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Differential Effects of Dopamine and AMPA Receptor Antagonists on the Expression of Conditioned Avoidance Responding in Rats

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AMPA receptor antagonists disrupt avoidance responding, but their day-to-day effect on this behavior has not been elucidated. This study compared the multisession effect of the AMPA/kainate receptor antagonist CNQX with that of the typical antipsychotic haloperidol on the expression of avoidance responding. Rats ($N = 199$) were trained to move to safety on presentation of a tone in one-way active conditioned avoidance and were tested across 5 sessions. Intracerebroventricular (icv) injection of CNQX (20-min injection–test interval) produced a dose-dependent, immediate block of avoidance responding, compared with the extinction-like decline of avoidance responding produced by haloperidol (intraperitoneal [ip], 60-min injection–test interval; icv, 60 but not 20-min injection–test interval). Previous exposure to CNQX significantly reduced its efficacy, illustrating that its effects may not be specific to the conditioned safety-related stimuli that control responding in conditioned avoidance, as proposed for antidopaminergic compounds. The new multisession profile of disrupted avoidance responding illustrated by CNQX suggests different roles for glutamatergic and dopaminergic neurotransmission in conditioned avoidance responding. Results are consistent with a role for AMPA receptors in maintaining the expression of learning.

Keywords: CNQX, haloperidol, glutamate, learning, antipsychotic

Animals learn to avoid aversive stimuli (Riess, 1971) as well as neutral stimuli that have previously been paired with an aversive stimulus (Mackintosh, 1983). In conditioned avoidance responding (CAR), animals are commonly trained to avoid a mild footshock by moving to the other side of a two-chambered box in response to the presentation of a signal (e.g., a tone that predicts shock). The successful avoidance response involves both classical and operant conditioning (Mowrer, 1947), which has made this paradigm useful in understanding the acquisition and expression of learning. CAR has also proven useful as a potent screen for novel antipsychotic compounds (Ahlenius, 1991), all of which affect brain dopaminergic neurotransmission (Seeman, Chau-Wong, Tedesco, & Wong, 1975). Recently, glutamatergic neurotransmission has also been implicated in CAR (Riedel, Platt, & Micheau, 2003).

CAR involves incentive learning, in which dopamine has been strongly implicated (Beninger, 1983; Berridge & Robinson, 1998; Miller, Wickens, & Beninger, 1990). Antidopaminergic agents produce a selective disruption of avoidance responding, impairing the ability of a rat to produce a conditioned avoidance response to a tone but not interfering with its ability to escape from a shock (Wadenberg & Hicks, 1999). In trained subjects, this disruption does not reach a maximum on the first trial but is manifested as an extinction-like loss of avoidance responding across multiple trials (Beninger, Phillips, & Fibiger, 1983). Previous results rule out the possibility that the gradual loss of responding produced by dopamine receptor antagonists can be explained by a buildup of drug after multiple injections (Wise, Spindler, de Wit, & Gerber, 1978). Rather, dopamine receptor antagonists lead to a gradual loss of the ability of safety-related stimuli to control responding (Beninger, 1989).

Glutamate has been implicated in incentive learning, both through its interactions with dopamine and on its own (Beninger & Gerdjikov, 2005; Riedel et al., 2003). From an extensive review of published studies, it appears that glutamatergic *N*-methyl-D-aspartate (NMDA) receptors play an important role in the acquisition and that α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate receptors play an important role in the expression of incentive learning (Beninger & Gerdjikov, 2005). When administered to trained rats in CAR studies, AMPA receptor antagonists produced a disruption of avoidance responding reminiscent of the effect seen with antidopaminergic compounds (Mathe, Fagerquist, & Svensson, 1999; Svensson & Mathe, 2000). However, the extinction-like effect normally seen with dopamine receptor antagonists has never been evaluated with AMPA/kainate receptor antagonists, as testing has been restricted to one session and data were averaged over trials.

In the present experiment, we assessed the effects of an AMPA/kainate receptor antagonist on the expression of CAR across multiple test sessions. Beninger and Gerdjikov (2004) have proposed a mechanism for incentive learning in which dopamine at D1-like receptors potentiates neurotransmission at AMPA receptors through the action of several signaling molecules. They suggested that AMPA receptors may be responsible for maintaining

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the expression of incentive learning. Thus, in the present study, we hypothesized that the blockade of AMPA/kainate receptors could result in an immediate and relatively constant block of avoidance responding across multiple testing sessions. For comparison, we included groups treated with haloperidol, a dopamine receptor antagonist that has previously been shown to produce the gradual extinction-like decline in avoidance responding typical of antipsychotic compounds in CAR.

Method

Subjects

Male Wistar rats ($N = 199$) from Charles River Laboratories (St.-Constant, Quebec, Canada) were maintained on a reverse daylight schedule (lights on from 1900 to 0700). The rats were given access to food (Lab Diet 5001; PMI Nutrition International, Brentwood, MO) and water ad libitum except during testing periods. The experiment was conducted in accordance with the guidelines of relevant provincial and federal legislation and was approved by the Queen's University Animal Care Committee.

Surgery

Some groups received intracerebroventricular (icv) injections (discussed later). Rats from these groups were anesthetized in an induction chamber with an inhalable anesthetic (5% isoflurane; Bimeda, Cambridge, Ontario, Canada) mixed with oxygen in a vaporizer system (Benson, Merkham, Ontario, Canada) and administered at 1.5 L/min. Anesthetized rats were fitted to a stereotaxic apparatus and administered isoflurane at a concentration of 2% or as needed to maintain anesthesia. For analgesia, buprenorphine hydrochloride in solution (0.15 mg/kg; Reckitt & Colman, Richmond, VA) was injected subcutaneously approximately 30 min before surgery. A 23-gauge (0.64 mm diameter) stainless steel guide cannula was implanted unilaterally into the right lateral ventricle, with coordinates 0.9 mm posterior to bregma, 1.4 mm lateral to the midline, and 3.5 mm ventral from the skull surface (Paxinos & Watson, 1998). Stainless steel wire stylets (0.31 mm diameter), flush with the end of the guide cannulas, were put in place to prevent occlusion. Ketoprophen (0.04 mg/kg; Merial, Baie d"Urfé, Quebec, Canada) was injected immediately after surgery and on 3 subsequent days postoperatively for analgesia. Rats were allowed approximately 1 week to recover before the start of behavioral testing.

Apparatus

A shuttle box (28.0 long × 81.5 wide × 35.5 cm high), divided in half by a partition, was constructed of black Plexiglas. The two sections of the shuttle box were connected through a rectangular opening (7.5 × 7.0 cm) in the partition. Both sections of the box had a clear Plexiglas hinged lid. Both sections had metal bars (3.0 mm in diameter), spaced approximately 1.0 cm apart, as flooring. The flooring in the shock side of the shuttle box could be electrified with a scrambled 0.5-mA AC (Lafayette Instruments Co., Layfayette, IN). A tone generator (Mallory, Indianapolis, IN) was mounted on the back wall of the shock compartment. Infrared emitters and sensors (Fairchild Semiconductor Co., South Portland, ME) were used to identify when a rat had passed through the partition. The shock, tone, and sensors were controlled by a computer.

Drugs

6-cyano-7-nitroquinoxaline-2,3-dione disodium salt (CNQX; Tocris, Ellisville, MO), a potent AMPA/kainate receptor antagonist was dissolved in distilled water (by gentle agitation and warming in a water bath at approximately 50 °C). It was administered in a volume of 1.0 ml/kg intraperitoneally (ip) at doses of 0.0, 1.0, 2.0, and 5.0 mg/kg, and in a volume of 2–3 μl icv at doses of 3.0, 54.0 and 81.0 μg. An upper solubility of 27 μg/μl was achieved by keeping the solution in the water bath; it was also necessary to purge the microinjector and tubing between each injection. Haloperidol (Janssen Pharmaceutica, Beerse, Belgium), a typical antipsychotic known to disrupt CAR, was dissolved in DMSO. It was administered at doses of 0.00, 0.04, 0.08, and 0.15 mg/kg ip in 1.0 ml/kg and at doses of 0.5, 10.0, 25.0 and 50.0 μg icv in 1.0 μl. Previous studies (e.g., Gerdjikov & Beninger, 2005) showed no behavioral effect of DMSO.

Procedure

Training was conducted during the early afternoon (during the rats' dark period) in a small room with overhead fluorescent lighting. Each training session consisted of 10 trials with an intertrial interval of 30 s. At the start of the first session, the rat was placed in the safe compartment of the shuttle box for 1 min. The rat was then removed from the safe compartment and placed in the shock compartment facing 180° from the partition. The trial began with the releasing of the rat and the simultaneous onset of the tone. If the rat moved to the safe compartment of the shuttle box within 10 s (judged as the rat's entire body, excluding tail, through the partition), the tone terminated and the trial was recorded as an avoidance. The trial length was recorded as the interval between the onset of the tone and the instant when the rat was through the partition. If the rat failed to move to the safe compartment of the shuttle box within the first 10 s of the trial, then the offset of the tone was contiguous with the electrification of the floor within the shock compartment. If the rat moved to the safe compartment of the shuttle box within the first 10 s of shock, then the trial was recorded as an escape. On the rare occasion when a rat failed to move to the safe compartment during the first 10 s of shock, it was gently pushed to safety. The dependent variable was the number of avoidance responses per session.

Each rat was trained for five days at 10 trials per day. Testing began the day after training was completed and consisted of five sessions spaced equally across five days. The procedure during testing was identical to that used during training.

Experiment 1: Systemic Control and Haloperidol

In Experiment 1A (systemic control and no-shock groups), one control subgroup ($n = 6$) was injected ip with distilled water 20 min before testing as a control for CNQX, and the second ($n = 6$) was injected ip with DMSO 60 min before testing as a control for haloperidol. A further group ($n = 13$) was given neither injections nor shock in test sessions examining the extinction of the avoidance response. In Experiment 1B (systemic haloperidol), groups

Table 1
The Number of Rats Included in the Analysis for Each Group Tested and the Average (± Standard Error) Percent Avoidance of Each Group on the Last Day of Training

Experiment and condition	Initial no. of rats	Illness or technical problem	Incorrect cannula placement	Final no. included in analysis	% Avoidance on last day of training	
					Average	*SE*
Experiment 1						
Experiment 1A						
Systemic control	12	0	0	12	97.50	1.79
No shock	13	0	0	13	96.92	1.33
Experiment 1B						
Systemic control	Same group as systemic control in Experiment 1A					
0.04 mg/kg haloperidol	13	0	0	13	98.46	1.04
0.08 mg/kg haloperidol	12	0	0	12	99.17	0.83
0.15 mg/kg haloperidol	13	0	0	13	99.23	0.77
Experiment 1C						
Central control	8	1	1	6	98.33	1.67
0.5 μg haloperidol	12	0	0	12	94.17	3.36
10.0 μg haloperidol	8	0	2	6	98.33	1.67
25.0 μg haloperidol	8	0	1	7	100.00	0.00
50.0 μg haloperidol	8	0	1	7	100.00	0.00
Experiment 1D						
50.0 μg haloperidol 20 min	Same group as 50.0 μg haloperidol in Experiment 1C					
50.0 μg haloperidol 60 min	8	1	2	5	98.00	2.00
Experiment 2						
Experiment 2A						
Systemic control	Same group as systemic control in Experiment 1A					
1.0 mg/kg CNQX	13	0	0	13	99.23	0.77
2.0 mg/kg CNQX	13	0	0	13	99.08	2.86
5.0 mg/kg CNQX	13	0	0	13	97.69	1.22
Experiment 2B						
0.04 mg/kg haloperidol	Same group as 0.04 mg/kg haloperidol in Experiment 1B					
5.0 mg/kg CNQX	Same group as 5.0 mg/kg CNQX in Experiment 2A					
0.04 mg/kg haloperidol and 5.0 mg/kg CNQX	13	0	0	13	99.23	0.77
Experiment 2C						
Central control	Same group as central control in Experiment 1C					
3.0 μg CNQX	8	0	1	7	94.29	2.02
54.0 μg CNQX	8	0	2	6	95.00	2.24
81.0 μg CNQX	8	0	1	7	98.57	1.43
Experiment 2D						
81.0 μg CNQX	Same group as 81.0 μg CNQX in Experiment 2C					
81.0 μg CNQX with home-cage injections	8	1	1	6	98.33	1.67
Total	199	3	12	184		

(*n*s = 12–13) were injected ip with 0.04, 0.08, or 0.15 mg/kg haloperidol 60 min before testing. In Experiment 1C (central haloperidol), groups (*n*s = 8–12) were injected icv with 0.5, 10.0, 25.0, or 50.0 μg haloperidol 20 min before testing. In Experiment 1D (comparison of central injection–test intervals), one group (*n* = 8) was injected with 50.0 μg haloperidol 60 min before testing.

Experiment 2: CNQX

In Experiment 2A (systemic CNQX), groups (*n*s = 13) were injected ip with 1.0, 2.0, or 5.0 mg/kg CNQX 20 min before testing. In Experiment 2B (systemic co-injection of haloperidol and CNQX), one group (*n* = 8) was co-injected ip with 5.0 mg/kg CNQX 20 min and 0.04 mg/kg haloperidol 60 min before testing. In Experiment 2C (central CNQX), groups (*n*s = 8) were injected icv with 3.0, 54.0, or 81.0 μg CNQX 20 min before testing. Last, in Experiment 2D (home-cage injections of CNQX between training and testing), one group (*n* = 8) was given two home-cage injections of 81.0 μg CNQX before testing with the drug. These injections were administered 24 and 48 hr after the last training session. On the third day after training, testing proceeded as usual, with five testing sessions each accompanied by an injection of 81.0 μg CNQX administered 20 min before testing. If the multisession profile of CNQX does not change with the addition of home-cage injections then the drug, like all antidopaminergic compounds, may produce its effect within CAR only when injections are paired with testing, suggesting a role for CNQX within the pairing of safety-related stimuli and control of responding.

Statistical Analyses

The number of avoidance responses on test days were expressed as a proportion of the number of avoidance responses on the last day of training for each rat (Table 1 shows the average level of

avoidance responding for each group on the last day of training). Separate two-way mixed-model analyses of variance (ANOVAs) were conducted to assess the differences among means for groups across test sessions. Drug group was classified as a between-subjects factor, and test session was classified as a within-subjects factor. Dunnett's C test was used for post hoc comparisons of the main between-subjects effect, as the largest variance was more than three times that of the smallest variance in the data set analyzed. We analyzed significant interactions using a simple effects ANOVA. In data sets that violated the assumption of sphericity, the Huynh–Feldt correction was used for the analysis of main effects and interactions involving within-subjects factors. For purposes of clarity, unadjusted degrees of freedom are reported.

Histology

On completion of the experiment, cannulated rats were euthanized with CO_2 gas. Brains were extracted, fixed in a 10% formalin solution, and placed in a refrigerator for at least 72 hr before slicing. We obtained coronal sections, 60 μm in thickness, from the cannulated region by slicing the brains on a cryostat at approximately −20 °C. The sections were mounted on gelatin-coated glass slides and stained with cresyl violet. Judgments about cannula placements were made by an observer who was not aware of the results for individual rats. Rats with cannula tips located in the lateral ventricle were included in subsequent analyses.

Results

Histology

We tested a total of 199 rats. Three rats failed to complete the study because of illness or technical problems. Of the remaining rats, 81 were injected centrally and underwent histological analysis. A rat was included in the statistical analysis if the tip of the cannula had pierced the corpus callosum and rested within the lateral ventricle (see Figure 1). Twelve rats were dropped from analysis because of inaccurate cannula placement (see Table 1).

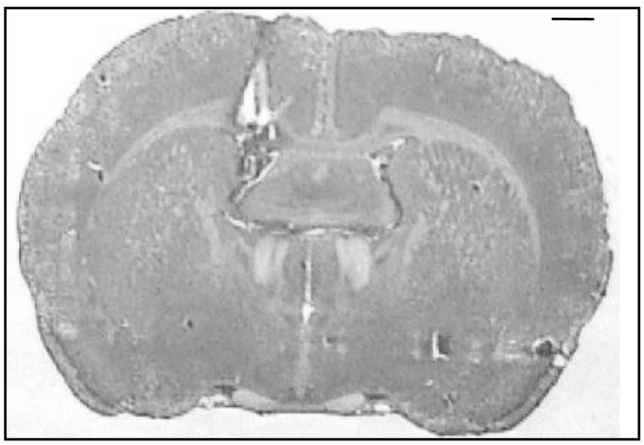

Figure 1. Coronal section showing representative unilateral cannula placement for icv-injected rats. The scale bar indicates 1 mm.

Experiment 1A: Systemic Control and No-Shock Groups

The 6 control rats that received water and the 6 that received DMSO continued to show avoidance responding during test sessions, and these groups did not differ significantly from each other, $F(1, 10) = 0.12$, $p = .73$, $\eta_p^2 = 0.01$. These groups were combined into a single systemic control group. When shock was no longer presented, avoidance responding decreased across testing days (see Figure 2A). An ANOVA comparing the systemic control and no-shock groups revealed a significant main effect for group, $F(1, 23) = 9.01$, $p = .01$, $\eta_p^2 = 0.28$; and interaction, $F(4, 92) = 3.33$, $p = .02$, $\eta_p^2 = 0.13$. Tests of simple main effect for each group revealed a session effect for the no-shock group, $F(4, 48) = 3.31$, $p = .02$, $\eta_p^2 = 0.22$; but not for the systemic control group, $F(4, 44) = 0.94$, $p = .45$, $\eta_p^2 = 0.08$. Thus, the no-shock condition led to a gradual decrease in avoidance responding.

Experiment 1B: Systemic Haloperidol

The systemic administration of haloperidol produced a dose-dependent decrease in proportion avoidance across testing sessions (see Figure 2A). An ANOVA comparing the systemic control and the three haloperidol dose groups revealed a significant main effect for group, $F(3, 46) = 12.91$, $p < .001$, $\eta_p^2 = 0.46$; and interaction, $F(12, 184) = 13.28$, $p < .001$, $\eta_p^2 = 0.46$. Tests of simple main effects for each group revealed a significant effect of session for the 0.04 mg/kg haloperidol group, $F(4, 48) = 2.85$, $p = .05$, $\eta_p^2 = 0.19$, reflecting the small decrease in avoidance responding during Session 3. There was also a significant simple main effect of test session for the 0.15 mg/kg haloperidol group, $F(4, 48) = 23.25$, $p < .001$, $\eta_p^2 = 0.66$, reflecting the decline in avoidance responding over sessions. The simple main effect of day was not significant for the control group, $F(4, 44) = 0.94$, $p = .45$, $\eta_p^2 = 0.08$; or for the 0.08 mg/kg haloperidol group, $F(4, 44) = 1.33$, $p = .28$, $\eta_p^2 = 0.11$. For the 0.08 mg/kg group, the failure to observe a significant session effect may reflect the lower avoidance score on Test Session 1 and the higher standard errors compared with the 0.04 mg/kg group. Thus, systemic administration of haloperidol produced a dose-dependent decrease in avoidance across testing sessions

Experiment 1C: Central Haloperidol

The central administration of haloperidol 20 min prior to testing produced a decrease in avoidance responding (see Figure 2B). An ANOVA revealed a significant main effect of group, $F(4, 33) = 3.70$, $p = .01$, $\eta_p^2 = 0.31$. However, in the post hoc analysis with Dunnett's C, no differences were large enough to reveal a significant effect. The decline in responding with some doses of haloperidol on Test Session 1 and the lack of a well-defined extinction-like decline in avoidance responding within this experiment illustrate that, at 20 min, haloperidol may be exerting its effects through nonspecific side effects and not through its interruption of the ability of safety-related stimuli to control CAR.

Experiment 1D: Comparison of Central Injection–Test Intervals

Changing the injection–test interval of 50.0 μg haloperidol from 20 min to 60 min produced a significant interaction when the two groups

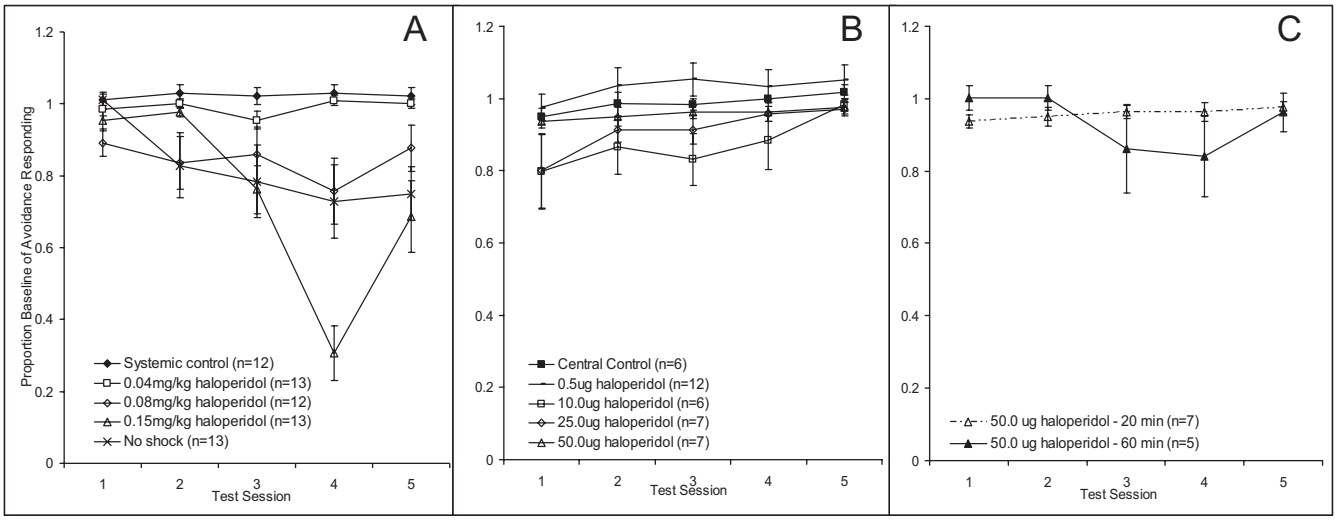

Figure 2. (A) Mean proportion (plus or minus standard errors of the mean) of avoidance responding for no-shock and systemic (control, 0.04, 0.08, and 0.15 mg/kg ip; 60-min injection–test interval) haloperidol groups in Experiments 1A and 1B. (B) Mean proportion (plus or minus standard errors of the mean) of avoidance responding for central (control, 0.5, 10.0, 25.0, and 50.0 μg icv; 20-min injection–test interval) haloperidol groups in Experiment 1C. (C) Mean proportion (plus or minus standard errors of the mean) of avoidance responding for 50.0 μg icv haloperidol groups with injection–test intervals of 20 or 60 min in Experiment 1D. Dashed lines indicate that a group has been shown in a previous graph.

were compared across testing sessions, $F(4, 44) = 3.32$, $p = .04$, $\eta_p^2 = 0.23$ (see Figure 2C). The 60-min injection–test-interval group showed an interruption of avoidance responding similar to the extinction-like effects shown in Experiment 1B, except that responding recovered in Test Session 5. A simple main effects ANOVA of days for the 60 min injection–test-interval group yielded an effect that approached significance, $F(4, 16) = 2.66$, $p = .07$, $\eta_p^2 = 0.40$, revealing the source of the significant interaction.

Experiment 2A: Systemic CNQX

The systemic administration of CNQX produced little change in avoidance across testing sessions (see Figure 3A). An ANOVA comparing the control and CNQX groups revealed no significant effects.

Experiment 2B: Systemic Co-Injection of Haloperidol and CNQX

The co-administration of haloperidol (0.04 mg/kg) and CNQX (5.0 mg/kg), like each dose alone, produced little change in avoidance responding across testing sessions (see Figure 3B). An ANOVA comparing the three groups yielded no significant effects.

Experiment 2C: Central CNQX

The central administration of CNQX produced a dose-dependent decrease in avoidance across testing sessions (see Figure 3C). An ANOVA revealed a significant main effect of group, $F(3, 22) = 22.59$, $p < .001$, $\eta_p^2 = 0.76$. Post hoc analysis found the 81.0 μg CNQX group to be significantly different from the saline and 3.0 μg CNQX groups and found the 54.0-μg CNQX group to be significantly different from the 3.0 μg CNQX group.

A significant interaction was found between group and test session, $F(12, 88) = 8.24$, $p < .001$, $\eta_p^2 = 0.53$. A simple main effect of day was found for the 81.0 μg CNQX group, $F(4, 24) = 19.90$, $p < .001$, $\eta_p^2 = 0.77$, confirming that responding was initially greatly impaired in this group but showed some recovery over test sessions.

Experiment 2D: Home-Cage Injections of CNQX Between Training and Testing

The addition of two 81.0-μg home-cage injections of CNQX between training and testing attenuated the effect of this dose on avoidance responding (see Figure 3D). An ANOVA comparing the home-cage-treated group with the group that received 81.0 μg of CNQX during testing in Experiment 2C revealed a significant main effect of group, $F(1, 11) = 12.30$, $p = .01$, $\eta_p^2 = 0.53$; and interaction, $F(4, 44) = 4.56$, $p < .01$, $\eta_p^2 = 0.29$. A simple effects ANOVA showed the groups to differ significantly on Test Session 1, $F(1, 11) = 80.52$, $p < .001$, $\eta_p^2 = 0.88$. Results show that previous treatment with CNQX attenuated its effects on avoidance responding. Similarly to the 54.0-μg CNQX group, the 81.0-μg CNQX home-cage-treated group differed from the 3.0-μg CNQX group, $F(1, 11) = 10.03$, $p = .01$, $\eta_p^2 = 0.48$, illustrating that the home-cage group maintained a significant reduction in avoidance responding.

Discussion

During training, all rats showed movement to the safe compartment of the shuttle box on presentation of the tone, indicating that they acquired the CAR. During testing in Experiment 1, the systemic administration of haloperidol produced a dose-dependent

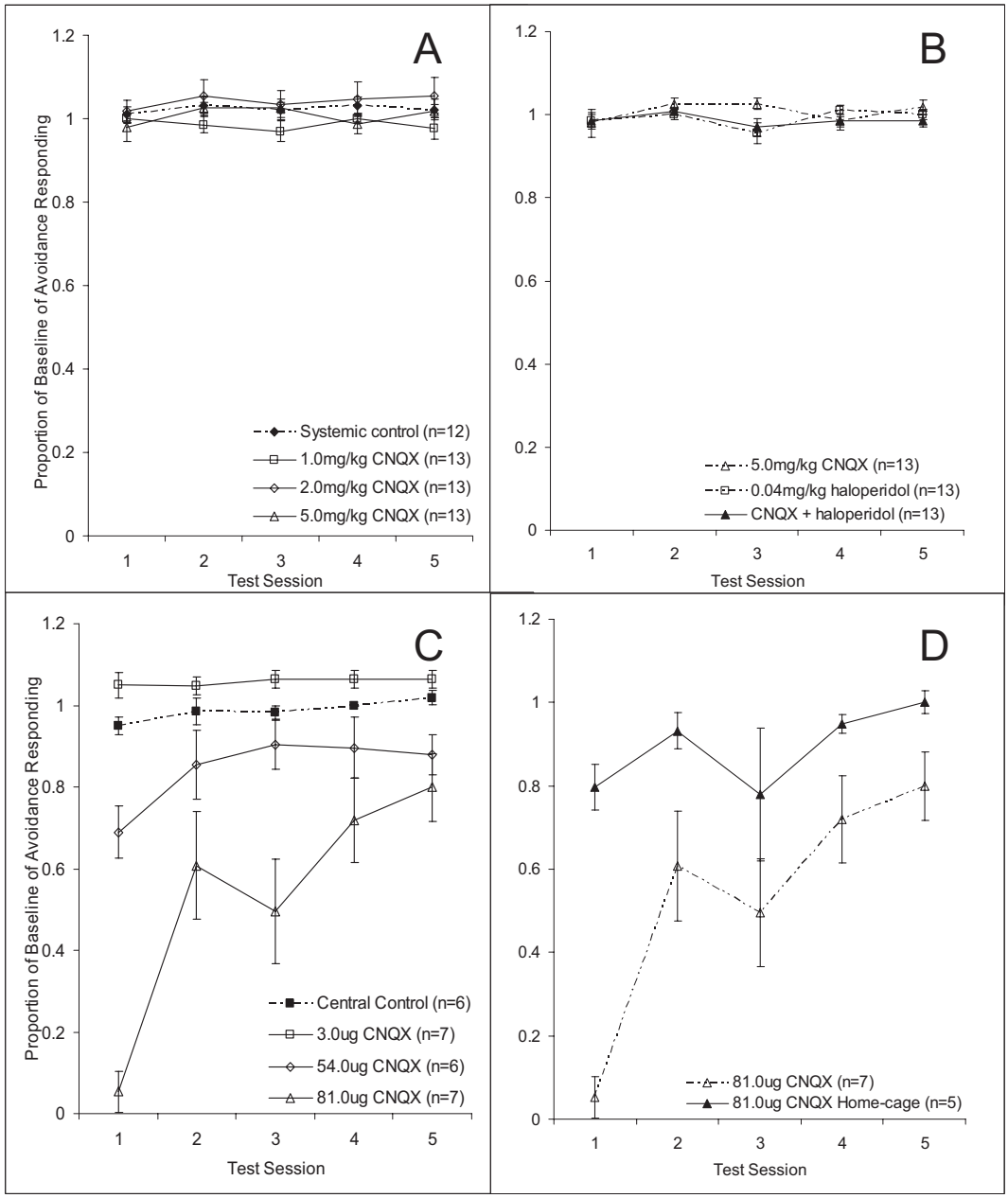

Figure 3. Mean proportion (plus or minus standard errors of the mean) of avoidance responding for (A) systemic control and CNQX (1.0, 2.0, and 5.0 mg/kg ip) groups in Experiment 2A; (B) systemic co-injection (5.0 mg/kg CNQX, 0.04 mg/kg haloperidol, and 5.0 mg/kg CNQX + 0.04 mg/kg haloperidol) groups in Experiment 2B; (C) central control and CNQX (3.0, 54.0, and 81.0 μg icv) groups in Experiment 2C; and (D) CNQX (81.0 μg) and CNQX home-cage (81.0 μg) injection groups in Experiment 2D. Dashed lines indicate that a group has been shown in a previous graph.

and extinction-like decrease in avoidance responding across testing sessions, replicating previous findings (Beninger et al., 1983). With a 60-min injection–test interval, the central administration of 50.0 μg haloperidol also showed an extinction-like decline in avoidance responding over the first four test sessions.

During testing in Experiment 2, the systemic administration of CNQX and the co-injection of CNQX with 0.04 mg/kg haloperidol showed little evidence of attenuating avoidance responding. The central administration of 54.0 μg and 81.0 μg CNQX led to a significant reduction in avoidance responding across testing sessions. This effect was most pronounced in the first test session. Two home-cage injections of 81.0 μg CNQX (administered 24 and 48 hr before testing) significantly reduced the Session 1 effect, but a decrease in avoidance responding that was significantly greater

than that in the low-dose CNQX group was still seen. Thus, CNQX produced a disruption of avoidance, but the effect did not increase over days like the effect of haloperidol.

When home-cage injections were included after training and before testing with antidopaminergic drugs (viz., pimozide), there was no significant difference between home-cage and non-home-cage groups tested with the same dose, indicating that the effects of these compounds depended on the environment in which they were given (Beninger et al., 1983). In contrast, previous exposure to CNQX significantly reduced its efficacy within conditioned avoidance, illustrating that its effects did not depend on the environment in which it was given. This effect may be partly due to the rats' tolerance to CNQX. However, no literature illustrating tolerance to the compound could be found. Further studies are needed to evaluate the tolerance of rats to CNQX. The effect of CNQX cannot be attributed entirely to drug novelty, because home-cage-injected rats still showed a reduction in CAR when tested with the drug. Results suggest that dopamine and AMPA receptors both play a role in the expression of CAR but that they are differentially involved.

The central administration of haloperidol 20 min before testing did not produce significant dose-dependent or extinction-like effects in CAR. With a 60-min injection–test interval, icv haloperidol produced an extinction-like decline in avoidance responding that more closely resembled that seen with systemic haloperidol. Thus, although injected directly into the brain, haloperidol required the same injection–test interval used in systemic studies to exert its effect on CAR. One possibility is that the effects of haloperidol depend in part on an active metabolite produced in the liver. Pharmacokinetic analysis of haloperidol has shown that it is heavily metabolized in the liver (Kudo & Ishizaki, 1999). However, there is no evidence that these metabolites are potent inhibitors of dopaminergic receptors. This holds for reduced haloperidol (Kirch, Palmer, Egan, & Freedman, 1985), which has been detected in the plasma of psychiatric patients at concentrations rivaling unchanged haloperidol (Someya, Shibasaki, Noguchi, Takahashi, & Inaba, 1992). Thus, it appears that the efficacy of haloperidol is not due to an active metabolite.

ICV haloperidol may still take some time to reach dopamine receptors because of slow uptake from the cerebrospinal fluid, accounting for the need of a 60-min injection–test interval. Systemically administered haloperidol enters the brain quite rapidly (Kapetanovic, Sweeney, & Rapoport, 1982), but its effects on glucose utilization take approximately 60 to 90 min to appear (Pizzolato, Soncrant, & Rapoport, 1984). Results suggest that haloperidol, icv or given systemically, reaches the brain quickly but requires some time to affect avoidance responding, possibly by taking time to block dopamine receptors in sufficient number.

In Experiment 2, little evidence for decreased avoidance responding was found with the systemic administration of CNQX. Although we used an injection–test interval consistent with previous research, systemic doses shown to attenuate avoidance responding at this interval were three to four times the largest dose used in the present study (Mathe et al., 1999; Svensson & Mathe, 2000). The high cost of CNQX limited our ability to test larger doses across multiple sessions. As an alternative, we used icv CNQX, allowing us to achieve high central concentrations with smaller quantities of drug.

Centrally administered CNQX was found to dose-dependently decrease avoidance responding across multiple test sessions. Results agreed with previous studies that used several AMPA receptor antagonists in a single test session (Mathe et al., 1999; Svensson & Mathe, 2000). This was the first study to evaluate the effects of an AMPA receptor antagonist on CAR over multiple sessions. The attenuation of avoidance responding by CNQX was most prominent on the first test session, with some recovery of responding evident in later test sessions. This was in contrast to the multisession extinction-like decline in CAR seen with haloperidol.

Co-injection of haloperidol (0.04 mg/kg) and CNQX (5.0 mg/kg), doses that produced no significant effects on their own, produced no significant effect. A synergistic effect might have been expected had both dopamine and AMPA receptors been involved in incentive learning in CAR. This result may contradict the hypothesis put forward by Beninger and Gerdjikov (2004). Alternatively, the dose of CNQX may have been too low and, for reasons outlined earlier, higher doses were not tested. Another possibility is that non-AMPA receptors are involved in incentive learning in CAR.

Because CNQX is not as selective for AMPA receptors as its quinoxalinedione counterparts, its effective site of action in mediating CAR may be debatable. CNQX and the NMDA glycine-site antagonist 7-chloro-4-hydroxy-3-(2-phenoxy)phenyl-2(1*H*)-quinolone have been shown to block the expression of amphetamine-induced conditioned place preference in rats, but the selective AMPA receptor antagonist 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(*f*)quinoxaline was unable to produce this effect, which illustrates that CNQX may act at glycine receptors to modify the expression of learning (Mead & Stevens, 1999). However, there is ample evidence dissociating NMDA and AMPA receptor function, implicating NMDA receptors in the acquisition and AMPA receptors in the expression of incentive learning (Beninger & Gerdjikov, 2005). The present observation that CNQX decreased the expression of CAR is consistent with previous findings from studies using AMPA receptor antagonists.

AMPA receptors are found throughout excitatory synapses in the brain, and their blockade may produce motor depression. For example, intra-amygdala infusions of CNQX have been shown to produce altered locomotor activity in rats (Mesches, Bianchin, & McGaugh, 1996). On the other hand, AMPA receptor antagonists produce marked disruption of avoidance responding without increasing catalepsy (Mathe et al., 1999; Svensson & Mathe, 2000). In the present study, the group receiving 81.0 μg CNQX without prior home-cage injections failed to escape on 49 of 70 trials during the first session, suggesting that motor capacity may have been affected. However, this appeared to be a transient effect, as fewer than 6 of 70 trails resulted in failure on subsequent sessions for this group. Escape failures were rare for all remaining sessions within all other drug groups ($M = 0.34\%$, $SE = 0.11\%$). Thus, CNQX appeared to have a motor effect on the first session at the largest central dose tested in this study, but this effect appeared minimal on all other test sessions and doses. Doses of other AMPA receptor antagonists in previous literature have illustrated comparable blocks of avoidance responding with no failure and no catalepsy (Mathe et al., 1999; Svensson & Mathe, 2000), arguing against the hypothesis that AMPA receptor antagonists produce their effects solely through a motor effect.

The differential effects of haloperidol and CNQX on avoidance responding may reflect the differential roles of dopamine and glutamate AMPA receptors in learning. Thus, dopamine acting at D1-like receptors may produce learning by changing the strength of glutamate synapses; this may be achieved through modification of AMPA receptor function or number (cf. Malenka & Bear, 2004; Tocco et al., 1991; for a review, see Beninger & Gerdjikov, 2004). How D1- and D2-like receptors cooperate in learning remains a topic of considerable debate (see Miller et al., 1990), and further studies are needed to work out this interaction.

The multisession profiles of haloperidol and CNQX outlined in the present study provide some support for the roles of dopamine and glutamate in incentive learning put forth by Beninger and Gerdjikov (2004). Haloperidol showed a consistent delay of onset and an extinction-like decline in avoidance responding, extending experimental evidence that implicates the dopaminergic system in the *conditioning* of safety-related stimuli to control responding. In contrast, CNQX, which blocks AMPA/kainate receptors, showed an immediate disruption of CAR followed by some recovery of responding. This suggests that AMPA/kainate receptors may maintain the expression of learning in CAR. The recovery of responding in later sessions may reflect the development of tolerance to repeated dosing, again through the potential modification of AMPA receptor function or number. Further studies that directly compare the behavioral effects of dopamine receptor antagonists with those of AMPA receptor antagonists are needed.

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