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Dissociable effects of the 5-HT_2 antagonist mianserin on associative learning and performance in the rabbit

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Abstract

Serotonin 5-HT_2 antagonists that significantly retard the acquisition of classically conditioned responses (CRs) also impair the performance of the unconditioned response (UR). Effects on the UR appear to be due to an inverse agonist action at the 5-HT_2 receptor. These findings raised the possibility that the learning deficits were either secondary to a performance deficit and/or that the retardation of learning was not due to actions at the serotonin 5-HT_2 receptor. In this study, we examined the effects of the 5-HT_2-receptor antagonists, namely mianserin and D-2-bromolysergic acid diethylamide hydrogen tartrate (BOL), on CR acquisition. We also determined whether the retarded acquisition of CRs produced by mianserin (a) was due to an action at the 5-HT_2 receptor and (b) was secondary to a performance deficit. Effects of drugs on CR acquisition, maintenance, and retention were determined during trace-conditioning of the rabbit's nictitating membrane (NM) response. BOL (0.058 to 5.8 μmol/kg) had no effect on CR acquisition, whereas mianserin (0.1 to 10 μmol/kg) produced a significant and dose-dependent retardation of CR acquisition. The retarded CR acquisition produced by mianserin (10 μmol/kg) was due to its actions at the 5-HT_2 receptor, because this effect was completely blocked by a dose of BOL (5.8 μmol/kg) that had no effect when given alone. Neither maintenance nor retention of learning was affected by mianserin treatment during acquisition. We conclude that mianserin acts as an inverse agonist at the serotonin 5-HT_2 receptor to produce both a retardation of CR acquisition and an impairment of the UR. However, the learning and performance effects of mianserin are separable.

Keywords: BOL; Classical conditioning; Inverse agonism; Learning; Mianserin; Nictitating membrane; Rabbit; Serotonin 5-HT_2 receptor antagonists

1. Introduction

Recently, several 5-HT_2 antagonists were examined in the rabbit for their effects on motor performance as measured by elicitation of the unconditioned nictitating membrane (NM) reflex as a function of unconditioned stimulus (US) intensity [6], and on learning as measured by Pavlovian conditioning of the NM response [12]. MDL-11,939, ritanserin, and mianserin (0.1 to 10 μmol/kg) significantly and dose dependently decreased the amplitude of the NM unconditioned response (UR) with no effect on UR threshold. By contrast, LY-53,857 (0.1 to 10 μmol/kg) and D-2-bromolysergic acid diethylamide hydrogen tartrate (BOL, 0.058 to 5.8 μmol/kg) had no significant effect on UR amplitude. The largest decrease in UR amplitude (60%) was produced by mianserin. The decrease in the amplitude of the NM reflex produced by mianserin was completely blocked by a dose of BOL that had no effect on UR amplitude when given alone (5.8 μmol/kg). This effect of mianserin and its blockade by BOL appears to be due to an action at 5-HT_{2A} or 5-HT_{2C} receptors. In rabbit cerebral cortex, mianserin and BOL have equivalent affinities for the 5-HT_{2A} receptor (mianserin k_i=1.46 nM; BOL k_i=0.13 nM) and BOL binds with a relatively high affinity at the 5-HT_{2C} receptor (k_i=4.40 nM) [1].

A previous study had demonstrated that MDL-11,939 and ritanserin produced a significant and dose-dependent retardation of associative learning while LY-53,857 had no effect [11,12]. These findings raised the possibility that the retarded acquisition of conditioned responses (CRs) to a tone conditioned stimulus (CS) produced by MDL-11,939 and ritanserin was due to impairment in the performance of the response. The present series of four experiments were designed to further explore this relationship between the motor and learning effects of 5-HT_2 antagonists. In Experiment 1 we examined the effects of BOL and mianserin on

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CR acquisition using a Pavlovian, trace-conditioning paradigm. Based on mianserin's ability to decrease the amplitude of the unconditioned NM response it was hypothesized that mianserin would produce a dose-dependent retardation of NM conditioning, while BOL, which had no significant effect on NM amplitude, should have no significant effect on learning. Because BOL blocked the effects of mianserin on the amplitude of the NM response, we also predicted, in Experiment 2, that BOL should block the effects of mianserin on CR acquisition.

The results of Experiments 1 and 2 supported the possibility that the retardation of learning produced by mianserin was due to its impairment of motoric function. Experiments 3 and 4 were designed to determine if mianserin was affecting CR acquisition due to its effects on performance of the NM response, or if mianserin was affecting associative learning independent of its effects on motor performance. These experiments were conducted in two stages. In stage 1 of both experiments, animals were treated with mianserin and trained to a preasymptotic level. Stage 2 was conducted following a 5-day drug washout period. In stage 2 of Experiment 3, CS–US pairings were continued, and animals were assessed for their ability to maintain conditioned responding in the absence of the drug. If mianserin had affected only the performance of the CR in stage 1, then previously drugged animals would be expected to show an immediate improvement in performance during stage 2. In stage 2 of Experiment 4, CS-alone presentations were used to assess retention of the CR in the absence of mianserin. The lack of a significant improvement in conditioned responding in previously drugged animals would suggest that decreased responding during acquisition could be attributed to interference with the learning process independent of any deficits in motor performance.

2. Materials and methods

2.1. Subjects

New Zealand White rabbits of both sexes, weighing 1.8 to 2.2 kg upon arrival, were housed individually with free access to rabbit chow and water under a 12 L:12 D cycle in an AAALAC-approved colony maintained at 22±1°C. Rabbits were given 5 days of adaptation to the laboratory before initiation of experiments. All animal experiments were carried out in accordance with the National Institute of Health guide "Principles of Laboratory Animal Care" (NIH publication No. 85-23, revised 1985).

2.2. Drugs

Mianserin hydrochloride was purchased from Research Biochemicals Int. (Natick, MA). BOL was supplied by the National Institute on Drug Abuse. Mianserin and BOL were dissolved in distilled water that also served as the vehicle control for these drugs. Because of its short duration of action [2], BOL was injected 20 min prior to testing, while mianserin was injected 1 h prior to testing. Injections of drug or vehicle were subcutaneous, between the scapulae, in a volume of 4 ml/kg. BOL was injected at doses of 0.058, 0.58, and 5.8 μmol/kg (0.032–3.2 mg/kg) while mianserin was injected at doses of 0.1, 1.0, and 10 μmol/kg (0.03–3.0 mg/kg). Dosages of drug, volume of injection, and the interval between injection and testing were identical with those employed in a previous study [6].

2.3. Apparatus

The conditioning apparatus, data acquisition system and general procedures have been described in detail elsewhere [7]. Briefly, each animal was placed in a Plexiglas restrainer and fitted with a headmount that supported a potentiometer that was directly coupled to a suture placed in the right NM. The headmount also supported a 2-mm diameter metal tube positioned 5–7 mm from the center of the right cornea for delivery of a 100-ms air puff US at a pressure of 200 g/cm^2, as measured at the end of the metal tube. A speaker, mounted in front and above the rabbit, was used to deliver a 100-ms, 90-dB, 1-kHz tone CS. Movements of the NM were transduced to DC voltages and digitized every 5 ms with a resolution of 0.03 mm of NM movement per analog-to-digital count. A response was defined as a 0.5-mm or greater extension of the NM, and its onset latency was calculated from the time at which the response first deviated from baseline by at least 0.03 mm. Prior to each experiment, animals were placed in the conditioning chambers for a 60-min adaptation session, during which no stimuli were presented and no drug was injected. Injections of drug or vehicle and behavioral training began on the day after this adaptation session. Training consisted of a trace-conditioning procedure with a CS–US interval of 500 ms, measured from the onset of the CS to the onset of the US. Responses occurring within the 500-ms period following CS onset were scored as CRs. Training or testing trials were presented at an average intertrial interval of 60 s (range: 55–65 s).

2.4. Design of experiments

Four experiments were carried out with separate sets of experimentally naïve animals. Experiment 1 employed separate sets of animals to assess the effects of mianserin and BOL on the rate of CR acquisition. Within each set animals were injected with vehicle ($n=6$) or with one of three doses of mianserin (0.1, 1.0, and 10 μmol/kg or 0.03, 0.3, and 3.0 mg/kg; ns=6, 6, and 5, respectively) or BOL (0.058, 0.58, and 5.8 μmol/kg or 0.032, 0.32, and 3.2 mg/kg; all $n=6$). Drugs were injected prior to each of eight acquisition sessions of 60 CS–US presentations per session. Experiment 2 employed four groups of animals to

determine if a 5.8-μmol/kg dose of BOL could antagonize the effects of a 10-μmol/kg dose of mianserin during acquisition training. Each of the four groups received two injections spaced 1 h apart. Thus, animals received vehicle + vehicle ($n = 14$), vehicle + BOL ($n = 14$), mianserin + vehicle ($n = 7$), or mianserin + BOL ($n = 8$). The second injection of vehicle or BOL occurred 20 min prior to initiation of training. Training consisted of eight sessions of 60 CS–US presentations per session. Experiment 3 assessed the maintenance of conditioned responding following acquisition training under vehicle ($n = 8$) or a 10 μmol/kg dose of mianserin ($n = 8$). Acquisition training consisted of six sessions composed of 60 CS–US presentations each and a seventh session of 30 CS–US presentations. The maintenance of conditioned responding was assessed 5 days after the seventh and last acquisition and drug injection session. The maintenance phase consisted of four sessions of 60 CS–US presentations per session. No drug or vehicle injections were administered during the maintenance phase. Experiment 4 assessed the retention of conditioned responding following acquisition training in the presence of vehicle ($n = 12$) or a 10-μmol/kg dose of mianserin ($n = 12$). Trace-conditioning took place during six sessions of 60 CS–US presentations per session. Retention of conditioned responding was assessed 5 days after the last acquisition and drug injection session. Retention testing took place during four sessions of 60 CS-alone presentations per session. No drug or vehicle injections were administered during retention testing.

2.5. Data analysis

The data were expressed as the percentages of CRs and were analyzed with repeated-measures analyses of variance using SYSTAT 7.0 for Windows (SPSS, Chicago, IL). Post hoc tests of between-group effects were carried out using either Tukey's HSD test or Dunnett's t-test, whichever was most appropriate. If Dunnett's t-test detected a significant difference between the drug dose and the vehicle control group, the rate of acquisition was further assessed by determining the number of trials required to achieve a criterion of 10 consecutive CRs. The trials-to-criterion measure was then analyzed with a one-way analysis of variance and significant effects followed up with a Dunnett's t-test.

3. Results

3.1. Experiment 1: mianserin and BOL dose response functions

As shown in Fig. 1, mianserin produced a dose-dependent retardation of CR acquisition. Vehicle-treated animals reached the highest asymptotic level of CRs, averaging 83% over the last 3 days of training. By contrast, the average percentages of CRs over the last 3 days for the 0.1, 1.0, and 10 μmol/kg dose groups were 64%, 41%, and 33%, respectively.

Although the 0.1 μmol/kg dose group showed a lower level of asymptotic CRs than the vehicle controls, reference to Fig. 1 indicates that this dose group and the vehicle group responded at comparable levels over the first five sessions of training. Fig. 1 also suggests that there was very little difference in the performance of the 1.0- and 10-μmol/kg dose groups throughout acquisition, with both groups showing a marked retardation in the rate of learning compared to vehicle controls. A repeated-measures analysis of the data depicted in Fig. 1 determined that there was a significant dose main effect, $F(3,19) = 5.44$, $p < 0.01$, a significant days main effect, $F(7,21) = 35.12$, $p < 0.001$, and a significant dose × days interaction, $F(7,133) = 1.85$, $p < 0.025$. Dunnett's test was used as a post hoc test for the dose main effect. Both the 1.0- and 10-μmol/kg dose groups showed significantly lower percentages of CRs than the vehicle controls.

To characterize further the mianserin dose effect function and its relationship to the rate of learning, the data for individual animals were expressed as the number of trials to achieve a criterion of 10 consecutive CRs. As shown in Fig. 2, there was a significant, dose-dependent increase in the number of trials required to achieve this criterion, $F(3,20) = 4.29$, $p < 0.05$. Animals receiving the highest dose of mianserin (10 μmol/kg) required 201 more trials than vehicle controls to achieve this criterion, and this difference was significant by Dunnett's test.

The effects of BOL on CR acquisition are presented in Fig. 3. Vehicle-treated animals reached an average of 67% CRs averaged over the last three acquisition sessions, compared to 55%, 76%, and 75% CRs for the 0.058-, 0.58-, and 5.8-μmol/kg doses, respectively. The analysis of variance determined that there were significant main effects of both dose, $F(3,20) = 3.74$, $p < 0.05$, and days, $F(7,140) = 33.83$, $p < 0.001$. There was no significant interaction between the two variables, $F(21,140) = 1.33$. A post hoc test of the dose main effect using Dunnett's t-test failed to detect a difference between any dose of BOL and the vehicle control group.

3.2. Experiment 2: antagonism of the effects of mianserin on CR acquisition by BOL

Acquisition rates for the four different treatment combinations are plotted in Fig. 4. Vehicle + vehicle control animals reached an average of 66% CRs averaged over the last three acquisition sessions. In agreement with the results of Experiment 1, the vehicle + mianserin (10 μmol/kg) group demonstrated a retarded rate of CR acquisition relative to controls, demonstrating 28% CRs across the last 3 days of acquisition. During the initial stage of training the vehicle + BOL (5.8 μmol/kg) group of animals appeared to be acquiring CRs at a faster rate than any other group. However, during the last 3 days of

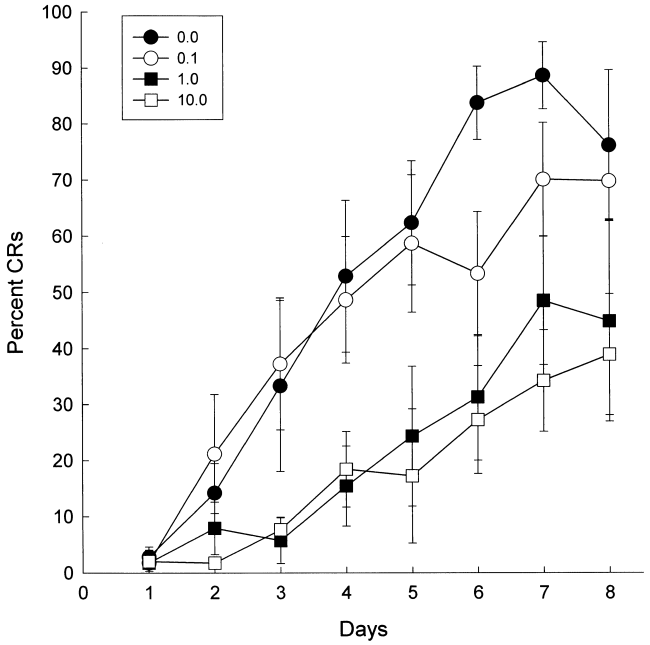

Fig. 1. Effect of mianserin on CR acquisition. Mean percentages of CRs are plotted for each acquisition session as a function of dose. Each session consisted of 60 CS–US pairings. Inset: dose of mianserin expressed as μmol/kg.

acquisition, the vehicle+BOL group demonstrated a level of CRs (67%) that was equivalent to that of the vehicle+-vehicle controls (66%). Most importantly, inspection of Fig. 4 indicates that BOL completely blocked the retardant effects of mianserin on CR acquisition. Thus, the mianserin+BOL group of animals demonstrated equivalent rates of acquisition and an equivalent level of CRs across the last 3 days of acquisition (65%) with that of the vehicle+vehicle controls.

The analysis of variance confirmed these observations. Thus, there were significant effects due to drug treatment, $F(3,38)=7.22$, $p<0.001$, days, $F(7,266)=48.06$, $p<0.001$, and the interaction, $F(21,266)=3.08$, $p<0.001$. The significant main effect of drug treatment was further analyzed using Tukey's HSD test. The mianserin+vehicle group averaged 20% CRs over all eight sessions and this was significantly lower than the 44% CRs exhibited by the vehicle+vehicle group. More importantly, BOL blocked the effect of mianserin such that mianserin+BOL animals averaged 42% CRs, a result that was not significantly different from that of vehicle+vehicle animals. Finally, mianserin+vehicle animals also responded at a significantly lower level than vehicle+BOL animals (20% vs. 57%).

3.3. Experiment 3: Maintenance of learning

In agreement with the results of Experiments 1 and 2, a 10-μmol/kg dose of mianserin retarded the rate of CR acquisition (top panel of Fig. 5). Vehicle-treated animals showed a large increase in the percentage of CRs beginning with the third acquisition session. On the final, half-session of acquisition training, the rate of conditioned responding in vehicle-treated animals was 56%. By contrast, mianserin-treated animals never exceeded a 10% rate of conditioned responding over the six full sessions of training and achieved less than a 14% rate on the final half-session of acquisition. Analysis of the acquisition data determined that there was a significant main effect of both drug, $F(1,14)=10.47$, $p<0.01$, and day, $F(5,70)=8.53$, $p<0.001$, as well as a significant interaction between the two variables, $F(5,70)=4.99$, $p<0.01$. The drug main effect for the half session on day 7 was also significant, $F(1,14)=9.27$, $p<0.01$.

Maintenance of learning was assessed during continued acquisition training, 5 days after the last drug or vehicle injection. No injections were administered during this maintenance phase. The relevant data are plotted over maintenance days 1–4 in the top panel of Fig. 5. Both groups of animals showed an increase in the percentage of CRs on the first maintenance session compared to the last acquisition session. Animals undergoing mianserin injections during the acquisition phase tended to show the greater increase in CRs when switched to the drug-free maintenance phase. Analysis of the data across maintenance sessions failed to detect any main or interaction effects. However, there was a nonsignificant ($p<0.06$) tendency for vehicle animals to perform at a higher level of CRs than mianserin animals.

The increase in the percentage of CRs between the two phases of training was subjected to a finer grained analysis by comparing the last block of 20 acquisition trials with the

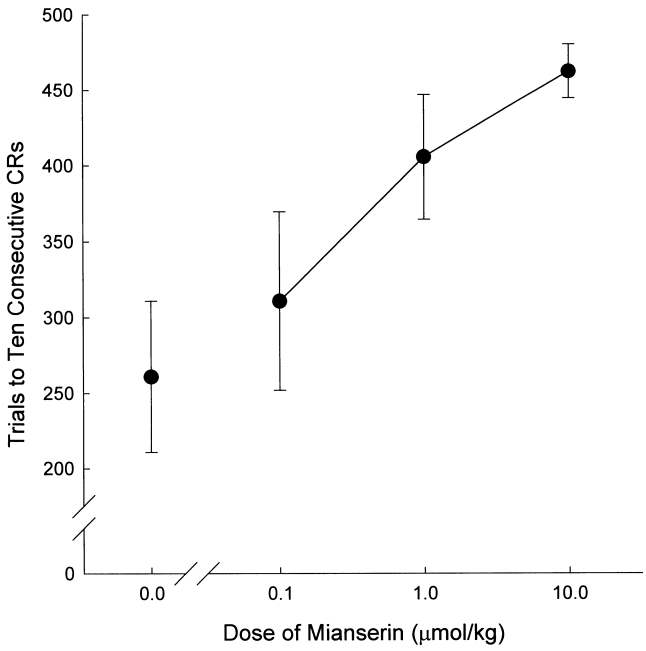

Fig. 2. Dose effect function of mianserin on rate of learning as assessed by the number of CS–US trials required to achieve a criterion of 10 consecutive CRs.

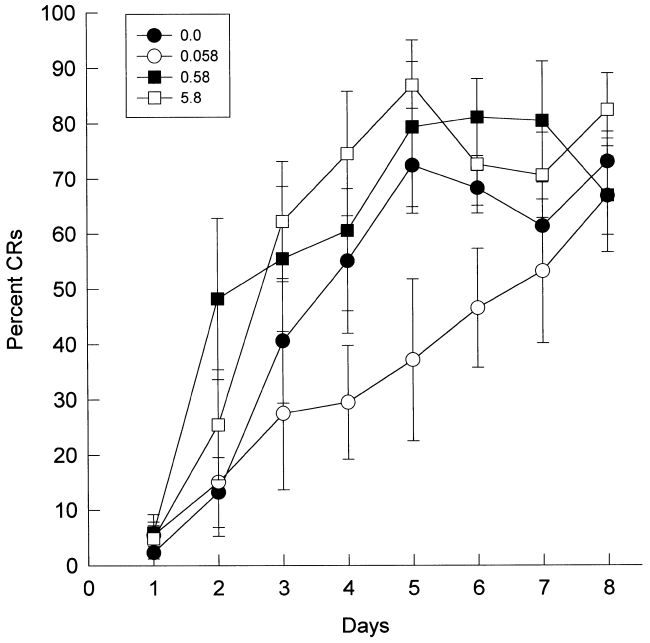

Fig. 3. Effect of BOL on rate of acquisition. Mean percentages of CRs are plotted for each acquisition session as a function of dose. Inset: dose of BOL expressed as μmol/kg.

first block of 20 maintenance trials. A pronounced increase in CRs between these two phases of training on the part of the mianserin group would suggest that performance variables were producing some of the apparent increase in learned behavior seen during the maintenance phase. The relevant data are shown in the bottom panel of Fig. 5. Between the last block of acquisition and the first block of maintenance, vehicle animals showed a 14% increase in the percentage of CRs compared to an 18% increase for mianserin animals. An analysis of variance on these data determined that the main effect of drug was significant, $F(1,14)=7.29$, $p<0.025$, as was the main effect of training phase, $F(1,14)=4.59$, $p<0.05$. The lack of a significant interaction between these two variables, $F(1,14)<1$, suggests that performance factors alone cannot account for the increase in CRs seen in mianserin animals during the drug-free training phase.

3.4. Experiment 4: Retention of acquisition

As shown in the top panel of Fig. 6, acquisition of CRs was again retarded following daily mianserin injections of 10 μmol/kg. The difference between mianserin- and vehicle-treated animals of this experiment was not as dramatic as in Experiment 3 (see Fig. 5), but similar to that of animals receiving the 10-μmol/kg dose of mianserin in Experiment 1 (see Fig. 1). Vehicle-treated animals showed an abrupt increase in the rate of conditioned responding between the first and second sessions and a terminal level of CRs of 44%. Mianserin-treated animals showed a much slower rate of increase in CRs and achieved a terminal level of CRs of 21%. Analysis of the acquisition data determined that there were significant main effects of both drug, $F(1,22)=11.8$, $p<0.01$, and days, $F(5,110)=9.09$, $p<0.001$. However, because the performance of mianserin-treated animals tended to parallel the performance of vehicle-treated animals, there was no significant interaction between drug condition and the repeated-measure, $F(5,110)=1.22$.

Retention of acquisition was assessed during four sessions of CS-alone trials conducted 5 days after the last injection and acquisition session. No drug or vehicle injections were administered during retention testing. The relevant data are plotted over retention days 1–4 in the top panel of Fig. 6. Both groups of animals showed a slight increase in the percentage of CRs relative to the last acquisition session but then showed orderly declines in CRs over subsequent sessions. There was no difference between the two groups of animals over the retention sessions, $F(1,22)=1.34$, nor did the acquisition drug treatment interact with the repeated-measure during retention testing, $F(3,66)<1$. However, a significant main effect of days was obtained, $F(3,66)=3.19$, $p<0.05$, indicating that both groups showed equivalent decreases in CRs over the four retention sessions.

A finer grained analysis of retention testing was conducted by comparing the last 20-trial block of acquisition training with the first 20-trial block of retention testing. The relevant data are shown in the bottom panel of Fig. 6.

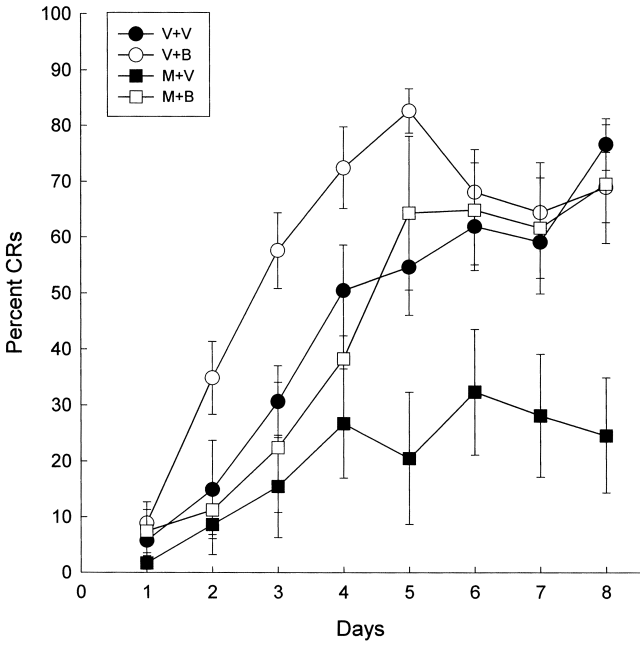

Fig. 4. Antagonism by BOL of the effects of mianserin on the rate of CR acquisition. Each animal was injected with either vehicle or mianserin (10 μmol/kg) 60 min prior to an injection of either vehicle or BOL (5.8 μmol/kg). Thus, there were four different treatment combinations: vehicle + vehicle (V+V), vehicle + BOL (V+B), mianserin + vehicle (M+V), and mianserin + BOL (M+B).

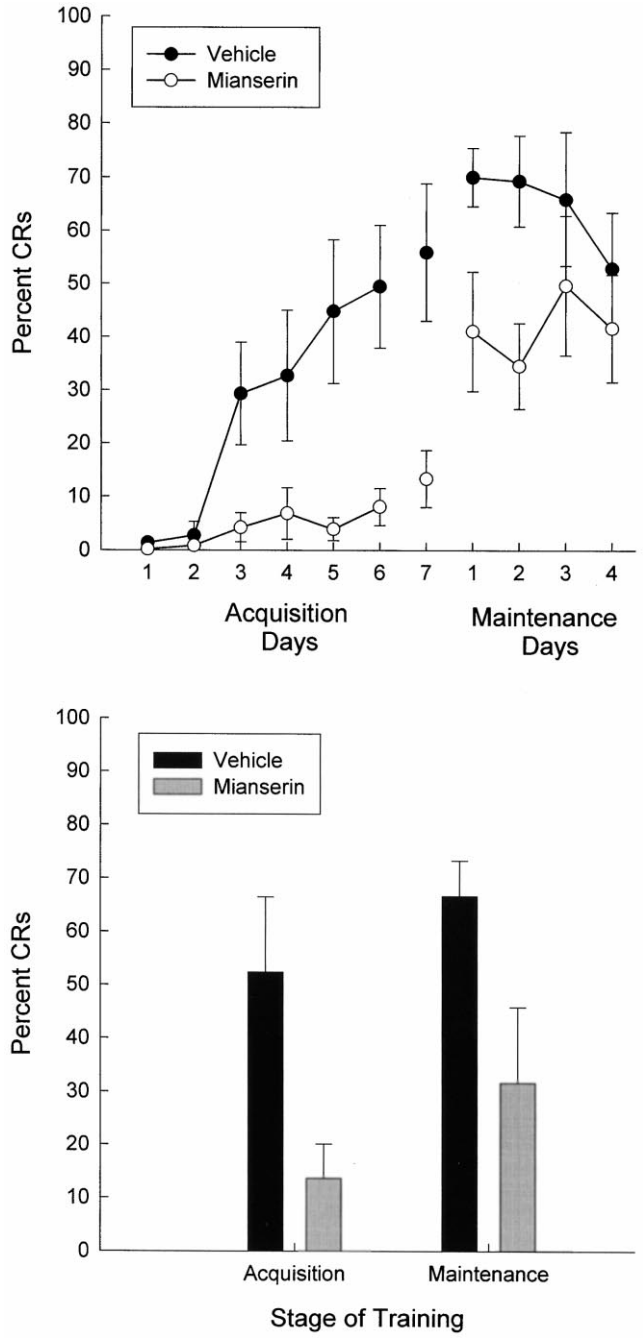

Fig. 5. (Top panel) Effect of mianserin (10 μmol/kg) on rate of acquisition and maintenance of conditioned responding. Day 7 of the acquisition phase was a half session consisting of only 30 CS–US pairings. Days 1–4 of the maintenance phase were conducted 5 days after the last acquisition and drug injection session. No drug or vehicle injections were administered during the maintenance phase. (Bottom panel) Mean percentages of CRs obtained during the last block of 20 acquisition trials vs. the first block of 20 maintenance trials.

Between the last block of acquisition and the first block of retention, vehicle animals showed an 8% increase in the level of CRs compared to a 9% increase for mianserin animals. An analysis of variance determined that the main effect of the drug treatment was the only significant source of variation, $F(1,22)=6.81$, $p<0.025$. The lack of a significant days effect and of a significant interaction between the treatment variable and the repeated measure suggests that the decreased percentage of CRs demonstrated by the mianserin group during acquisition was most likely due to a decrease in learning rather than to some effect on performance.

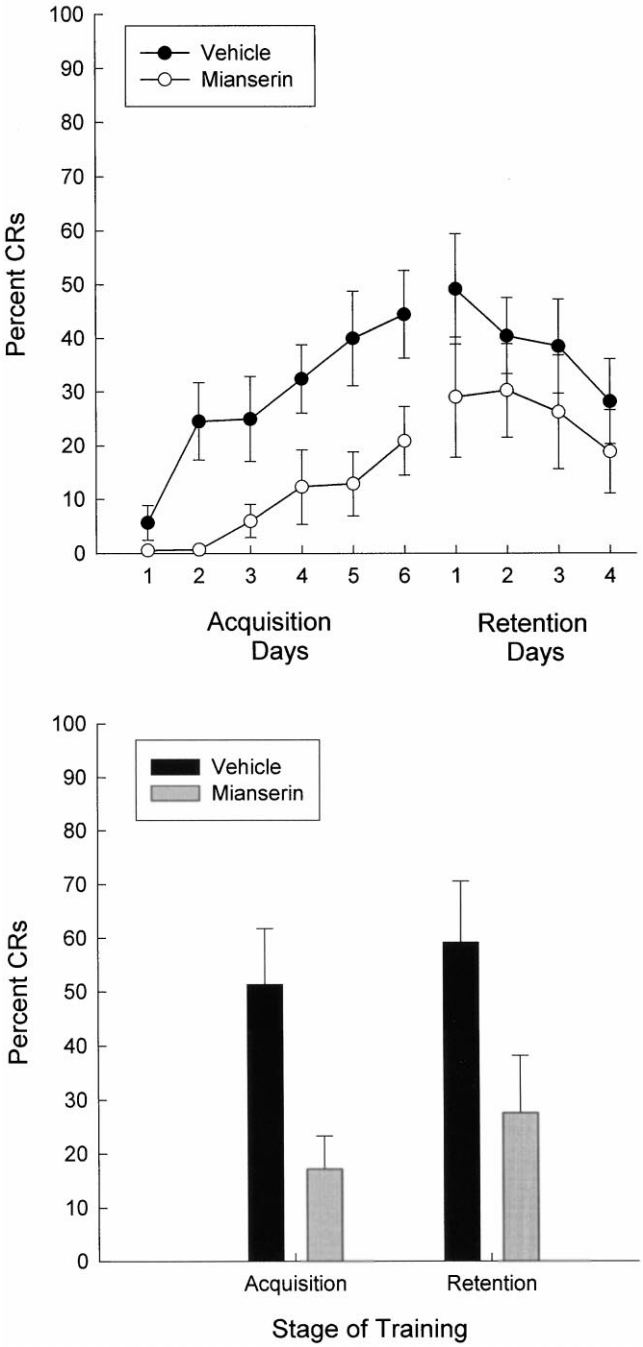

Fig. 6. (Top panel) Effect of mianserin (10 μmol/kg) on rate of acquisition and retention testing. Retention days 1–4 took place 5 days after the last acquisition and drug injection session. Retention was assessed during a drug-free series of CS-alone trials. (Bottom panel) Mean percentages of CRs obtained during the last block of 20 acquisition trials vs. the first block of 20 retention trials.

4. Discussion

4.1. Mianserin retarded learning through an action at the 5-HT$_2$ receptor

The present study extends our previous work with 5-HT$_2$ agonists and antagonists by demonstrating that the 5-HT$_2$ receptor plays an important role in associative learning and in the performance of motor responses. Agonists at the 5-HT$_2$ receptor enhance associative learning as measured by the rabbit's NM response [4,7,9,11] and increase the amplitude of the unconditioned NM reflex [5,7–10]. The results of this and previous studies indicate that antagonists at the 5-HT$_2$ receptor fall into two categories. LY-53,857 [12] and BOL (Experiment 1 of this study) could be classified as neutral antagonists in that they had little or no effect on learning. These drugs also had little or no effect on motor performance as measured by their effects on the amplitude of the UR elicited by varying intensities of the US [6]. By contrast, ritanserin and MDL-11,939 [11,12] and mianserin (this study) could be classified as inverse agonists because they significantly retarded learning. All three drugs also reduced the amplitude of the unconditioned NM reflex [6] at the same doses that retarded learning. The reduction in reflex amplitude [6] and the retarded rate of learning (this study) produced by mianserin (10 μmol/kg) was completely antagonized by the 5-HT$_2$ antagonist BOL (5.8 μmol/kg). These results provide strong evidence that mianserin was producing both the learning and performance effects through an action at the 5-HT$_2$ receptor. These results also suggest that mianserin's retardation of learning and impairment of the NM reflex, effects opposite to those of 5-HT$_2$ agonists, were due to an inverse agonist action at the 5-HT$_2$ receptor.

Although BOL had no significant effect on the rate of learning compared to vehicle controls, inspection of Fig. 3 suggests that BOL tended to retard the rate of learning at the lowest dose while having little effect at the higher doses. It should be noted that pizotifen, another 5-HT$_2$ antagonist, was also reported to attenuate the rate of eyeblink conditioning in rabbits in a reverse, dose-dependent fashion [3]. The basis for this low dose effect of either BOL or pizotifen remains unclear.

4.2. Mianserin's retardation of learning was not due to performance variables

The lack of an effect of mianserin on the maintenance of learned responding in Experiment 3 indicates that mianserin was not merely interfering with the performance of the learned response during the acquisition phase. If interference with motor performance was the sole mechanism by which mianserin affected acquisition of the CR, then an immediate increase in the percentage of CRs should have occurred at the beginning of the maintenance phase. However, a comparison of the last block of acquisition trials with the first block of maintenance trials indicated that there was no immediate increase in learned responding between the two stages of training, which could be attributed to the acquisition treatment condition. The two groups of animals showed equivalent increases in learned responding as they entered the maintenance phase. It might be argued that mianserin-treated animals showed a savings effect given the large increase in learned responding between the last acquisition session and the first maintenance session. However, the magnitude of that increase was comparable to the increase seen in vehicle animals between the second and third acquisition sessions, making the argument for a savings effect untenable.

Retention of the acquired CR was perfect, regardless of the acquisition treatment condition. Both groups of animals actually showed a modest increase in the percentage of CRs during the first 20 trials of retention testing compared to the last 20 trials of acquisition training, even though acquisition and retention testing were separated by 5 days of rest. The retention results provide further evidence that the deficit in learned responding cannot be attributed solely to the known motor impairments produced by mianserin [6]. Furthermore, because retention was completely unaffected by mianserin treatment during acquisition, the deficit in acquisition cannot be attributed to a state-dependent phenomenon.

In summary, these experiments indicate that mianserin was retarding learning and affecting performance through an inverse agonist action at the 5-HT$_2$ receptor, but that the two behavioral effects were separable. These results suggest that 5-HT$_2$ agonists and antagonists are acting on independent neuronal systems involved in learning and in performance of the NM response.

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