Available online at www.sciencedirect.com
SCIENCE DIRECT®
Neurobiology of Learning and Memory 81 (2004) 105–114
Neurobiology of Learning and Memory
www.elsevier.com/locate/ynlme

NMDA receptors and voltage-dependent calcium channels mediate different aspects of acquisition and retention of a spatial memory task

B.L. Woodside,[a] A.M. Borroni,[b] M.D. Hammonds,[c] and T.J. Teyler[d,*]

[a] *Department of Psychology, Baldwin-Wallace College, Berea, OH 44017, USA*
[b] *Oberlin College, Oberlin, OH 44074, USA*
[c] *Department of Neurobiology and Pharmacology, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272, USA*
[d] *WWAMI Medical Education Program, Health Sciences Building, University of Idaho, Moscow, ID 83844-4207, USA*

Received 20 March 2003; revised 24 July 2003; accepted 27 October 2003

Abstract

Activity dependent calcium entry into neurons can initiate a form of synaptic plasticity called long-term potentiation (LTP). This phenomenon is considered by many to be one possible cellular mechanism underlying learning and memory. The calcium entry that induces this phenomenon can occur when *N*-methyl-D-aspartate receptors (NMDARs) and/or voltage-dependent calcium channels (VDCCs) are activated. While much is known about synaptic plasticity and the mechanisms that are triggered by activation of these two Ca^{2+} channels, it is unclear what roles they play in learning. To better understand the role activation of these channels may play in learning we systemically administered pharmacological antagonists to block NMDARs, VDCCs, or both during training trials and retention tests in a radial arm maze task. Wistar rats injected with the NMDAR antagonist MK-801 (0.1 mg/kg) were impaired in the acquisition of this task. In contrast, rats injected with verapamil (10 mg/kg), an antagonist to VDCCs, acquired the task at the same rate as control animals, but were impaired on a 10-day retention test. A group of animals injected with both antagonists were unable to learn the task. The results suggest that each of the calcium channels and the processes they trigger are involved in a different stage of memory formation or expression.

Keywords: LTP; Long-term potentiation; nmdaLTP; vdccLTP; Spatial memory; Spatial learning

1. Introduction

Synaptic plasticity refers to neural connections that change in strength in response to development, experience, and pathology. These synaptic gain changes affect neural communication and may underlie the behavioral changes exhibited in learning (Bliss & Collingridge, 1993; McNaughton & Morris, 1987; Teyler & DiScenna, 1987). The best known increase in synaptic efficacy is long term potentiation (LTP) induced by Ca^{2+} entry into the post-synaptic cell via activation of NMDARs (nmdaLTP; Dunwiddie & Lynch, 1979). A mechanistically different form of LTP is mediated by Ca^{2+} entry through VDCCs (vdccLTP; Grover & Teyler, 1990). While both forms require Ca^{2+} entry and result in a potentiated post-synaptic response, their cellular mechanisms of induction and expression differ.

These two forms of LTP have similar characteristics as well as distinct differences, suggesting that they may work together in the overall process of memory formation, but serve different roles within that process. Both forms of LTP, which can be co-expressed as compoundLTP (Grover & Teyler, 1994), are similar in that they are input specific, induced by increased afferent activity, associative, and are long-lasting (Cavus & Teyler, 1996; Grover & Teyler, 1990, 1995; Levy & Steward, 1979; McNaughton, Douglas, & Goddard, 1978)—all characteristics that support an underlying role in the memory process. Calcium influx through NMDARs triggers a serine–threonine kinase signaling pathway that results in phosphorylation of existing AMPA receptors and inser-

* Corresponding author.
E-mail address: tteyler@earthlink.net (T.J. Teyler).

1074-7427/$ - see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/j.nlm.2003.10.003

tion of new AMPA receptors in the post-synaptic membrane (Fukunaga, Muller, & Miyamoto, 1996; Lu et al., 2001; Malinow, Mainen, & Hayashi, 2000), processes that result in a reversible form of LTP. In contrast, calcium influx through VDCCs results in tyrosine kinase activation (Grover & Teyler, 1995) and an irreversible form of LTP (Morgan, Coussens, & Teyler, 2002). Application of tyrosine kinase inhibitors blocks vdccLTP, but has no effect on nmdaLTP (Cavus & Teyler, 1996), whereas nmdaLTP is completely blocked by serine–threonine kinase inhibitors that have no effect on vdccLTP (Cavus & Teyler, 1996; Grover & Teyler, 1995). The neurotrophic factors BDNF and NT-3 are selectively released with the induction of vdccLTP (Patterson, Grover, Schwartzkroin, & Bothwell, 1992) and there is an increase in *trk*B receptor expression (Cavus, Grover, & Teyler, 1993; Teyler et al., 1994). This evidence indicates that Ca^{2+} influx through NMDARs and VDCCs trigger different cellular processes.

A sizeable body of research exists to support the role of Ca^{2+} entry via NMDARs in behavioral learning and memory. Competitive and non-competitive antagonists of the NMDAR which block nmdaLTP in vitro (Grover & Teyler, 1990) and in vivo (Morgan & Teyler, 1999) also can impair acquisition of a number of behavioral tasks including the radial arm maze (Caramanos & Shapiro, 1994) and water maze (Packard & Teather, 1997), fear conditioning (Blair, Schafe, Bauer, Rodrigues, & Le Doux, 2001), conditioned taste aversion (Escobar, Alcocer, & Bermudez-Rattoni, 2002), and simple odor discriminations (Staubli, Thibault, DiLorenzo, & Lynch, 1989). Little research, however, exists examining the role of Ca^{2+} entry via VDCCs in memory formation.

In this experiment we used the 4/8 radial arm maze task, a spatial task known to be hippocampally dependent (Jarrard, 1993), in conjunction with pharmacological antagonists of the NMDA and VDCC Ca^{2+} channels, to examine the distinct role that each may play in the acquisition and storage of spatial information. The radial arm maze task allows for the examination of the time course of memory formation by evaluating performance within a trial, between trials, and after a delay at retention (Olton & Papas, 1979). Animals were trained to obtain a food reward from four consistently baited arms of the 8-arm maze over a period of weeks. To solve this problem, animals must remember from day to day which four arms are baited (reference memory, RM), and within a trial must not re-enter an arm just visited (working memory, WM). Following achievement of criterion by the control group, all animals were left in their home cages for 10 days without training or drugs, and were then tested for retention.

To assess the role of each calcium channel separately, prior to each trial animals were injected (IP) with either the NMDAR antagonist MK-801, at a dose that blocks nmdaLTP in vivo (Abraham & Mason, 1988) but has no effect on vdccLTP, or the VDCC antagonist verapamil, at a dose that blocks vdccLTP in vivo without affecting nmdaLTP (Morgan & Teyler, 1999). In addition, a double drug group received both MK-801 and verapamil at doses shown to block both forms of LTP (Morgan & Teyler, 1999), and a control group received physiological saline. It was hypothesized that blocking calcium influx through NMDARs would impair acquisition, as has been previously demonstrated, while blocking VDCCs would impair long-term memory formation, as suggested by the cellular processes initiated by calcium influx through VDCCs. It was also anticipated that blocking both calcium channels would seriously impair performance in this spatial task.

2. Methods

Fifty-two 70-day-old male Wistar rats, individually housed in clear Plexiglas cages and kept on a 12/12 light/dark cycle, were mildly food deprived to maintain approximately 85% of ad lib weight during the experiment.

The apparatus used was an 8-arm radial arm maze. The maze was elevated 85 cm from the floor and consisted of an octagonal start area (53.5 cm across) with 10 cm by 30 cm arms (with clear plastic sides 20 cm high) radiating outward. Each arm contained a reward cup (3.25 cm high by 4.25 cm in diameter) located 2.5 cm from the distal end of the arm and centered between the side walls. Pneumatically controlled gates separated the start area from the arms. The arms could be removed from the central start area and were periodically interchanged with one another. The maze was located in a 3 m by 3 m room that featured distinct spatial cues on the walls. All animals were initially shaped for 12 days (Table 1) to complete a task in which all 8 arms were rewarded (8/8) with 1/2 of a Kelloggs Froot Loop. Animals received one trial per weekday in all phases of the experiment.

After shaping, animals were randomly assigned to one of four treatment groups: saline ($n = 13$), MK-801 ($n = 13$), verapamil ($n = 13$), or both MK-801 and verapamil ($n = 13$). The 4/8 radial arm maze task was used for this phase of the experiment. The same four arms were rewarded for each animal throughout the experiment. Rewarded arms were varied between animals and balanced across groups. The acquisition phase was run for 40 days. Each animal was injected (IP) approximately 1 h before its acquisition trial with either saline, MK-801 (0.1 mg/kg), verapamil (10 mg/kg), or both MK-801 (0.1 mg/kg) and verapamil (10 mg/kg). At the start of a trial, each animal was placed in the center of the maze with the gates closed. The gates were then opened and the animal was al-

lowed to enter an arm. A choice was recorded when the hindquarters of the animal crossed the entry gate of the arm. The seven other gates were then closed, and when the animal emerged from the chosen arm, that gate closed. After a 5-s delay all gates were opened again and the choice procedure was repeated. The trial was complete when either all four rewards were acquired or when 10 min had elapsed.

At the end of the acquisition phase a 10-day retention period was observed during which no training took place, no drugs were administered, and the animals remained in their home cages (Table 1). On days 50–52 retention tests were run under the same conditions as the acquisition training. On day 60, a drug reversal trial was run to evaluate whether any retention impairments could be explained as drug-induced encoding or retrieval failures. For the drug reversal trials the saline animals were randomly reassigned to one of the three original drug groups, MK-801 ($n = 4$), verapamil ($n = 5$), or both MK-801 and verapamil ($n = 4$), and received the respective IP injections. Except for the drug reversals, this retention trial was identical to conditions during acquisition. On days 66–68, to test whether or not animals were relying on distal spatial cues to solve the task, all groups were retrained under the original conditions, including drug administration. After the 3 days of retraining, a single retention trial was administered (day 69) with white curtains enclosing the maze so that all distal spatial cues were effectively removed.

A working memory (WM) error was recorded when an animal re-entered any arm during a trial. A reference memory (RM) error was recorded when an animal entered a non-rewarded arm for the first time during a trial. Time to complete the trial, and the order of the arms entered, were recorded for each subject for all phases of the experiment. All training and retention trials were videotaped.

Table 1
Experiment 2: Timetable

Day	Treatment
−12 to 0	Shaping (12 days) No drugs 8/8 Arms baited
1–40	Acquisition (40days) Four treatment groups Saline control MK-801 Verapamil Both MK-801 and verapamil 4/8 Arms baited
41–50	Retention Period (10 days) Home cage, no drugs, no training
50–52	Retention testing (3 days) Four treatment groups as above 4/8 Arms baited
53–59	Home Cage (7 days) No drugs, no training
60	Drug reversal test (1 day) Drug-reversed groups Saline to verapamil Saline to MK-801 Saline to MK-801 and verapamil All original drug groups to saline 4/8 Arms baited
61–65	Home cage (5 days) No drugs, no training
66–68	Retraining (3 days) Original treatment groups Retrain under original conditions
69	Cue removal test (1 day) Distal spatial cues removed

3. Results

The results for the experiment are divided into four phases: acquisition, retention, drug reversal, and spatial cue removal. One animal from the verapamil group continually failed to produce data and was subsequently dropped from the experiment.

3.1. Acquisition

The acquisition phase lasted 40 days. The data for RM errors during the acquisition phase is shown in the first 8 blocks of five trials each in Fig. 1. A repeated-measures ANOVA, drug group × acquisition trial block (1 through 8), for reference memory errors was significant for trial block, ($F_{7,329} = 40.49$; $p < .001$), drug group ($F_{3,47} = 5.15$; $p < .01$), and the interaction of drug across trial block ($F_{21,329} = 3.03$; $p < .001$). A post hoc S–N–K test indicated that the significant difference between groups was evidenced by the subgroups saline and verapamil vs. the subgroups MK-801 and the double drug group. Fig. 1 shows that while most groups appear to show some improvement with training, the group that received both MK-801 and verapamil was severely impaired in acquisition. For this group, the number of RM errors was not significantly different between the first and last training trials (paired t, $p > .1$) indicating that these animals failed to learn the task. The MK-801-treated group was not noticeably different from the group that received both drugs until the eighth block of trials, at which point there was some improvement in performance. This improvement, however, did not reach the level of the verapamil group or the saline control group. A series of one-way ANOVAs for drug group were performed for each of the 8 trial blocks. Significant differences were found for trial blocks 6 through 8: block 6, $F_{3,47} = 4.96$, $p < .01$; block 7, $F_{3,47} = 6.20$, $p < .01$; and block 8, $F_{3,47} = 7.77$, $p < .001$. An S–N–K post hoc

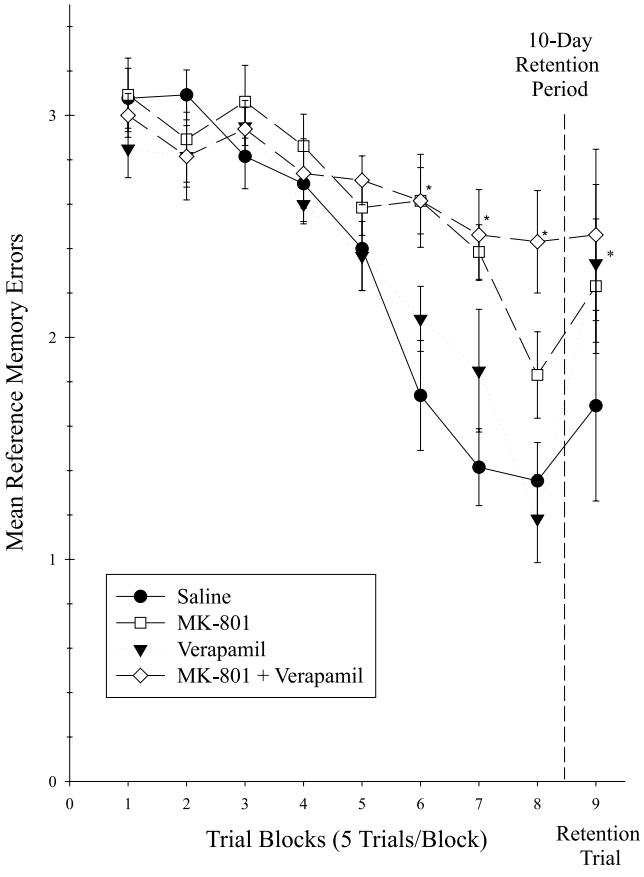

Fig. 1. Reference memory errors. Mean reference memory errors (first entry into a non-baited arm) during acquisition training (one trial per day, days 1–40, data plotted in 8 five-trial blocks) and for the retention test (day 50) following the 10-day retention period, for each group of animals. Male Wistar rats were trained in the 4/8 radial arm maze task and were assigned to the following groups: saline (control, $n = 13$), MK-801 (0.1 mg/kg, $n = 13$), verapamil (10 mg/kg, $n = 12$), or both MK-801 and verapamil (0.1 and 10 mg/kg respectively, $n = 13$). All drugs were given IP 60 min prior to training and retention testing. The animals that received MK-801 were significantly impaired in acquisition at blocks 6 and 7, while animals that received both drugs were impaired at blocks 6, 7, and 8 as compared to the saline control or verapamil groups. The animals injected with verapamil were not different from controls during acquisition, but demonstrated significant forgetting at the retention test.

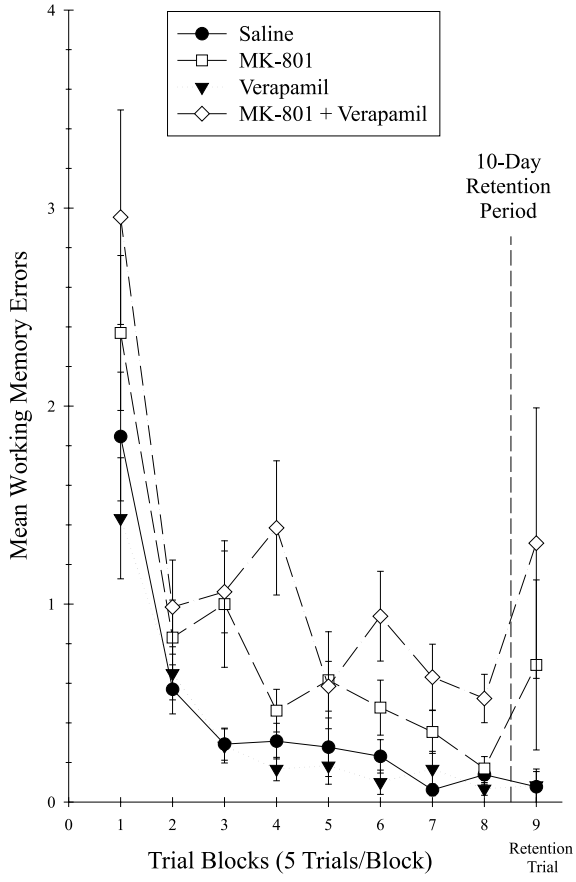

Fig. 2. Working memory errors. Average working memory errors are presented for each block of training trials during acquisition (1st 8 blocks) and for the retention test. A working memory error was recorded when the rat would re-enter any arm within a trial. There was no significant difference between groups during acquisition or retention. After trial block 1, all groups averaged <1.5 WM errors per trial (with the exception of an increase at trial block 4 and at retention for the double drug group).

test on each trial block showed significance was supported by differences between the subgroups MK-801 and double drug vs. the saline and verapamil groups on trial blocks 6 and 7, but only by the double drug group on trial block 8.

The data for WM errors during acquisition is shown in Fig. 2. A repeated-measures ANOVA, drug group × acquisition trial block, for working memory errors was significant for trial block ($F_{7,329} = 28.57$; $p < .01$), and group ($F_{3,47} = 4.13$; $p < .05$), but not for the interaction, ($F_{21,329} = 0.90$; $p = .597$). After the first block of trials, all groups averaged <1.5 WM errors per trial (with the exception of an increase at trial block 4 for the double drug group).

3.2. Retention

The data for the first retention test for RM errors and WM errors is also shown in Figs. 1 and 2, respectively. The sharp increase in RM errors on the retention test for the verapamil group shown in Fig. 1 (and Fig. 3) represents a 97% increase in reference memory errors from the last block of training to the first retention test. In comparison, saline animals' RM errors increased 25%, MK-801 increased 22%, and the double drug group increased only 1% over the retention period (reflecting their failure to learn the task).

A repeated-measures ANOVA was run for the last training trial × the first retention test. There was significance for trial, $F_{1,47} = 8.171$; $p = .006$, and for drug group, $F_{3,47} = 4.964$; $p = .004$, and the interaction of trial by drug group approached significance at $F_{3,47} = 2.692$; $p = .057$. Since all groups were run to a criterion determined by the mean performance score of

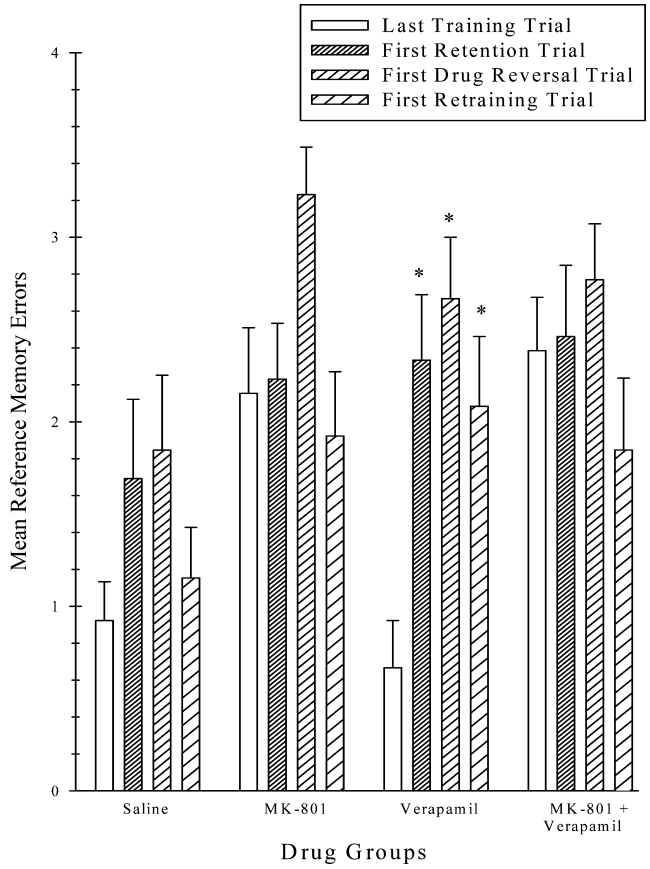

Fig. 3. Retention period—reference memory errors. Average reference memory errors are presented for each group for the last training trial, and three retention trials. These retention trials occurred after a period in which the subjects received no exposure to the experimental task, drugs, or environment. The trials consist of the 1st retention test at the end of the 10-day retention period, the 1st drug reversal test which occurred after a 7-day retention period, and the 1st retraining trial which occurred after a 5-day retention period. Only the animals that received verapamil were significantly impaired at all three retention tests. The MK-801 group demonstrated a significant difference in performance between the 1st drug reversal trial and the 1st retraining trial. The increase in errors at the 1st drug reversal trial could have been due to state-dependent effects.

the control group, their acquisition scores were compared to those of the control group at the end of 40 days to assess their ability to acquire the task. However, this meant that the different groups acquired the task to different levels of proficiency. This, in turn, caused the retention scores to reflect inaccurate levels of forgetting based on comparative performance scores between groups at retention, as opposed to the actual difference within a group between performance at the end of training and at retention. For this reason a difference score for reference memory errors was computed for each animal for performance between the last training trial and the first retention trial. A one-way ANOVA for drug group was performed using the difference score data which demonstrated significance at $F_{3,47} = 3.133$, $p = .034$ and an S–N–K post hoc demonstrated a significant difference in means for the verapamil group.

In the design of this experiment there are three measures of retention after a period with no exposure to the experimental task or environment. The first retention test on days 50–52 is after a 10-day retention period (see Table 1). The first drug reversal trial on day 60 is after a 7-day retention period, and the first retraining trial (on day 66) for subsequent cue removal is after a 5-day retention period. The effects of blocking calcium entry through VDCCs may be best illustrated by examining the difference in performance between groups at these three retention tests. A one-way ANOVA (for trial) with reference memory error data from last training trial, the first retention trial, the first drug reversal trial, and the first retraining trial, was run independently for each drug group (Fig. 3). There was no significant difference in reference memory errors for the saline or double drug groups, $F_{3,48} = 1.629$, $p = .195$ and $F_{3,48} = 1.238$, $p = .306$, respectively. There was a significant difference for trial for the MK-801, $F_{3,48} = 3.309$, $p = .028$, however, a tukey HSD post hoc showed that this difference was only significant between the drug reversal and the first retraining trial (see Fig. 3). The spike in errors for MK-801 animals at drug reversal could be due to state-dependent effects (see Section 4). In contrast to these results, the verapamil group demonstrated a significant impairment at all three retention trials, $F_{3,44} = 6.944$, $p = .001$; tukey HSD: last trial vs. 1st retention test $p = .005$, last trial vs. 1st drug reversal trial $p = .001$, last trial vs. 1st retraining trial $p = .022$ (Fig. 3).

A repeated-measures ANOVA, drug group × trial, (the last training block and the retention test), for working memory errors showed significance for group ($F_{3,47} = 3.03$; $p < .05$), but not for trial ($F_{1,47} = 2.40$; $p = .128$), or the interaction ($F_{3,47} = 1.00$; $p = .401$). The control and verapamil groups maintained their levels of WM errors across the retention period (Fig. 2), demonstrating that an increase in working memory errors did not contribute to the poor RM retention performance of the verapamil group.

3.3. Drug reversal

The drug reversal test was conducted to examine saline animals for impairments in retention when injected with one of the original drug dosages. The saline animals were randomly divided into three groups; one received the double drug injections ($n = 4$), one received MK-801 ($n = 4$), and the last received verapamil ($n = 5$). The results of this drug reversal test are shown in Fig. 4. No group differed significantly from their performance on the first retention test and, most notably, the saline animals receiving verapamil did not

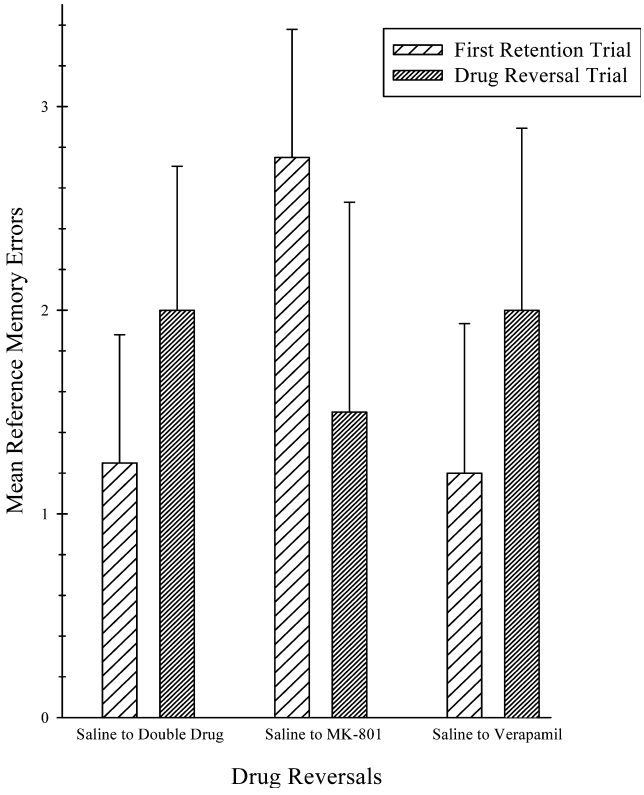

Fig. 4. Drug reversal. For the drug reversal trial the saline animals were randomly reassigned to one of the three original drug groups, MK-801 ($n = 4$), verapamil ($n = 5$), or both MK-801 and verapamil ($n = 4$), and received the respective IP injections. Except for the drug reversal, this retention trial was identical to conditions during acquisition. Shown here are the subjects average reference memory errors for the 1st retention test (light bars) compared to their average reference memory errors for the 1st drug reversal trial (dark bars). There were no significant differences between trials or groups. Most significant is that saline animals that received verapamil in the drug reversal trial did not demonstrate a retention impairment, suggesting that the original verapamil group's poor retention performance was at encoding, not at retrieval.

show a significant impairment in retention. A repeated-measures ANOVA, for drug group × trial (the first retention test verses the drug reversal retention test) showed no significance for drug group ($F_{2,10} = 0.28$; $p = .761$), trial ($F_{1,10} = 0.03$; $p = .865$), or interaction ($F_{2,10} = 1.35$; $p = .303$).

To determine any drug effects on activity, the average time spent in each arm across acquisition training was compared, resulting in a significant effect for group ($F_{3,24} = 9.6$; $p < .001$). An inspection of group means indicated that the MK-801 and double drug groups were spending less time in each arm (21 and 22 s, respectively) than were saline and verapamil groups (26 and 27 s, respectively). These results indicate that there was no motor impairment and that groups receiving MK-801 ran the maze faster than the control or verapamil groups. There was no significant difference between the verapamil and control groups.

3.4. Cue removal

In the final phase of the experiment, all animals were first retrained for 3 days under the original training conditions, and then tested for performance with white curtains around the maze to eliminate all distal spatial cues. A two-way, repeated measures ANOVA, for drug group × trial (the average retraining errors verses the cue removal test) showed significance only for trial ($F_{1,47} = 16.33$; $p < .001$), but not for drug group ($F_{3,47} = 0.38$; $p = .769$), or the interaction ($F_{3,47} = 1.58$; $p = .207$). As can be seen in Fig. 5, even though the interaction was not significant, removal of spatial cues appeared to have a negative effect on performance of the saline control and verapamil groups compared to the performance of the MK-801 and the double drug group.

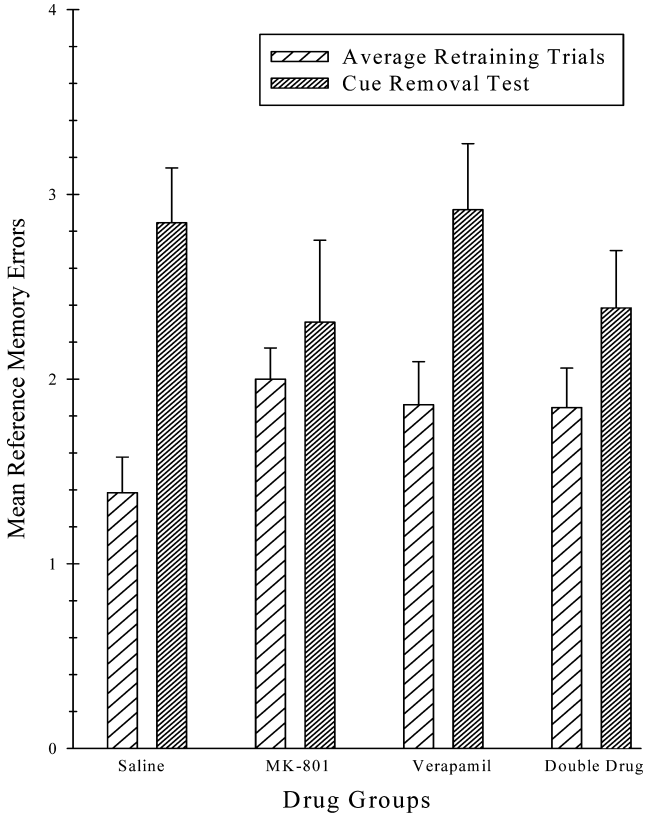

Fig. 5. Cue removal. The cue removal test was conducted to determine if the groups which had successfully acquired the 4/8 task had relied on distal spatial cues. All groups were retrained in the task for 3 days under original conditions, including respective IP drug injections. On the 4th day the maze was surrounded by a white curtain which eliminated all distal spatial cues. Other than the cue removal, all conditions of the trial were the same as training. Shown here are the mean reference memory errors for each group averaged over the three days of retraining (light bars) and the average reference memory errors per group for the cue removal test (dark bars). The difference between average retraining performance and cue removal performance did not reach a level of statistical significance for any of the groups, however, the saline and verapamil groups did demonstrate a noticeable increase in reference memory errors when the cues were removed.

4. Discussion

The results suggest that the two Ca^{2+} channels examined are involved with different aspects of memory formation. Blocking Ca^{2+} influx via NMDARs impaired the animals' ability to acquire the task, while blocking VDCCs had no affect on acquisition, but impaired retention. These results suggest a synergistic role for both NMDARs and VDCCs in memory formation as rats given both drugs demonstrated no significant learning during the acquisition period.

4.1. Acquisition phase

Reference memory (memory storage over a 24-h period) was impaired for the MK-801 group during acquisition, and was essentially blocked for the group receiving both drugs (blocking both NMDARs and VDCCs). The RM improvement in the MK-801 group in block 8 (Fig. 1) suggests that this group is capable of learning, but at a slower rate, and perhaps through mechanisms initiated by calcium influx through VDCCs. The fact that the double drug group showed no learning during the acquisition phase lends further support to the proposition that limited acquisition in the MK-801 group may have relied on calcium influx through VDCCs, and suggests that Ca^{2+} influx through at least one of these Ca^{2+} channels is necessary for spatial learning to occur. These results support and extend findings in previous studies in which MK-801 was shown to have little or no effect on WM, but impaired acquisition of RM in the radial arm maze (Caramanos & Shapiro, 1994).

The verapamil group acquired the task at the same rate as the saline control group and both groups reached criterion by day 40. The lack of any impairment during acquisition for the verapamil animals suggests that daily exposure to the task was sufficient to retain the memory trace through NMDAR mediated processes.

4.2. Retention phase

The most interesting result was demonstrated by the effect of the VDCC antagonist, verapamil, on the long-term retention of reference memory. While the verapamil group acquired the task at the same rate as the saline control group, there was significant memory loss at the end of the retention period (Figs. 1 and 3). There was some forgetting in the saline and MK-801 groups at retention, however, only the verapamil animals demonstrated significant impairment with a 97% increase in errors at retention. Data for the double drug group was included in this phase only as a reference since those animals failed to acquire and therefore would not have a comparison for retention.

It could be argued that all drug groups were impaired at retention since there is no significant difference in their performance on the retention trial (Fig. 1). However, since only the verapamil and saline groups achieved criterion, each group's performance across the retention period is best evaluated as a difference score. The performance of the saline control and MK-801 groups was similar when comparing difference scores as is evidenced by their parallel performance graphs (Fig. 1) indicating the slight memory impairment was due to the time period and not the MK-801. The verapamil group was the only group that demonstrated significance in the difference scores.

A second measure which more clearly defines the dissociation between the effects of MK-801 and verapamil can be seen in Fig. 3. In this graph the performance on the last day of training is compared to the first retention trial, the drug reversal trial, and the first day of retraining for the cue removal test. These data points represent each group's retention performance after a 10-day retention period, a 7-day retention period, and a 5-day retention period, respectively. The repeated but spaced exposure to the training in the post-acquisition phases of this experiment was sufficient to ameliorate any deficits in retention performance for the animals in all groups except the verapamil group. These animals were still significantly impaired after the third retention interval, suggesting that VDCCs may play a role in encoding spatial information over long intervals.

4.3. Working memory

Working memory performance was not significantly impaired in either the acquisition or retention phase of the experiment, a result consistent with earlier studies showing little effect of MK-801 on WM (Caramanos & Shapiro, 1994). However, the data in Fig. 2 shows that the double drug and the MK-801 groups did commit more WM errors during acquisition and at retention than the verapamil and control groups. This may have been due to the hyperactivity displayed by the groups receiving MK-801. Nonetheless, these results suggest that either other processes than those mediated by NMDARs or VDCCs were involved in working memory, or that the drug blockade was incomplete. The drug concentrations chosen for this experiment were sufficient to block tetanus induced nmdaLTP and vdccLTP in CA1 hippocampus in vivo (Morgan & Teyler, 1999) without producing debilitating behavioral side effects from higher concentrations. However, the involvement of extra-hippocampal brain areas in this task, and their sensitivity to these antagonist concentrations is unknown.

Other forms of synaptic plasticity have been described (Christie & Abraham, 1994; Harris & Cotman, 1986; Kapur, Yeckel, Gray, & Johnston, 1998), but they

are unlikely candidates because they are either afferent to the hippocampal CA1 region (the radial maze is a hippocampally dependant task), or are also blocked by the drugs employed in this study. It is possible, of course, that unknown forms of synaptic plasticity are also operative and support within-trial performance. The lack of pronounced working memory effects with these drugs offers support for the contention that this short-term form of memory is served by forms of synaptic plasticity not affected by manipulations of NMDARs or VDCCs (Borroni, Fichtenholtz, Woodside, & Teyler, 2000).

4.4. Drug reversal and activity data

Saline animals, injected with verapamil and tested again for retention during the drug reversal phase of the experiment, showed no significant impairment compared to their first retention test (Fig. 4). These results suggest that the retention impairment of the original verapamil group was due to a failure to properly encode or consolidate the information, rather than a problem during the retrieval process.

The activity data showed that the MK-801 and double drug groups spent less time exploring arms than the saline or verapamil groups. The verapamil and saline groups did not differ in this measure, indicating that the poor retention of verapamil animals does not relate to differences in activity levels. The significantly faster maze performance displayed by the MK-801 and double drug groups probably reflects the hyper-activity effects seen at low doses of MK-801. However, in the drug reversal phase, control animals injected with MK-801 or both drugs showed no significant impairment in reference memory performance (Fig. 4), indicating that the side-effect of hyperactivity was most likely not responsible for the acquisition impairments.

4.5. Cue removal test

Each treatment group was tested for their reliance on distal cues in the cue removal phase of the experiment. The results indicated that both saline control and verapamil groups experienced more RM errors following removal of distal cues (Fig. 5), signifying that they were relying on distal spatial cues to solve the task. The MK-801 and the double drug group (MK-801 and verapamil), however, did not noticeably increase their errors following removal of distal cues, suggesting that they were relying on proximal, intra-maze cues. To the extent that NMDAR-dependent processes are associated with spatial processing, it follows that disabling this system will result in animals switching to an alternative strategy supported by other cellular processes.

4.6. General discussion

Considerable evidence exists to support the role of NMDARs in spatial learning (Caramanos & Shapiro, 1994; Morris, Anderson, Lynch, & Baudry, 1986; Ward, Mason, & Abraham, 1990). However, there are also studies demonstrating that when animals are pre-exposed to procedural training, place learning in the Morris water maze can be accomplished while blocking NMDARs and, consequently, nmdaLTP (Bannerman, Good, Butcher, Ramsay, & Morris, 1995; Saucier & Cain, 1995). Our findings showed that animals injected with MK-801, even with pre-drug procedural shaping, were impaired in this spatial task. There is some acquisition in the MK-801 group, however, and this learning, as well as the spatial learning in the studies cited above, may be partially attributable to VDCC-mediated processes. The verapamil group, in contrast, acquired the task normally, but was deficient in retention.

Since both NMDARs and VDCCs gate Ca^{2+} into the post-synaptic cell, how can the influx of Ca^{2+} into a neuron result in different physiological responses (and behavioral performance as these results have shown)? Two possible explanations have been offered (Teyler et al., 1994) and are briefly reviewed here. First, different afferent activation parameters are required for induction of the two forms of plasticity, and these differences could result in varying concentrations of intracellular calcium (Lisman, 1989). A difference in intracellular calcium concentration combined with different calcium binding affinities for kinases and phosphatases (Kasai, 1993) could mediate differential cellular responses (Artola & Singer, 1993; Lisman, 1989) underlying the behavioral differences observed.

The second hypothesis for different physiological responses to an increase in intracellular Ca^{2+} is based on the possibility that calcium is compartmentalized in the post-synaptic cell. This is supported by the fact that NMDA receptors are found primarily in the post-synaptic density, while voltage-dependent calcium channels are found in highest concentrations on the soma, proximal dendrites, and at the base of dendritic spines (Muller & Connor, 1991; Westenbroek, Ahlijanian, & Catterall, 1990). This separation of NMDARs and VDCCs on the post-synaptic cell may allow for calcium influx through these channels to act on different, location-specific kinases or phosphatases. Some evidence for this is provided by a study that investigated *c-fos* activation after LTP induction (Lerea & McNamara, 1993). NMDAR or VDCC activation can induce *c-fos* expression, but each uses a different Ca-dependent intracellular signaling pathway. There are additional sources of intracellular Ca^{2+} that may mediate cell response, such as intracellular stores of Ca^{2+} that are released by activation of metabotropic glutamate receptors. It may

be that both the hypotheses above play a role in the differentiated cellular responses to calcium influx and more may yet be elaborated.

The results of this study add to the evidence suggesting that NMDARs may mediate a form of plasticity designed to store information about cellular activity for a period of hours to days, whereas activation of VDCCs may initiate cellular processes that result in more permanent alterations in synaptic strength (Borroni et al., 2000). It is likely that both processes work synergistically, in a parallel or serial fashion, to form, consolidate, and possibly re-consolidate long-term memory. In the absence of channel blocking drugs, increases in afferent activity lead to the sequential induction of the two forms of LTP. NmdaLTP appears at modest levels of post-synaptic depolarization, and, given sufficient postsynaptic depolarization from enhanced afferent activity, reduced GABAergic activity, or neuromodulatory influences, vdccLTP is also induced, giving rise to a compound-LTP (both forms present) (Cavus & Teyler, 1996). While LTP was not measured in this experiment, under normal conditions each form of LTP, or some combination of the two (compoundLTP), may have been induced as a result of the behavioral training experiences. However, whether this is actually the case remains to be demonstrated in future experiments.

In summary, these results support the hypothesis that Ca^{2+} influx through NMDARs and VDCCs trigger processes that underlie different aspects of memory formation. The NMDAR antagonist MK-801 impaired rats' ability to acquire the task, while verapamil, the VDCC antagonist, impaired long-term retention. Subjects that received MK-801 did show some learning that may have been mediated by VDCCs. Animals given verapamil acquired as well as controls, demonstrating that NMDAR-mediated or other cellular processes were sufficient to maintain a stable representation with repeated exposure to the task. The group of animals given both drugs demonstrated no significant learning during the acquisition period suggesting that Ca^{2+} influx through at least one of these channels is required for spatial learning to occur. This data adds to the theory that multiple forms of synaptic plasticity in the hippocampus, and elsewhere, are involved in memory formation and storage.

Acknowledgments

We acknowledge the support of the Herbert W. Hoover Foundation and the NIH (MH57892). In partial fulfillment for the Ph.D degree (B.L.W.). Please send reprint requests to T.J. Teyler (tteyler@earthlink.net). We thank L. Jarrard and L. Brown-Croyts for critically reading the ms.

References

Abraham, W. C., & Mason, S. E. (1988). Effects of the NMDA receptor/channel antagonists CPP and MK-801 on hippocampal field potentials and long-term potentiation in anesthetized rats. *Brain Research, 462*, 40–46.

Artola, A., & Singer, W. (1993). Long-term depression of excitatory synaptic transmission and its relationship to long-term potentiation. *Trends in Neuroscience, 16*(11), 480–487.

Bannerman, D. M., Good, M. A., Butcher, S. P., Ramsay, M., & Morris, R. G. M. (1995). Distinct components of spatial learning revealed by prior training and NMDA blockade. *Nature, 378*, 182–186.

Blair, H. T., Schafe, G. E., Bauer, E. P., Rodrigues, S. M., & Le Doux, J. E. (2001). Synaptic plasticity in the lateral amygdala: A cellular hypothesis of fear conditioning. *Learning & Memory, 5*, 229–242.

Bliss, T. V., & Collingridge, G. L. (1993). A synaptic model of memory: Long-term potentiation in the hippocampus. *Nature, 361*, 31–39.

Borroni, A. M., Fichtenholtz, H., Woodside, B. L., & Teyler, T. J. (2000). Role of vdccLTP and nmdaLTP in spatial memory. *Journal of Neuroscience, 20*, 9272–9276.

Caramanos, Z., & Shapiro, M. L. (1994). Spatial memory and *N*-methyl-D-asparate receptor antagonists APV and MK-801: Memory impairments depend on familiarity with the environment, drug dose, and training duration. *Behavioral Neuroscience, 108*, 30–43.

Cavus, I., Grover, L. M., & Teyler, T. J. (1993). Enhanced neurotrophin receptor expression following long-term potentiation. *Society for Neuroscience Abstracts, 19*, 376.11.

Cavus, I., & Teyler, T. J. (1996). Two forms of long-term potentiation in area CA1 activate different signal transduction pathways. *Journal of Neurophysiology, 76*, 3038–3047.

Christie, B. R., & Abraham, W. C. (1994). L-type voltage-sensitive calcium channel antagonists block heterosynaptic long-term depression in the dentate gyrus of anesthetized rats. *Neuroscience Letters, 167*, 41–45.

Dunwiddie, T. V., & Lynch, G. (1979). The relationship between extracellular calcium concentrations and the induction of hippocampal long-term potentiation. *Brain Research, 169*, 103–110.

Escobar, M. L., Alcocer, I., & Bermudez-Rattoni, F. (2002). In vivo effects of intracortical administration of NMDA and metabotropic glutamate receptors antagonists on neocortical long-term potention and conditioned taste aversion. *Behavioral Brain Research, 129*, 101–106.

Fukunaga, K., Muller, D., & Miyamoto, E. (1996). CaM kinase II in long-term potentiation. *Neurochemistry International, 28*(4), 343–358.

Grover, L. M., & Teyler, T. J. (1990). Two components of long-term potentiation induced by different patterns of afferent activation. *Nature, 347*, 477–479.

Grover, L. M., & Teyler, T. J. (1994). Activation of NMDA receptors in hippocampal area CA1 by low and high frequency orthodromic stimulation and their contribution to induction of long-term potentiation. *Synapse, 16*, 66–75.

Grover, L. M., & Teyler, T. J. (1995). Different mechanisms may be required for maintenance of NMDA receptor-dependent and independent forms of long-term potentiation. *Synapse, 19*, 121–133.

Harris, E. W., & Cotman, C. W. (1986). Long-term potentiation of guinea pig mossy fiber responses is not blocked by *N*-methyl-D-aspartate antagonists. *Neuroscience Letters, 70*, 132–137.

Jarrard, L. E. (1993). On the role of the hippocampus in learning and memory in the rat. *Behavioral and Neural Biology, 60*, 9–26.

Kapur, A., Yeckel, M. F., Gray, R., & Johnston, D. (1998). L-type calcium channels are required for one form of hippocampal mossy fiber LTP. *Journal of Neurophysiology, 79*, 2181–2190.

Kasai, H. (1993). Cytosolic Ca2+ gradients, Ca2+ binding proteins and synaptic plasticity. *Neuroscience Research, 16*(1), 1–7.

Levy, W. B., & Steward, O. (1979). Synapses as associative memory elements in the hippocampal formation. *Brain Research, 175*, 233–245.

Lu, W., Man, H., Ju, W., Trimble, W. S., MacDonald, J. F., & Wang, Y. T. (2001). Activation of synaptic NMDA receptors induces membrane insertion of new AMPA receptors and LTP in cultured hippocampal neurons. *Neuron, 29*(1), 243–254.

Lerea, L. S., & McNamara, J. O. (1993). Ionotropic glutamate receptor subtypes activate c-fos transcription by distinct calcium-requiring intracellular signaling pathways. *Neuron, 10*(1), 31–41.

Lisman, J. (1989). A mechanism for the Hebb and the anti-Hebb processes underlying learning and memory. *Proceedings of the National Academy of Sciences of the United States of America, 86*(23), 9574–9578.

Malinow, R., Mainen, Z. F., & Hayashi, Y. (2000). LTP mechanisms: From silence to four-lane traffic. *Current Opinion in Neurobiology, 10*(Jun), 352–357.

McNaughton, B. L., Douglas, R. M., & Goddard, G. V. (1978). Synaptic enhancement in fascia dentata: cooperativity among coactive afferents. *Brain Research, 157*, 277–293.

McNaughton, B. L., & Morris, R. G. M. (1987). Hippocampal synaptic enhancement and information storage within a distributed memory system. *Trends in Neurosciences*, 408–415.

Morgan, S. L., Coussens, C. M., & Teyler, T. J. (2002). Depotentiation vdccLTP requires NMDAR activation. *Neurobiology of Learning and Memory, 76*, 229–238.

Morgan, S. L., & Teyler, T. J. (1999). VDCCs and NMDARs underlie two forms of LTP in CA1 hippocampus in vivo. *Journal of Neurophysiology, 82*(2), 736–740.

Morris, R. G. M., Anderson, E., Lynch, G. S., & Baudry, M. (1986). Selective impairment of learning and blockade of long-term potentiation by an *N*-methyl-D-asparate receptor antagonist AP5. *Nature, 319*, 774–776.

Muller, W., & Connor, J. A. (1991). Dendritic spines as individual neuronal compartments for synaptic Ca2+ responses. *Nature, 354*(6348), 73–76.

Olton, D. S., & Papas, B. C. (1979). Spatial memory and hippocampal function. *Neuropsycologia, 17*, 669–682.

Packard, M. G., & Teather, L. A. (1997). Intra-hippocampal estradiol infusion enhances memory in ovariectomized rats. *Neuroreport, 14*, 3009–3013.

Patterson, S. L., Grover, L. M., Schwartzkroin, P. A., & Bothwell, M. (1992). Neurotrophin expression in rat hippocampal slices: A stimulus paradigm inducing LTP in CA1 evokes increases in BDNF and NT-3 MRNAs. *Neuron, 9*, 1081–1088.

Saucier, D., & Cain, D. P. (1995). Spatial learning without NMDA receptor-dependent long-term potention. *Nature, 378*, 186–189.

Staubli, U., Thibault, O., DiLorenzo, M., & Lynch, G. (1989). Antagonism of NDMA receptors impairs acquisition but not retention of olfactory memory. *Behavioral Neuroscience, 103*, 54–60.

Teyler, T. J., Cavus, I., Coussens, C., DiScenna, P., Grover, L., Lee, Y. P., & Little, Z. (1994). Multideterminant role of calcium in hippocampal synaptic plasticity. *Hippocampus, 4*(6), 623–634.

Teyler, T. J., & DiScenna, P. (1987). Long-term potentiation. *Annual Review of Neuroscience, 10*, 131–161.

Ward, L., Mason, S. E., & Abraham, W. C. (1990). Effects of the NMDA antagonists CPP and MK-801 on radial maze performance in rats. *Pharmacology Biochemistry and Behavior, 35*, 785–790.

Westenbroek, R. E., Ahlijanian, M. K., & Catterall, W. A. (1990). Clustering of L-type Ca2+ channels at the base of major dendrites in hippocampal pyramidal neurons. *Nature, 347*(6290), 281–284.