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Pharmacology, Biochemistry and Behavior 69 (2001) 585–593

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

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MK-801 induced retrieval, but not acquisition, deficits for passive avoidance conditioning

S.B. Harrod[a,*], R.W. Flint[b], D.C. Riccio[c]

[a]*Department of Psychology, University of Kentucky, Lexington, KY 40506, USA*
[b]*Department of Psychology, The College of Saint Rose, Albany, NY 12203, USA*
[c]*Department of Psychology, Kent State University, Kent, OH 44242, USA*

Received 27 October 2000; received in revised form 26 March 2001; accepted 6 April 2001

Abstract

Two experiments using a state-dependent retention (SDR) design determined whether MK-801 blocked the acquisition and retention of an avoidance response. In Experiments 1 and 2, rats were trained and tested 30 min after injections of either saline or MK-801 (0.05 and 0.10 mg/kg, respectively). Two minutes after training, subjects were immediately tested, and in both experiments, the avoidance response was acquired. The 24-h retention tests for Experiment 1 revealed that the data marginally supported a SDR interpretation. In Experiment 2, the dose of MK-801 was increased to 0.10 mg/kg, and the results showed that MK-801 rendered passive avoidance (PA) state-dependent. These experiments indicate that neither the 0.05 nor 0.10 mg/kg doses of MK-801 prevented acquisition of the avoidance response and that the latter dose rendered memory for PA training state-dependent. It is suggested that doses of MK-801 that did not impair PA learning can function as a cue state and influence expression of memory for PA.

Keywords: MK-801; NMDA receptor antagonist; State-dependent retention; Passive avoidance; Learning; Memory; Rat

1. Introduction

Numerous experiments have demonstrated that testing human and nonhuman subjects in a stimulus environment that is altered from training, i.e., shifted context, results in significant retrieval deficits (Kissinger and Riccio, 1995; Spear et al., 1980; Smith, 1979; Hinderliter et al., 1975; Perkins and Weyant, 1958). Contemporary theories of memory retrieval suggest that contextual cues can mediate the expression of memory. Thus, according to this hypothesis, a conditioned response has a higher probability of being performed in the presence of specific contextual cues, namely those present during the learning episode (Bouton, 1994; Spear and Riccio, 1994).

As with changes in external contextual cues, altered ''internal'' or interoceptive cues can lead to profound impairments in memory retrieval as well. Such memory impairments have been examined by administering various pharmacological agents, hypo- and hyperthermic conditions, and electroconvulsive shock prior to training and/or testing (Mactutus et al, 1980; Miller and Springer, 1972; Thompson and Neely, 1970; Overton, 1978). As is the case with shifts of external contextual cues, the memory loss in subjects tested with an altered internal state has been considered to result from retrieval failure. That is, the mechanism of forgetting in these particular manipulations is related to the absence of cues elicited by the pharmacological or thermal stimulus during acquisition. Thus, the similarity of the interoceptive contextual cues present during acquisition and testing determines the probability that the target response is retrieved (Spear and Riccio, 1994). Retrieval that depends on the congruence of internal contextual cues at training and testing has been referred to as state-dependent retention (SDR). Early studies utilized a 2×2 design in which subjects received either saline or a drug prior to both training and testing (Overton, 1978). The design yields the saline–saline (S–S), drug–drug (D–D), saline–drug (S–D), and drug–saline (D–S) groups.

Many studies have investigated the SDR effects of various agents by using a single-trial passive avoidance (PA) procedure (Spear and Riccio, 1994). In these experi-

* Corresponding author. Tel.: +1-859-257-6019; fax: +1-859-323-1979.

E-mail address: sharr2@pop.uky.edu (S.B. Harrod).

0091-3057/01/$ – see front matter
PII: S0091-3057(01)00565-2

ments, SDR was observed when the "state" of the organism during testing matched that of training. Conversely, mismatches between training and testing state resulted in forgetting. The aim of the present set of experiments was to examine whether MK-801, a noncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonist, rendered memory for PA conditioning state-dependent.

It is well accepted that MK-801 impairs the acquisition, and hence memory, of various behavioral tasks. For instance, MK-801 has been reported to impair memory for passive (Benvenga and Spaulding, 1988; Venable and Kelly, 1990; Mondadori and Weiskrantz, 1993; Nakagawa and Iwasaki, 1996; Kim and McGaugh, 1992) and active avoidance learning (Delay, 1996; Xu et al., 1998) and the acquisition and extinction of Pavlovian fear conditioning (Baker and Azorlosa, 1996; Johnson et al., 2000). Furthermore, MK-801 impedes the acquisition of taste-potentiated odor aversion (Robinson et al., 1989) and the acquisition and extinction of a hypoalgesic response (Cox and Westbrook, 1994). MK-801 has been reported to impair spatial learning in both the radial eight-arm maze (Shapiro and O'Conner, 1992) and water maze tasks (Robinson et al., 1989; Whishaw and Auer, 1989; Heale and Harley, 1990; Filliat and Blanchet, 1995) and also to block tolerance to stress-induced analgesia (Vaccarino and Clavier, 1997).

Interestingly, one study suggested that NMDA antagonists induced SDR effects. Using a food-rewarded lever-pressing task, Jackson et al. (1992) trained rats to lever-press on a fixed ratio 10 (FR 10) schedule of reinforcement following injections of MK-801, PCP, ketamine, CPP, or saline. Rats achieved criterion when they met the FR 10 requirement within the first 120 s of the training session. Prior to the 24-h retention test, animals were administered the NMDA antagonists or saline (i.e., the D–S, S–D, and S–S groups), and the percentage of animals that completed the FR 10 requirement within the first 120 s of the training session was recorded. The retention test measured whether the response requirement transferred to the same or different drug state. Although Jackson et al. (1992) did not include a D–D group to assess retention, there was a failure to transfer earlier learning in the mismatched conditions for all four NMDA receptor antagonists. In these experiments, doses of the NMDA receptor antagonists lower than those that prevent normal acquisition of the FR 10, 120-s requirement were used. All animals trained on one of NMDA antagonists met the FR 10 criterion as well as subjects trained on saline. These results suggest that NMDA antagonists, given at doses lower than those which impart memorial or performance deficits, produce SDR for a simple schedule of reinforcement.

The present set of experiments used a standard PA procedure to investigate whether MK-801 induced SDR. Prior experiments using the PA paradigm have suggested that MK-801 blocked acquisition of the avoidance response (Benvenga and Spaulding, 1988; Venable and Kelly, 1990). The results of those experiments showed that animals treated with MK-801 prior to training did not exhibit good PA performance compared to saline controls during the 24-h retention test. In those experiments, however, immediate tests were not administered to determine whether the avoidance response had been acquired. Although the experiments ruled out the possibility that motoric problems, analgesia, or increases in locomotion accounted for poor performance, it is not clear whether the deficit imparted by MK-801 reflected an acquisition deficit or later retrieval failure, since testing did not occur until 1 day after training.

In order to determine if MK-801 prevents acquisition of a single-trial PA response in the present experiments, animals were tested immediately (i.e., 2 min) after training. The dependent measure for the immediate test was whether rats crossed into the compartment previously paired with footshock, and if so, how quickly (latency). Regardless of the outcome, subjects were tested again 24 h after training, following injections of either MK-801 or the vehicle, in accord with a SDR design. If MK-801 impairs acquisition of the PA response as other experiments have suggested (Venable and Kelly, 1990), it was predicted that during the immediate test, subjects would exhibit short latencies to traverse the PA apparatus. This outcome would preclude a SDR explanation and would replicate other findings showing that administration of MK-801, in accord with a SDR design, resulted in poor memory for the response (Baker and Azorlosa, 1996; Nakagawa and Iwasaki, 1996; Cox and Westbrook, 1994; Kim and McGaugh, 1992). However, long latencies or failure to cross into the chamber paired with footshock would imply that rats learned the PA response. This outcome would suggest that the impairments observed in studies using a single-trial training procedure that did not measure acquisition might have been due to retrieval failure rather than impaired acquisition.

2. Experiment 1

2.1. Methods

2.1.1. Subjects

Sixty, 100–250-day-old, male Sprague–Dawley rats, purchased from Hilltop, served as subjects in the present experiment. All animals were housed individually in hanging wire-mesh cages and were maintained on a 15:09-h light/dark cycle. Food and water were available ad libitum.

2.1.2. Apparatus

The training and testing apparatus was a Plexiglas rectangular box ($38 \times 18 \times 21$ cm) divided into two identical-size chambers. One chamber was painted white and fitted with a clear lid, and the walls and lid in the opposite chamber were painted black. An (8×10 cm) guillotine doorway divided the apparatus, which allowed subjects to move freely throughout the two chambers. A 15-W light bulb was suspended from the ceiling 30 cm above the white

compartment; no other light source was present in the training/testing room. The floor of the entire apparatus was made up of 2.5-mm stainless-steel rods, evenly spaced 1 cm apart. The noncontingent footshock (NCFS) apparatus, located in an adjacent room, was a clear, rectangular, Plexiglas chamber. The chamber was situated on stainless-steel rods, similar to those of the training apparatus. NCFS were delivered through with a matched impedance shock source. White noise was presented throughout the experiment to reduce any potential auditory disturbances.

2.1.3. Procedure

Animals were randomly assigned to one of six groups designated by whether they received saline or MK-801 prior to training and testing. Thus, in accord with the 2×2 SDR design, the following groups were used in Experiment 1: the S–S ($n = 12$), MK–MK ($n = 12$), S–MK ($n = 12$), and MK–S ($n = 12$) groups. Also, NCFS controls received "pseudo-training." These subjects were included in order to determine whether MK-801 impaired the animal's ability to traverse the apparatus and whether it changed the rats' natural preference to remain in the black compartment. In general, rats given nonreinforced access to the black chamber show a preference for the dark compartment. If the drug changes a rat's preference for the dark compartment, more time spent on the white side of the apparatus could be interpreted as having fear for that chamber (e.g., amnesia). Thus, the pseudo-trained control groups allow an evaluation of the behavior of animals receiving the drug, footshock, and equal exposure to the chamber in the absence of explicit conditioning of fear to the black side of the apparatus. Subjects in the NCFS control groups were given saline (NCFS S–S $n = 10$) or MK-801 (NCFS MK–MK $n = 10$) 30 min prior to training and testing. All animals were handled for 2 min daily, 2 days prior to the beginning of the experiment.

2.1.3.1. Training. On Day 1, all subjects were given an intraperitoneal (ip) injection of saline or MK-801 (0.05 mg/kg) 30 min prior to training or pseudotraining. During training, animals were removed from their home cages, held for 10 s in the training/testing room, and then placed facing away from the guillotine door on the white side of the training apparatus. After 20 s, the door was raised allowing subjects to cross to the black chamber. Upon crossing, the door was lowered and a 3-s, inescapable, 1.15-mA footshock was delivered through a matched impedance shock source. Subjects were immediately removed from the apparatus. Animals in the NCFS condition were given pseudotraining in the training context. Specifically, 30 min following injections, subjects were placed in the training apparatus for 20 s and given nonreinforced exposure to the black compartment. Animals were placed in a holding cage for 15 min and then placed into the NCFS apparatus where they received a 3-s, 1.15-mA footshock. Fifteen minutes were interpolated between pseudotraining and NCFS to prevent any association between footshock and the two contexts. Following footshock, all rats were immediately removed from the shock apparatus and placed in a holding cage. If rats failed to make the crossover response within 5 min of placement, subjects were removed from the apparatus and were not used in the study.

2.1.3.2. Immediate testing. Two minutes after training, subjects were placed on the white side of the apparatus for 20 s and given access to the black chamber for 60 s. Rats not crossing within that period were removed and given a latency score of 60 s. Similarly, the pseudotrained rats were given the immediate test 2 min after NCFS. Latency to cross into the black compartment was the dependent measure.

2.1.3.3. 24-h retention tests. Twenty-four hours after training, animals were given injections of either saline or MK-801. Thirty minutes after injection, subjects were placed in the white chamber for 20 s after which the door was opened and they were given access to the black portion of the apparatus for 300 s. The latency to cross into the black compartment and the time spent in the black chamber were the dependent variables. The time spent in the black compartment was converted to a spatial aversion score, which represented the proportion of time spent in the white (safe) relative to the black (footshock) chamber. The aversion score was derived from taking the total time spent on the white side minus the total time on the black side divided by the total time allotted for testing. A positive aversion score indicates that animals remained on the white side for the majority of testing, whereas a negative score reflects choice of the black compartment and indicates poor avoidance behavior. Scores of zero indicate an equal preference for both compartments. Footshocks were not delivered during testing.

2.2. Statistics

Data taken from avoidance experiments generally result in skewed distributions, which represent both the maximum and minimum scores. Therefore, one-way, nonparametric Kruskal–Wallis ANOVA and subsequent Mann–Whitney U comparisons were used to analyze the training latencies and immediate test latencies in Experiments 1 and 2 and the 24-h latency scores in Experiment 2. Since transformations tend to normalize scores, parametric 2×2 factorial ANOVA and planned t tests were used for the 24-h latency and spatial aversion scores from Experiment 1 and the immediate latency and 24-h spatial aversion scores from Experiment 2. The 24-h latency scores from Experiment 1 were subjected to a log transformation, whereas when computing the aversion scores, the data were transformed as all scores fall between 1.0 and -1.0. Bonferroni corrections were used for both the Mann–Whitney U and t tests to protect against type 1 errors. The Bonferroni correction was computed by dividing $\alpha = .05$ by the number of comparisons made. Thus, significance was derived only if the probability was less than the computed α value (Kirk, 1995).

Table 1
Median and the mean log crossover latencies from Experiment 1

Group	*n*	Training latency median (IQ)	Immediate test latency median (IQ)	24-h test latency mean ± S.E.M.
S–S	12	7.5 (11.0)	60.0 (0.0)	2.3 ± 0.1
MK–MK	12	11.5 (7.0)	60.0 (0.0)	2.0 ± 0.2
S–MK	12	8.5 (17.0)	60.0 (0.0)	1.8 ± 0.2
MK–S	12	7.5 (11.0)	60.0 (0.0)	1.6 ± 0.2[a]
S–S (NCFS)	8	4.0 (12.0)	4.0 (5.0)[a]	0.7 ± 0.2
MK–MK (NCFS)	8	3.0 (2.0)	11.5 (15.0)[a]	0.5 ± 0.1

[a] $P < .01$, different from S–S.

2.3. Results and discussion

2.3.1. Training latencies

The median training latencies are presented in Table 1. Latencies to cross into the black chamber during training (i.e., the response that resulted in footshock) were recorded to determine whether MK-801 influenced response time. A Kruskal–Wallis one-way ANOVA indicated that training latencies did not differ across the six groups examined ($H = 0.446$, $P > .05$), suggesting that MK-801 did not alter crossover latencies.

2.3.2. Immediate test

Immediate test data are presented in Table 1. A Kruskal–Wallis one-way ANOVA revealed that there were differences in subject's latencies to enter the shock-related compartment ($H = 54.07$, $P < .01$). Table 1 shows that the NCFS groups crossed quickly, whereas the S–S, MK–MK, S–MK, and S–MK groups exhibited median scores of 60 s (maximum). All of the scores in the MK–MK, S–MK, and MK–S groups were 60 s, and the S–S group had only one score less than 60 s. To decrease the number of comparisons, the S–S groups' immediate test latencies were compared to those of the NCFS groups. Mann–Whitney *U* tests confirmed that the NCFS groups' latencies were performed more quickly than those of the S–S group. These findings are not surprising given that animals, which received NCFS training, were, by definition, not "trained." Thus, shorter latencies to cross reveal that the NCFS groups did not acquire fear to the black compartment. Comparison of the two NCFS groups revealed that their crossover latencies at the immediate test were not different, indicating that MK-801 did not increase locomotor activity.

2.3.3. 24-h latency

The mean log latencies to cross into the black compartment are presented in Table 1. A 2×2 (Train × Test) ANOVA revealed a significant Train × Test interaction [$F(1,44) = 4.41$, $P < .05$] but no main effect of train or test [$F(1,44) = 1.36$, $P > .05$; $F(1,44) = 0.17$, $P > .05$, respectively]. Subsequent planned comparisons revealed that the latency scores did not differ between the S–S and MK–MK, MK–MK and MK–S, or S–MK and MK–S groups. However, comparisons between the S–S and MK–S groups were significantly different, indicating that when MK-801 (0.05 mg/kg) was present at training, but not at testing, PA performance was impaired. Performance of the two NCFS groups was not different, suggesting that MK-801 (0.05 mg/kg) did not change pseudotrained rats' latencies to cross into the darkened chamber.

2.3.4. 24-h aversion

Fig. 1 represents the mean aversion to the white and black chambers when tested 24 h after training. A 2×2 (Train × Test) ANOVA conducted on the preference scores revealed a significant Train × Test interaction [$F(1, 44) = 4.18$, $P < .05$] but no main effect of train or test [$F(1,44) = 0.17$, $P > .05$; $F(1,44) = 0.02$, $P > .05$, respectively). Subsequent planned comparisons of the spatial aversion scores did not differ between the S–S and MK–MK, MK–MK and MK–S, or S–MK and MK–S groups. Although the spatial aversion score of the MK–S group was substantially lower than the S–S group's score, the analysis did not reach statistical

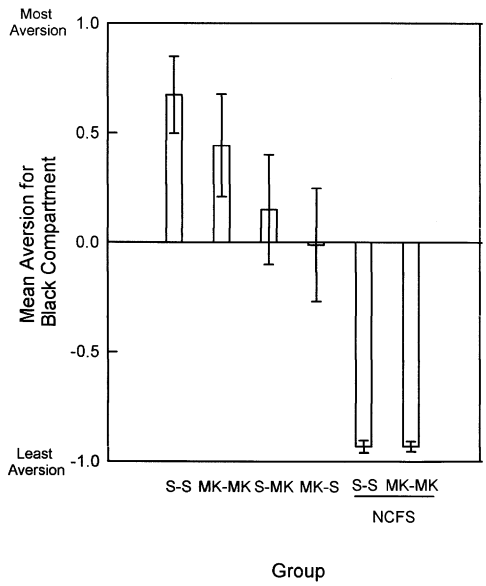

Fig. 1. Mean ± S.E.M. aversion for either the white or black side of the PA apparatus in Experiment 1. Positive numbers represent an aversion to the black side, whereas negative numbers indicate a preference for the black compartment. S–S, MK–MK, MK–S, and S–MK represent animals administered with MK-801 (0.05 mg/kg) or saline before training and/or testing.

significance ($.05 > P > .01$). Performance of the two NCFS groups was not different, suggesting that MK-801 (0.05 mg/kg) did not change pseudotrained rats' preference for the darkened chamber.

The results of the present study show that the 0.05 mg/kg dose of MK-801 injected 30 min prior to training did not impair acquisition of the PA response. Examination of the immediate test data shows that when tested 2 min after training, the S–S, MK–MK, S–MK, and MK–S groups exhibited long latencies to cross into the black compartment and demonstrated the spatial aversion response. The 24-h latency data suggest that although all rats learned the PA response, the MK–S group crossed into the fear-related chamber more quickly than the S–S group. The impairment observed in the present experiment is consistent with findings from other studies demonstrating that a similar dose of MK-801 impairs latency to cross into the black compartment 24 h after training (Benvenga and Spaulding, 1988; Venable and Kelly, 1990). Interestingly, in the present experiment, there was no difference in latency between the S–S and MK–MK groups. This differential performance among groups is not attributable to extinction effects from the acquisition test, since all groups refrained from crossing into the darkened compartment during the 60-s test. Although these data suggest that MK-801 induced SDR, the difference between the MK–MK and MK–S groups did not reach statistical significance. To unequivocally determine that MK-801 induces SDR, the MK–S group must also differ from the MK–MK group.

The 24-h aversion data indicate that although performance of the MK–MK and S–S groups did not differ, the MK–S group did not perform differently than either S–S or MK–MK groups. However, although the comparison between the MK–S and S–S groups did not reach traditional levels of statistical significance, the marginal effect is consistent with previously reported findings (Benvenga and Spaulding, 1988; Venable and Kelly, 1990) that a similar dose of MK-801, administered 30 min prior to training but not at a 24-h retention test, results in a deficit in PA performance.

The results of Experiment 1 suggest that MK-801 (0.05 mg/kg) did not induce ataxia or hyperactivity and did not impair acquisition of the PA response. Although the 24-h data indicate that a shift from MK-801 at training impaired latency to cross into the black compartment, the spatial aversion scores reflect this impairment less clearly. One explanation for these discrepant results may be that the 300-s test was not sensitive enough to detect the MK-801-induced impairment.

3. Experiment 2

Experiment 1 demonstrated that MK-801 (0.05 mg/kg) did not impair acquisition of the PA response. Experiment 2 examined if a higher dose of MK-801 (0.10 mg/kg) impaired the acquisition of PA and whether it produced reliable SDR effect. To remove any possible influence of the within-subjects design used earlier, subjects were tested only once, at the 24-h interval, and separate groups were tested for acquisition. Unlike the previous experiment, this study did not utilize the full 2×2 SDR design. In the prior experiment, the S–MK group's performance was similar to that of the S–S and MK–MK groups'. This result implied that if SDR processes were mediating PA performance, then it was likely due to an asymmetrical SDR effect (Spear and Riccio, 1994) in which only the shift between drug to no-drug conditions yields impairment. Since asymmetrical SDR effects are not uncommon in the literature, the more critical comparison of state dependency involves the shift from drug to no-drug (e.g., MK–S). Accordingly, the present experiment included the S–S, MK–MK, and MK–S groups only. Two separate groups of animals were used to assess acquisition with an immediate test. Also, a substantially longer test condition (900 s) was used to explore further the issue of whether hesitancy to cross into the black compartment was due to acquisition of the PA response or an artifact of freezing to the experience of prior footshock only (see Kim et al., 1992). The S–S, MK–MK, and MK–S groups were trained and administered a 600-s test 24 h after training. To determine if MK-801 affected crossover behavior, NCFS groups were pseudotrained and tested either in the presence or absence (saline) of MK-801 (0.10 mg/kg). The 24-h retention test was extended from 300 to 600 s to increase the sensitivity of the measure.

3.1. Methods

3.1.1. Subjects

Fifty-eight, 100–250-day-old, male Sprague–Dawley rats, purchased from Hilltop, served as subjects in the present experiment. All animals were housed individually in hanging wire-mesh cages and were maintained on a 15:09-h light/dark cycle. Food and water were made available ad libitum.

3.1.2. Apparatus

The training, testing, and NCFS apparatus were identical to those of Experiment 1. White noise was present throughout the experiment to reduce any potential auditory disturbances.

3.1.3. Procedure

Animals were randomly assigned to one of seven groups designated by whether they received saline or MK-801 prior to training and testing. Thus, the following groups were used in Experiment 2: the S–S ($n = 10$), MK–MK ($n = 10$), and MK–S ($n = 10$) groups. Unlike Experiment 1, these subjects were not given an immediate test. Rather, two separate groups, the S–IMM ($n = 7$) and the MK–IMM ($n = 7$), were given saline or MK-801 30 min prior to training. Animals in these two conditions were given an immediate test 2 min after training.

NCFS controls received pseudotraining in order to determine whether MK-801 impaired the animals' ability to

traverse the apparatus and whether it changed rats' natural preference to remain in the black compartment. Subjects in the NCFS controls were given saline (NCFS–S–S $n=7$) or MK-801 (NCFS–MK–MK $n=7$) 30 min prior to training and testing. All animals were handled for 2 min daily, 2 days prior to the beginning of the experiment.

3.1.3.1. Training. On Day 1, all subjects were given an intraperitoneal injection of saline or MK-801 (0.10 mg/kg) 30 min prior to training. Training was identical to that in Experiment 1.

3.1.3.2. Immediate testing. Two minutes after training, subjects in the S–IMM and MK–IMM groups were placed on the white side of the apparatus for 20 s and given access to the black chamber for 900 s. Latency (in seconds) to cross into the black compartment and relative aversion were the dependent measures. In Experiment 1, subjects were administered an immediate acquisition test and were tested 24 h later with a retention test. The immediate tests were short in duration to reduce any extinction that might have occurred with an extended testing procedure. The S–IMM and MK–IMM immediate test groups in Experiment 2 were trained and tested 2 min later, whereas animals in the S–S, MK–MK, and MK–S groups were trained and tested 24 h later. This approach allowed the S–IMM and MK–IMM groups' latency and aversion scores to be measured independently.

3.1.3.3. 24-h retention tests. Twenty-four hours after training, animals in the S–S, MK–MK, and MK–S groups were given injections of either saline or MK-801. Thirty minutes after injection, subjects were placed in the white chamber for 20 s and given access to the black portion of the apparatus for 600 s. An aversion score, the proportion of time spent in the white (safe) relative to the black (footshock) chamber was measured, as well as the latency to cross into the black compartment.

3.2. Results and discussion

3.2.1. Training latencies

The training latencies from Experiment 2 are presented in Table 2. A Kruskal–Wallis one-way ANOVA revealed that there were no differences in subject's latencies to reenter the shock-related compartment [$H=8.016$, $P>.05$]. Furthermore, the S–IMM and MK–IMM groups' latencies to cross into the black chamber did not differ ($U=18.00$, $P>.05$), suggesting that MK-801 (0.10 mg/kg) did not affect crossover latencies.

3.2.2. Immediate test

Comparison of the S–IMM and MK–IMM groups revealed no differences in latency to cross into the black compartment ($U=23.5$, $P>.05$). Thus, animals treated with MK-801 prior to training learned the PA response similarly to the S–IMM group as reflected in performance on the extended 900-s test. These data are presented in Table 2.

3.2.3. 24-h retention test

3.2.3.1. 24-h latency. A Kruskal–Wallis one-way ANOVA revealed that there were differences in subject's latencies to enter the shock-related compartment [$H=18.64$, $P<.001$]. The 24-h latency data are presented in Table 2. Planned comparisons indicated that the S–S and MK–MK groups' latencies to cross into the black compartment were not different. However, further comparisons revealed that the MK–S group was different from the S–S group, although the MK–S group did not perform significantly different than the MK–MK group. Performance of the S–S and MK–MK NCFS groups did not differ, suggesting that MK-801 (0.10 mg/kg) did not affect crossover latencies. These data replicate the findings from Experiment 1 and demonstrate that although rats acquired the PA response, the absence of the drug at training impaired performance on the 24-h retention test. Furthermore, while the pattern of data in Table 2 is consistent with a SDR explanation, the lack of a significant difference between the MK–MK and MK–S groups weakens the interpretation with respect to the latency measure.

3.2.3.2. 24-h aversion. A one-way ANOVA for the aversion scores was significant, suggesting differential aversion scores [$F(4,43)=6.39$, $P<.01$]. The 24-h aversion data are presented in Fig. 2. Planned comparisons revealed that the S–S and MK–MK groups' aversions were not different, suggesting that both groups spent the majority of time in the white compartment avoiding the black chamber. Further

Table 2
The median crossover latencies and mean aversion scores from Experiment 2

Group	*n*	Training latency median (IQ)	Immediate test latency median (IQ)	Immediate test aversion mean ± S.E.M.	24-h test latency median (IQ)
S–S	10	10.0 (7.0)	–	–	600.0 (0.0)
MK–MK	10	9.5 (11.0)	–	–	600.0 (0.0)
MK–S	10	5.5 (3.0)	–	–	79.0 (160.0)[a]
S–S (NCFS)	7	13.0 (12.0)	–	–	10.0 (11.0)
MK–MK (NCFS)	7	16.0 (9.0)	–	–	96.0 (164.0)
S–IMM	7	7.1 (2.0)	900.0 (0)	0.5 ± 0.4	–
MK–IMM	7	11.9 (8.0)	900.0 (0)	0.5 ± 0.3	–

[a] $P<.01$, different from S–S.

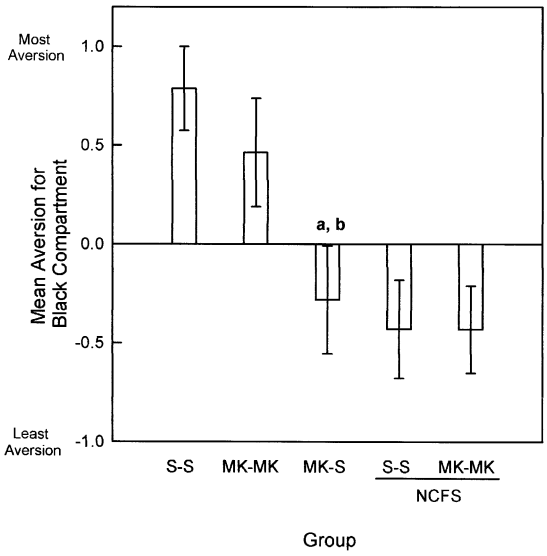

Fig. 2. Mean ± S.E.M. aversion for either the white or black side of the PA apparatus in Experiment 2. Positive numbers represent an aversion to the black side, whereas negative numbers indicate a preference for the black compartment. S–S, MK–MK, MK–S, and S–MK represent animals administered with MK-801 (0.10 mg/kg) or saline before training and/or testing. S–IMM and MK–IMM represent animals administered with saline or MK-801 (0.10 mg/kg) prior to training. Both groups were tested 2 min later. [a] $P < .02$, different from S–S. [b] $P < .02$, different from MK–MK.

comparisons indicated that the MK–S group exhibited less aversion to the black compartment than both the S–S and the MK–MK groups, indicating the MK–S group exhibited poor PA performance. The two NCFS groups' performance did not differ from each other, indicating that MK-801 (0.10 mg/kg) did not change pseudotrained rats' preference for the darkened chamber.

The aversion data support a SDR explanation of PA performance. Both the MK–MK and S–S groups exhibited an aversion to the black side, and both conditions were statistically different from the MK–S group. This finding suggests that if subjects were given MK-801 both before training and testing, their performance was similar to subjects trained and tested on saline, thus providing further evidence that MK-801 did not impair rats ability to learn the PA response. The MK–S group showed poor retention of the PA response apparently because of the mismatch in drug state at the time of testing. While this replicates earlier findings that MK-801 impairs PA performance, the deficit appears to be one of retention rather than acquisition of the response.

4. General discussion

The present experiments used a test given immediately following the training trial to determine whether MK-801 blocked the acquisition of a PA response. In the first experiment, pretraining administration of MK-801 (0.05 mg/kg) did not prevent the acquisition of the PA response, as rats exhibited avoidance of the compartment associated with footshock. The data from the immediate test in the second experiment replicated these results and showed that subjects given an extended 900-s test exhibited long latencies to traverse the apparatus, regardless of treatment, and showed an aversion for the black compartment. Observation of the animals behavior during the extended test revealed that the rats did not remain frozen during the 900-s period but explored the white compartment and extended their bodies into the darkened chamber without completely crossing over. These observations further imply that the aversion for the black compartment and the long latencies to traverse the PA apparatus reflect memory for a punished crossover response. Taken together, the immediate test data from Experiments 1 and 2 suggest that the subjects in the saline and MK-801 groups acquired the fear response.

Interestingly, although rats acquired the response, pretraining administration of MK-801 resulted in impaired performance when subjects were tested without the drug 24 h after training. These findings replicate previously reported findings demonstrating that pretraining administration of MK-801 impairs memory for training when testing occurs in the absence of the drug (Benvenga and Spaulding, 1988; Venable and Kelly, 1990). However, the previously reported studies did not determine whether rats acquired the PA response when training occurred in the presence of MK-801. Thus, although performance deficits were recorded 24 h after training, it is unclear whether MK-801 had blocked the original acquisition or whether the change in drug state impaired expression. The present set of experiments investigated acquisition of single-trial PA conditioning following administration of MK-801 and found that MK-801 did not impair acquisition but a shift in drug state did impair retrieval.

This dissociation between the acquisition and retention of the avoidance response exhibited by subjects treated with MK-801 can be accounted for by a SDR explanation (Overton, 1964; Spear and Riccio, 1994). The implication of a SDR finding is that animals administered pretraining injections of MK-801 or saline acquired the PA response. However, if rats were trained after administration of MK-801 and tested with saline, expression of the response was impaired. One explanation for these results is that MK-801 induces a discriminable internal state that acts as a retrieval cue. According to a SDR explanation, PA was acquired in the presence of the MK-801-induced cue state, and retrieval of the response was contingent on whether MK-801 or saline was present during testing.

Although internal cue states are not directly quantifiable, there exist many examples of drugs that induce SDR (Spear and Riccio, 1994). The present results suggest that MK-801 induces similar state-dependent properties. However, the mechanism by which MK-801 produces an internal cue state is not well understood. Regardless, there are many studies, which report that MK-801 induced distinct "internal cue states." Thus, MK-801 is self-administered (Carlezon and Wise, 1996) and has been shown to reinstate cocaine self-administration (De Vries et al., 1998), suggesting that

MK-801 produces reinforcing effects in rats. Furthermore, drug discrimination studies indicate that rats readily discriminate MK-801 from vehicle across a dose range that includes the doses used in the present set of experiments (Willetts and Balster, 1988; Zajaczkowski et al., 1996). Taken together, these findings suggest that MK-801 produces a discriminable cue state in rats.

Long-term potentiation (LTP), a long-lasting form of synaptic plasticity displayed in the hippocampus and amygdala, has been advanced as the candidate substrate for learning and memory processes (Teyler and DiScenna, 1986; Chapman et al., 1990; Grover and Teyler, 1990; Bliss and Collingridge, 1993; Maren, 1996). This hypothesis is supported by findings, which show that NMDA antagonists that block LTP in vitro also disrupt the acquisition of Pavlovian fear-conditioning procedures (Baker and Azorlosa, 1996; Maren et al., 1996; Gewirtz and Davis, 1997). Moreover, a number of experiments have demonstrated that pretraining, but not posttraining or pretesting, administration of MK-801 impairs memory. These results further suggest that activation of NMDA receptors is critical for the acquisition of various tasks (Robinson et al., 1989; Heale and Harley, 1990; Venable and Kelly, 1990; Delay, 1996). The results of the present experiments only partially support these suggestions. The present findings indicate that pretraining administration of MK-801 did not impair acquisition. Moreover, when rats were administered MK-801 prior to training and were tested in the absence of the drug, retention was poor.

Other studies have examined fear conditioning using the 2×2 SDR design but, in contrast to the present findings, did not conclude that MK-801 induced SDR (Kim and McGaugh, 1992; Baker and Azorlosa, 1996; Nakagawa and Iwasaki, 1996). For example, Kim and McGaugh (1992) administered MK-801 or vehicle prior to training rats on a modified PA task. The findings showed that MK-801 did not prevent acquisition of the response. However, the results of the retention tests revealed that MK-801 impaired memory for training but did not induce SDR. Importantly, there are procedural differences between the present experiments and those used in the Kim and McGaugh's (1992) study. For example, a single-trial PA procedure was used in the present experiments, whereas Kim and McGaugh (1992) used a continuous multiple-trial inhibitory avoidance task. In the latter procedure, a rat crossed into the dark compartment and was allowed to escape back into the illuminated chamber following punishment. If the subject remained in the illuminated compartment for 100 s after punishment, then the subject was removed from the apparatus and training was completed. If the rat made a second crossover response, footshock was delivered until the subject escaped into the illuminated compartment. Thus, in this procedure, the rat could make multiple crossover responses in order to acquire the task. Kim and McGaugh (1992) reported that rats administered MK-801 or vehicle prior to training required approximately two to four acquisition trials to learn the response. This distinction is important since overtraining has been shown to prevent the development of SDR (Spear and Riccio, 1994). Furthermore, in the present experiments, rats were given intraperitoneal injections of MK-801 or saline, whereas Kim and McGaugh (1992) administered MK-801 or vehicle directly into the amygdala. In contrast to peripheral administration, intraamygdala infusions of MK-801 may prevent the drug-induced cue state suggested to be responsible for SDR.

Nakagawa and Iwasaki (1996) used a single-trial PA task and showed that pretraining administration of 0.2 mg/kg MK-801, but not 0.05 or 0.10 mg/kg, resulted in poor PA performance. When MK-801 (0.2 mg/kg) was administered according to the 2×2 SDR design, poor performance was observed regardless of whether MK-801 was administered before training and testing (MK–MK) or exclusively prior to training (MK–S). However, in contrast to previously reported findings (Benvenga and Spaulding, 1988; Venable and Kelly, 1990) and to the results of the present experiments, Nakagawa and Iwasaki (1996) did not observe poor performance using doses of 0.05 or 0.10 mg/kg MK-801.

Interestingly, Baker and Azorlosa (1996) examined if MK-801 had an effect on the rate of extinction using a Pavlovian fear-conditioning procedure. The results demonstrated that extinction was blocked regardless of whether MK-801 was administered prior to extinction and the extinction tests (MK–MK) or exclusively before extinction (MK–S). These findings suggest that MK-801 impaired extinction of Pavlovian fear conditioning.

A general procedural difference between the present report and other studies examining MK-801 and PA conditioning is the use of the spatial aversion score. The present experiments measured the latency to cross into the black compartment and the spatial aversion score, whereas prior experiments measured crossover latencies (Benvenga and Spaulding, 1988; Venable and Kelly, 1990; Kim and McGaugh 1992; Nakagawa and Iwasaki, 1996). When the relative time spent in each compartment was the dependent measure, our results showed that MK-801 rendered PA memory state-dependent. A similar pattern, although only reaching a marginal level of significance, was seen in the latency measurements.

The spatial aversion data suggest that the retrieval and expression of an avoidance response in animals trained on a dose of MK-801 (0.10 mg/kg) was dependent on the presence of MK-801 during testing. The results of the present experiments extend the findings that the NMDA receptor antagonist MK-801 impairs memory during a retention test but suggest that the mechanism for this outcome is SDR. These findings are consistent with the hypothesis that drugs, which induce internal cue states, can affect memory through retrieval cue properties (Carlezon et al., 1995; Jackson et al., 1992).

Acknowledgments

The research reported here was supported in part by National Institute of Mental Health Grant MH37535 to David Riccio, and the care and use of animals was approved

by Kent State University's Animal Care and Use Committee (ACUC).

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