneous locomotor activity. This experiment indicated that the current doses of CNQX had little effects on horizontal activity, but decreased rearing significantly at the highest 3 mg/kg dose. Although the latency to initiate responding during the first interval was not affected by CNQX, we saw an

increased latency to the first CS presentation after the 3 mg/kg CNQX dose. Therefore, we cannot exclude the possibility that attenuation of responding after the highest 3 mg/kg dose was due in part to motor impairing effects by CNQX. Data from the locomotor activity experiment indicated also that CNQX was pharmacologically active during the second 15-min interval, and therefore CNQX effects during the first and second interval can be dissociated. The differential effects of CNQX on cocaine responding during the first two intervals suggest that CNQX effects on "cocaine-seeking" and "cocaine-taking" could be separable (for discussion of these concepts, see Everitt and Robbins 2000). However, arguments of CNQX effects on cocaine intake based on the 120-min latter half of the session under FR4(FR7:S) cannot be made, because we do not have data on the time course of the pharmacological activity of CNQX during the whole 120-min section.

Our present results with systemic CNQX injections are in agreement with the demonstration that injections of a selective AMPA/kainate antagonist LY293558 into the nucleus accumbens decreased responding for cocaine-associated cues under a second-order schedule (Di Ciano and Everitt 2001). They also extend findings from other behavioural paradigms in which CNQX has been used previously for examining the involvement of the AMPA/kainate receptors in drug-environment conditioning. For example, intra-accumbal or intra-ventral subiculum injections of CNQX blocked responding on the drug-associated lever in the conditioned reinforcement paradigm (Burns et al. 1994; Hitchcott and Phillips 1997), and systemic injections of CNQX prevented expression of conditioned place preference induced by amphetamine (Mead and Stephens 1999). Expression of cocaine- and amphetamine-induced place preference has reported to be attenuated also by other AMPA/kainate antagonists, including DNQX, GYKI 52466, and NBQX (Layer et al. 1993; Kaddis et al. 1995; Tzschentke and Schmidt 1997). Many lines of evidence indicate that behavioural sensitisation induced by psychomotor stimulants is mediated partly through AMPA receptors. Intra-accumbal AMPA infusions induced a greater motor response in animals that received cocaine in the test environment than in those that had received saline, and cocaine elevated accumbal glutamate levels only in sensitised subjects (Bell and Kalivas 1996; Pierce et al. 1996). Conversely, blocking nucleus accumbens AMPA/kainate receptors with CNQX attenuated cocaine-induced locomotor activity in animals that had received their daily cocaine in the testing environment, while it failed to affect activity in other treatment groups (Bell et al. 2000). These findings suggest that AMPA receptor mediated glutamate transmission is particularly important in expression of behavioural sensitisation if specific drug-environment associations have a role in the sensitisation process.

The degree to which AMPA receptors are involved in drug-environment conditioning could also explain some observed discrepancies between different studies. For example, expression of psychomotor stimulant-induced behavioural sensitisation has been shown to be attenuated by NBQX and GYKI 52466 (Tzschentke and Schmidt 1997; Mead and Stephens 1998), but failures of NBQX and LY293558 to affect expression have also been reported (Li et al. 1997; Carlezon et al. 1999). An additional difficulty in interpreting results from studies in which the current AMPA antagonists have been used is the relative non-selectivity of these compounds for AMPA receptors. The series of quinoxalinedione compounds, CNQX, DNQX and NBQX, as well as a member of another class of antagonists, LY293558, have also inhibitory activity at kainate receptors (Bleakman and Lodge 1998). Moreover, CNQX and DNQX have been reported to show affinity for the glycine site of the NMDA receptor (Sheardown et al. 1990). Therefore, it has not always been easy to show whether the modulation of drug-environment conditioned behaviours by these antagonists has been related to their action on AMPA/kainate receptors or the glycine site (Mead and Stephens 1999).

Our present work with systemic CNQX injections does not suggest any candidate neuroanatomical systems where modulation of glutamatergic neurotransmission through AMPA receptors could affect cue-controlled behaviour. However, such candidate systems have been described previously. Both human and animal studies have implicated the anterior cingulate cortex and basolateral amygdala in processing of drug-associated stimuli (See 2002). It has been postulated that expression of drug-environment conditioned responses requires transfer of information about the drug-related stimuli from the anterior cingulate cortex and basolateral amygdala via glutamatergic projections to the nucleus accumbens core, where interactions between glutamatergic and dopaminergic transmission would mediate the information further to the motor system (Everitt et al. 1999). This circuit would not be specific for the expression of cocaine-related conditioned responses. However, it is also possible that long-term cocaine self-administration causes adaptive alterations in the glutamatergic system that would further contribute to the ability of cocaine-associated cues to control behaviour. For example, these changes could include long-lasting depression of excitatory synaptic transmission in the nucleus accumbens and/or long-term potentiation of excitatory inputs on ventral tegmental dopamine neurons, which would both imply changes either in AMPA receptor number or function (Thomas et al. 2001; Ungless et al. 2001).

In summary, we developed a second-order schedule of cocaine reinforcement in Wistar rats, under which animals responded in the characteristic scalloped pattern and showed monotonic increases in the number of active lever responses during the first and second intervals with an increase in the cocaine unit dose, as well as a concomitant decrease in the latency to the first CS presentation. In rats responding under this schedule, systemic injections of the AMPA/kainate receptor antagonist CNQX dose-dependently decreased the number of the first interval responses without affecting responding

after the first cocaine infusion was delivered. Attenuation of the first interval responding could not be entirely ascribed to any motor effects by the antagonist. These findings support and extend earlier studies that have suggested a role for the AMPA/kainate receptors in the mediation of cocaine seeking controlled partly by cocaine-associated cues.

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