Differential contribution of NMDA receptors in hippocampal subregions to spatial working memory

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***N*-methyl-D-aspartate (NMDA) receptor–dependent synaptic plasticity in the mammalian hippocampus is essential for learning and memory. Although computational models and anatomical studies have emphasized functional differences among hippocampal subregions, subregional specificity of NMDA receptor function is largely unknown. Here we present evidence that NMDA receptors in CA3 are required in a situation in which spatial representation needs to be reorganized, whereas the NMDA receptors in CA1 and/or the dentate gyrus are more involved in acquiring memory that needs to be retrieved after a delay period exceeding a short-term range. Our data, with data from CA1-specific knockout mice, suggest the possibility of heterogeneous mnemonic function of NMDA receptors in different subregions of the hippocampus.**

N-methyl-D-aspartate receptors (NMDARs) in the hippocampus have been extensively studied for their involvement in synaptic plasticity and memory[1–6]. Development of a selective knockout technique for the NMDARs of CA1 in the hippocampus[7–10] has allowed the shift of focus from the global role of NMDARs in the whole hippocampus in memory to their specific involvement in subregions of the hippocampus. Although computational models for hippocampal subregions have emphasized a unique function of each subregion in memory[11–15], pharmacological manipulations of NMDARs traditionally manipulated the whole hippocampus[3–6].

Detailed descriptions exist on subregion-specific functions derived from computational models[11–16], yet information is scarce regarding subregion-specific manipulation of NMDARs in the hippocampus. There is little data on the involvement of NMDARs in CA3, the subregion emphasized, by computational models[11–16], physiological evidence[17] and a behavioral experiment[18], in mnemonic processing of information. Physiological evidence[17] demonstrates that synchronized activation of CA3 produces Hebbian potentiation of recurrent collateral synapses independently of external stimulation, producing a so-called 'attractor state'[16], which can be inhibited by an NMDAR antagonist. We propose that setting up the attractor state for a mnemonic item within CA3 depends on the induction of synaptic plasticity such as long-term potentiation (LTP) via NMDARs[16]. Retrieving the item, however, may not necessitate NMDARs, as expression of LTP depends on non-NMDA receptors in the hippocampus[1,2,19]. Here we provide behavioral evidence for a functional difference in NMDARs among hippocampal subregions for spatial working memory, using localized subregion-specific injections of an NMDAR antagonist, D-(–)-2-amino-5-phosphonovaleric acid (APV).

Results

Spatial working memory in a novel spatial environment

Rats learned to choose between a visited arm (study arm) and an unvisited arm (choice arm) with a short-term delay (10 seconds) imposed between the two arms. Bilateral cannulas were then implanted in CA3 (**Fig. 1**). Additional spread-control groups were used with cannulas implanted in either the CA1 or the dentate gyrus (DG) region (**Fig. 1**). A within-subject analysis revealed no difference between the PBS injection and the APV injection into any of the subregions ($p > 0.5$) in performing the task for a block of 16 trials in the familiar room (**Fig. 2b**).

Each cannula group (CA1, CA3 and DG) was then divided into a PBS group (CA1-PBS, CA3-PBS and DG-PBS) and an APV group (CA1-APV, CA3-APV and DG-APV) and was tested in a novel room. The PBS groups' data were combined to produce one control group (CT-PBS). An analysis of variance (ANOVA) with a repeated-measures design performed on the data from the APV groups and the PBS groups showed a significant effect of groups ($F_{3,21} = 33.3$; $p < 0.0001$) and blocks ($F_{3,63} = 24.4$; $p < 0.0001$). There was also a significant interaction effect (blocks × groups; $F_{9,63} = 5.21$; $p < 0.0001$). A *post hoc* analysis (Tukey HSD) demonstrated that only the CA3-APV group was significantly impaired in performing the task in the novel room throughout the four blocks compared to the CT-PBS group ($p < 0.0001$ for blocks 1 and 2; $p < 0.01$ for blocks 3 and 4; **Fig. 2c**), the CA1-APV group and the DG-APV group ($p < 0.0001$ for blocks 1 and 2, $p < 0.01$ for blocks 3 and 4).

Analysis of the study-arm duration and the choice latency (see Methods) revealed that all APV-injected groups (including the CA3-APV group) were not significantly different from the CT-PBS group in either study-arm duration ($p > 0.1$) or in choice latency ($p > 0.5$) in the novel room (**Fig. 2d**). Thus, the

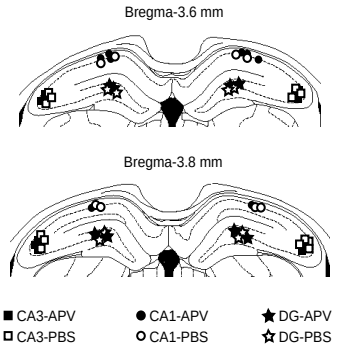

Fig. 1. Histologically verified cannula placements for the behavioral experiment (CA3-APV, n = 6; CA3-PBS, n = 7; CA1-APV, n = 4; CA1-PBS, n = 4; DG-APV, n = 4 and DG-PBS, n = 4). Modified sections from ref. 38.

impairment observed in the CA3-APV group cannot be attributed to a sensorimotor problem following the APV injection. Perfect performance of the CA3-APV in the familiar room, with two days of APV injections, after rats returned from the novel room, provides further evidence that the CA3-APV impairment demonstrated in the novel room was not due to a sensorimotor deficit (**Fig. 2e**).

Spatial memory with novel intermediate-term delays

To test more specifically whether APV injected into CA3 (as well as into CA1 or DG) would produce a spatial working memory deficit in the familiar spatial environment with a delay longer than a short-term range, we randomly intermixed four intermediate-delay (5-min) trials with four original short-delay (10-s) trials, thus forming a block of eight trials per day. The data from the PBS-injected groups (CA3-PBS, CA1-PBS and DG-PBS) were combined (CT-PBS), because these groups performed similarly ($p > 0.1$). The animals injected with APV in CA3 exhibited an initial impairment at five-minute delays, but they showed improvement in their performance (**Fig. 3a**). However, APV injection into either CA1 or DG markedly impaired performance at five-minute delays. Neither of the groups showed any deficit at 10-second delays (**Fig. 3b**). An ANOVA performed with groups as a between-subject variable and both delays and blocks as two within-subject variables revealed a significant effect of groups ($F_{3,20} = 9.0$; $p < 0.01$) and delays ($F_{1,20} = 150.9$; $p < 0.0001$). There was a significant interaction effect between delays and blocks ($F_{3,60} = 3.6$; $p < 0.05$). There were also significant interaction effects between groups and within-subjects variables: 'groups × delays' ($F_{3,20} = 8.4$; $p < 0.01$), 'groups × blocks' ($F_{9,60} = 2.5$, $p < 0.05$) and 'groups × delays × blocks' ($F_{9,60} = 3.5$, $p < 0.01$). Further *post hoc* analysis demonstrated that between the CT-PBS and the CA3-APV subjects, there was a significant difference ($p < 0.01$) in performance for the first block, whereas the remaining three blocks were not statistically significant. The CA1-APV group was impaired in performance from blocks 2 to 4, compared to the CT-PBS group (p values < 0.05, < 0.01 and < 0.05 for blocks 2, 3 and 4, respectively) at five-minute delays. The DG-APV group was impaired in blocks 3 and 4 (p-values < 0.05 and < 0.01, respectively) compared to the CT-PBS group in 5 minute–delay trials. The CA3-APV group differed from the CA1-APV group in blocks 3 and 4 (p-values < 0.05) and significantly differed from the DG-APV group in block 4 (p < 0.05). For the 10 second–delay trials given with intermixed 5-minute trials within the same day, there was no significant difference among the groups ($p > 0.1$, **Fig. 3b**).

The CA3-APV group was not significantly different in the study-arm duration and the choice-latency ($p > 0.5$ for both) from the CT-PBS group (**Fig. 3c**). However, the CA1-APV and the DG-APV subjects spent more time on study arms than both the CT-PBS and the CA3-APV groups, regardless of the upcoming delay period (10 seconds or 5 minutes) when the trials included 10-second delays intermixed with 5-minute delays (**Fig. 3c**), although an ANOVA revealed that only the difference between the CA1-APV and the CT-PBS subjects was statistically significant ($F_{1,13} = 20.4$; $p < 0.01$). With respect to choice latency, neither the CA1-APV group nor the DG-APV group was different ($p > 0.1$) from the CT-PBS group, which suggests that sensorimotor functions are not likely to be impaired. Therefore, the increased study-arm duration suggests that the animals tried to process familiar spatial cues more to achieve such a goal.

Selective blockade of CA3 NMDARs

To test whether our drug-injection parameters for the CA3 region estimated from a previous autoradiographic study[20] selectively blocked the NMDAR-dependent synaptic plasticity in CA3, we made acute electrophysiological recordings simultaneously from different hippocampal subregions with unilateral injection of APV or vehicle solution (PBS) into the dorsal CA3 region. Evoked neural responses were recorded from each subregion (CA3, CA1 and DG) of the dorsal hippocampus in anesthetized animals independent of the behavioral experiment (**Fig. 4a**). After the induction of LTP through the perforant path, the APV injection into CA3 resulted in almost complete inhibition of the LTP induction in CA3, although it also slightly affected the induction of LTP in CA1 (**Table 1**, **Fig. 4b**). However, the CA1 region was still able to produce a 143.9 ± 12.7% of baseline response within 30 minutes after tetanic stimulation (**Table 1**), suggesting a relatively intact ability of CA1 to produce LTP with the APV injection in CA3. The LTP induction in DG was not affected (**Table 1**, **Fig. 4b**). With the subregions (CA1, DG and CA3), the drug (APV versus PBS) and the time (0 minutes versus 30 minutes) after the tetanic stimulation as three within-subject variables, an ANOVA was performed on the percent of baseline-evoked response data. The ANOVA showed significant effects of the drug ($F_{1,3} = 37.3$; $p < 0.01$) and the time ($F_{1,3} = 18.4$; $p < 0.05$), as well as a significant interaction effect between the two variables ($F_{1,3} = 18.3$; $p < 0.05$). There was no significant effect of the subregions and the interaction effects between the subregions and other within-subject variables (p-values > 0.05), possibly due to subregional variability in evoked responses. However, when the evoked responses in CA3 alone were analyzed with the use of the drug and the time as two within-subject variables, the APV injected into CA3 produced less potentiation in CA3 immediately after the tetanic stimulation ($F_{1,3} = 12.6$; $p < 0.05$) as well as within 30 minutes after the stimulation ($F_{1,3} = 18.3$; $p < 0.05$) compared to PBS (**Table 1**). The same ANOVA separately performed on the data from the other two subregions (CA1 and DG), however, did not exhibit a significant difference between the PBS and the APV conditions either immediately after the stimulation or 30 minutes after the stimulation (p-values > 0.1). There was no significant difference between baseline-evoked responses and responses 0 minutes or 30 minutes after the tetanic stimulation in rats injected with APV into CA3 (**Table 1**) ($p > 0.1$). These results strongly suggest that there was relatively selective blockade of the induction of LTP in CA3 with our CA3 drug-injection parameters used for the behavioral testing.

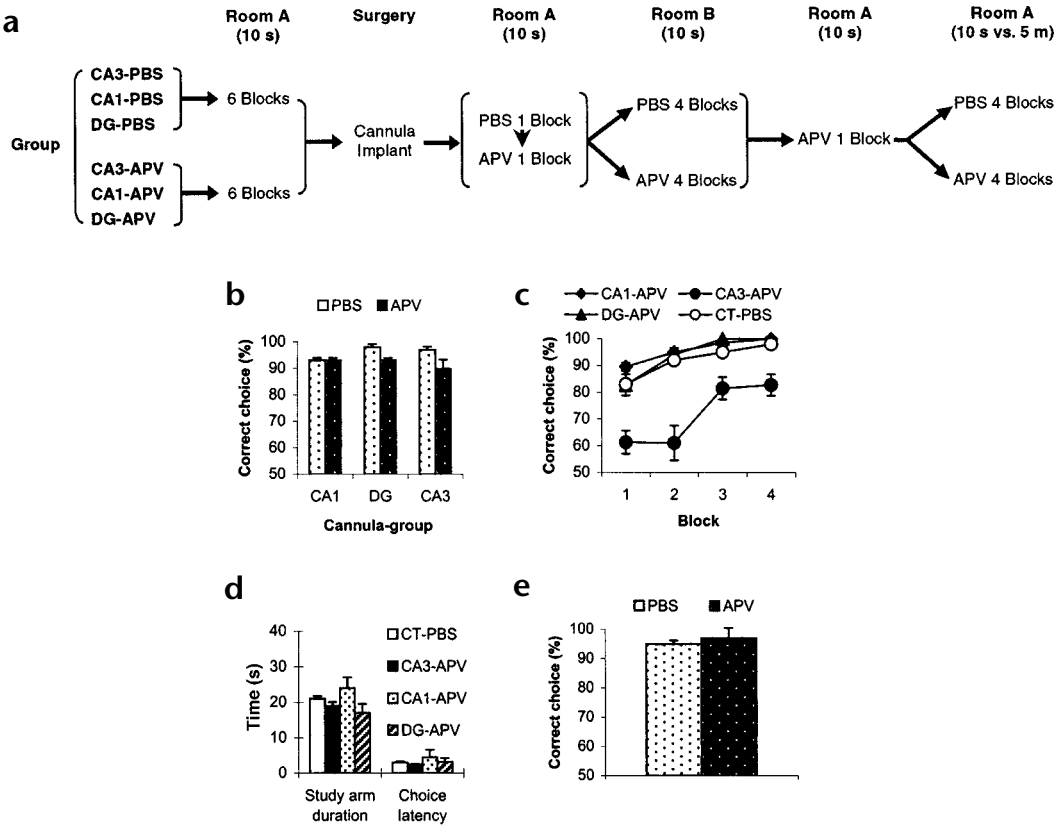

Fig. 2. Performance in the familiar spatial environment versus the novel spatial environment. (**a**) Procedure used for the behavioral experiments. Rooms A and B were different rooms with 8-arm mazes surrounded by distinctively different extra-maze cues (room A, familiar spatial environment; room B, novel spatial environment). Delays used for different rooms are shown for each room assignment. One block, 2 days of 16 trials. (**b**) Comparison of within-subjects performance of different groups implanted with cannulas in different hippocampal subregions in the familiar room under the influence of PBS (2 days of 16 trials) followed by APV (2 days of 16 trials). None of the groups exhibited significant differences in performance between the two conditions (PBS versus APV). (**c**) Acquisition of the novel spatial environment for different cannula groups. The PBS-injected groups (CA1-PBS, CA3-PBS and DG-PBS) showed no difference, and were hence regrouped as one control group (CT-PBS). The CA3-APV group exhibited marked impairment of acquisition for the first two blocks compared to other groups (CA1-APV, DG-APV and CT-PBS), which improved in the remaining two blocks (blocks 3 and 4). (**d**, **e**) Control data for a possible sensorimotor problem in the CA3-APV group in the novel environment. (**d**) Activity measures. Study arm duration, the total time spent on a study arm; choice latency, the latency to retrieve a food reward at the end of each arm from the onset of a choice phase. There were no significant differences between the CA3-APV and the CT-PBS groups in both measures. (**e**) Performance of the CA3-cannula group (CA3-APV and CA3-PBS) in the familiar room for 1 block, after the 4 blocks of testing in the novel room. All the animals in those groups were first tested with PBS injection (1 block) followed by APV injection (1 block). There was no difference between the two drug conditions, and performance was comparable with the post-surgery performance in the same room (**b**).

In the later phase of the testing (especially in the variable-delay protocol with the 10-second versus 5-minute delays), can the LTP induction still be blocked by APV while the animals perform the task? To test this, we induced LTP 40 minutes after the injection of APV in CA3 (**Fig. 5**, APV-LTP-40m); compared to induction after 10 minutes (**Fig. 5**, APV-LTP-10m), this approach was less effective in blocking LTP induction. However, the potentiated response reached baseline within 60 minutes after the APV injection (20 minutes after tetanic stimulation, **Fig. 5**), which suggests that potentiated responses in CA3 by tetanic stimulation given 40 minutes after the APV injection into CA3 could not be sustained for enough amount of time to be categorized as an LTP response.

Discussion

Our results suggest that the NMDARs in CA3, but not in CA1 and DG, are involved in acquisition of spatial working memory in a new spatial environment and are not critical for performing the task in a familiar spatial environment. In addition, blocking NMDARs in different subregions of the hippocampus affected spatial working memory differentially depending on mnemonic demands of test components. In other words, blocking the NMDARs in CA3 produced a deficit when the rats were transferred to the novel spatial environment to perform the same spatial working memory task and produced a deficit initially when the rats were required to remember familiar spatial cues for a longer period. Blocking the NMDARs in CA1 and DG did not affect performance in the novel spatial environment, but produced a sustained impairment of performance in trials with intermediate delays, while sparing performance with short delays.

The NMDARs in CA3 and spatial reorganization

Hippocampal cells tend to exhibit dynamic changes in their spatial representation (for example, place field) when changes occur in a spatial environment[26–29]. In our behavioral protocol, when the rats were transferred from the familiar room to the room with novel spatial cues, there were certainly novel spatial features to

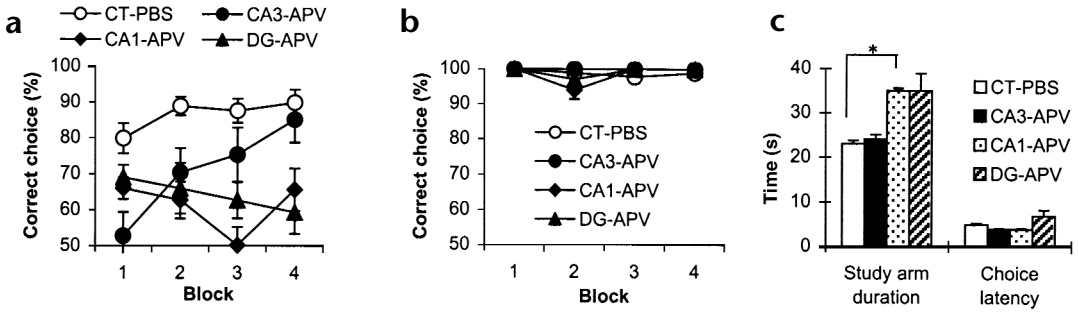

Fig. 3. Performance with variable delays in the familiar room. Four 5 min–delay trials were randomly intermixed with four original 10 s–delay trials. (**a**) Comparison of performance of different cannula groups in trials with 5-min delays. Note sustained impairment of choice accuracy in the animals injected with APV in CA1 (CA1-APV) or in DG (DG-APV) compared to the CT-PBS group (CA1-PBS, DG-PBS and CA3-PBS groups) and also note constantly improved performance of the CA3-APV group. (**b**) Comparison of performance of the same cannula groups shown in (**a**), but in trials with 10-s delays. All groups had perfect performance. (**c**) Control data for a possible sensorimotor problem in the CA3-APV group in both 10-s and 5-min delay trials. Study arms were explored for a longer duration in the DG-APV and the CA1-APV groups (*$p < 0.05$), yet there was no difference among the groups in choice latency.

be encoded to perform the task. Therefore, it would be reasonable to assume that changes in spatial representation in the hippocampus occurred. However, there were also invariant components (such as a similar radial 8-arm maze and the rectangular shape of the room) of the task that can be carried over to the novel room. The high choice accuracy shown by the controls when they were transferred to the novel room (**Fig. 2c**) supports such a possibility. Therefore, hippocampal cells might maintain the common spatial representation across different rooms, yet might have to reorganize or remap[27] the familiar spatial representation developed in one room when the rats are transferred to the novel room. The intact performance of the animals with APV injected into CA3 in the familiar spatial environment, but the impaired performance in the novel spatial environment (that is, the novel room; **Fig. 2c**) suggests that such a reorganization of spatial representation may require the NMDARs in CA3.

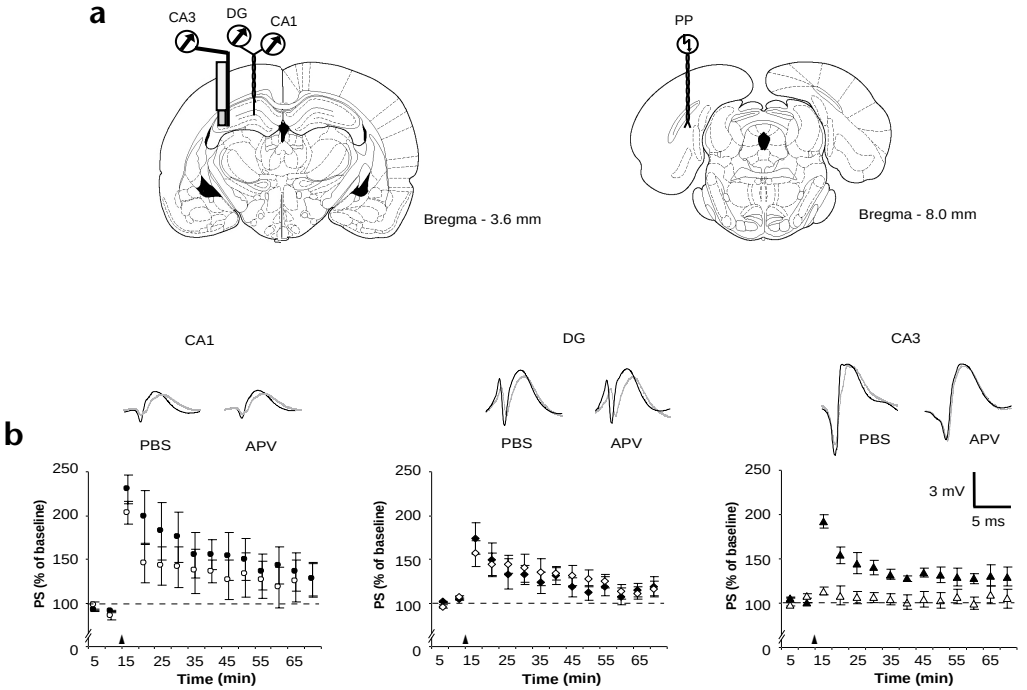

Fig. 4. Selective inhibition of the LTP induction in CA3 with APV injections into the CA3 subregion. (**a**) Electrode arrangements, a guide cannula (22G) attached with a monopolar electrode for CA3 and a twisted bipolar electrode for both DG and CA1 for recording evoked responses, after stimulating the perforant path (PP) through a twisted bipolar electrode in the angular bundle. (**b**) Within-subjects comparison of evoked responses simultaneously recorded in different hippocampal subregions after either PBS injection or APV injection (0-min) followed by LTP induction (shown as a black triangle at 10 min) by stimulating the perforant path (4 trains of 8 pulses at 400 Hz at 20-s intervals). The amplitude of the evoked population spike (PS) recorded in each subregion was measured. In DG, the APV injection (white diamonds) into CA3 produced comparable LTP with the one produced by the PBS injection (black diamonds) into CA3. In CA1, the APV injection (white circles) into CA3 caused a small decrease in potentiation of evoked responses compared to the PBS injection (black circles). Note almost complete inhibition of the LTP induction in CA3 with APV injection (white triangles) into CA3 compared to the PBS injection (black triangles). Insets show representative evoked responses from the different subregions before (gray traces) and after (black traces) the LTP induction with either the PBS or the APV injection within a subject.

Table 1. Percent of baseline (mean ± s.e.m.) population spikes simultaneously recorded in different subregions of the dorsal hippocampus after APV (or PBS) injection into CA3.

Time post-tetanus (*t*)	0 min		30 min	
Subregions	**PBS**	**APV**	**PBS**	**APV**
CA3	192.4 ± 8.3%	110.5 ± 4.3%	121.3 ± 3.4%	97.5 ± 8.2%
CA1	235.8 ± 15.9%	209.1 ± 12.8%	162.4 ± 16.7%	143.9 ± 12.7%
DG	175.6 ± 18.7%	157.4 ± 15.3%	130.9 ± 10.6%	133.6 ± 7.3%:

t, minutes elapsed after tetanic stimulation of the perforant path.

The impaired performance of the CA3-APV group (**Fig. 3a**) when the intermediate delay (5 minutes) was introduced in the familiar room also suggests that the NMDARs in CA3 could be essential for triggering the reorganization of a familiar spatial representation in response to the introduction of a novel task rule (novel intermediate-term delay)[30]. That is, in our variable delay protocol, the change produced in the mnemonic demand (the delay) could necessitate modification of the existing spatial representation ultimately to reproduce a suitable type of spatial representation that can be maintained and retrieved after a longer period of time. The CA3 NMDARs might be involved in the detection of such a mnemonic demand and initiation of representational reorganization. The reorganization might be achieved by incorporating more detailed spatial information and/or by elaborating the original spatial representation. Alternatively, the existence of non–NMDAR dependent systems in the CA3 network might cause only the initial impairment in the CA3-APV group; non–NMDAR dependent synaptic plasticity in CA3 might compensate for the deficient NMDAR-dependent synaptic plasticity. Opioid receptors have especially been suggested to be critical in producing LTP in CA3 (ref. 31).

NMDARs in CA1 and intermediate-term memory

In contrast to the CA3-APV group, rats with APV injected in CA1 or DG were unimpaired in the trials when they were transferred to the novel spatial room. However, the CA1-APV and the DG-APV groups were impaired in executing the trials with intermediate delays (5 minutes) even in the familiar room with no improvement across 8 days contrary to the rapid improvement of the CA3-APV group. The perfect performance of those animals in the short-delay (10-second) trials within the same day refutes an assignment of a generic sensorimotor deficit associated with the APV-injection. Therefore, it seems that the NMDARs in CA1 and/or DG become essential when animals have to acquire a spatial memory that needs to be maintained and retrieved after a delay period exceeding a short-term range (such as in 10 seconds in our experiment).

Both electrophysiologically and behaviorally, the current experiment was unable to dissociate the NMDAR system in CA1 from the one in DG in the DG-APV group. This seemed to be due to an upward spread of APV to CA1 along a cannula track following an APV injection into DG. Although the CA1-APV and the DG-APV groups showed similar patterns of deficit, especially in 5-minute delay trials in the familiar spatial environment, we believe the NMDARs in CA1 are more important than those in DG in producing deficits at intermediate-term (5-minute) delays, for the following reasons. The development of the technology for CA1-specific NMDAR knockout mice has recently driven research regarding the role of NMDARs in the CA1 region[7–10]. The NMDAR-dependent plasticity in CA1 is not critical for hippocampal recognition of previously encountered environments in the knockout mice lacking in the *NMDAR1* gene in CA1 (ref. 8), which, in turn, further confirms our data demonstrating that the CA1-APV group was not impaired when they were transferred to the novel room with short delays. However, the knockout mice exhibit impairments in memory in a situation in which information should be maintained during a delay period (30 seconds in ref. 7 and 14–29 days in ref. 9) exceeding a short-term range (such as several seconds). Intrahippocampal injections of an NMDAR antagonist in the CA1 region of the hippocampus also produces deficits in retrieving either spatial working memory[20] after an intermediate-term delay (2 hours), but not after a short-term delay (15 seconds), or spatial contextual memory[32] after a long-term delay (24 hours), yet sparing immediate retrieval. Our data suggest that NMDARs in CA1 (and/or in DG) are essential for acquiring a spatial memory that needs to be maintained and retrieved after an intermediate or a long delay period.

Electrophysiological assessment of the drug diffusion

Although we initially estimated our drug-injection parameters based on a previous autoradiographic study[20] carried out to determine the spread of intracranially injected APV, it was still unknown whether detecting the diffusion range of a radioisotope-labeled drug diffusion (^{3}H-D-AP5) can be equated with assaying the range of functional influence of the drug in the system. To address the latter question, we tested the functional consequence of local diffusion of APV in CA3 to each subregion of HIPP electrophysiologically *in vivo* with the estimated injection parameters. Because the main reason to diffuse NMDAR antagonist in CA3 in our experiment was to block the induction of LTP within CA3 while minimally affecting the induction of LTP in other subregions (CA1 and DG), we tested the amount of decrease in the induction of LTP in different subregions as a result of diffusion of APV in CA3.

The marked inhibition of the LTP induction in CA3 demonstrated that the main site of action of APV was localized within CA3 in the CA3-APV group. Although CA3 had some residual

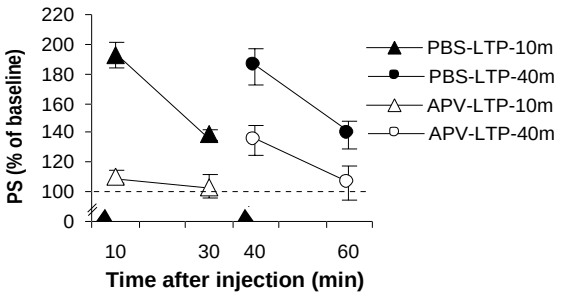

Fig. 5. Sustained blockade of LTP induction in CA3 by APV injection into CA3. Evoked responses were measured in CA3 immediately and 30 min after tetanic stimulation (shown as black triangles) that was given either 10 min (PBS-LTP-10m and APV-LTP-10m) or 40 min (APV-LTP-40m and PBS-LTP-40m) after the drug injection (PBS or APV) into CA3. Inefficient LTP induction occurred in CA3 when tetanic stimulation was given even 40 min after the APV injection (APV-LTP-40m), compared to the robust LTP induction with the PBS injection (PBS-LTP-10m and PBS-LTP-40m) into CA3. Dotted line, baseline-evoked response.

capability to be potentiated immediately following the tetanic stimulation after the APV injection, compared to the PBS-injected condition, almost complete blockade of long-term synaptic potentiation occurred in CA3 (**Table 1**). In addition, DG was unaffected by the APV injection into CA3.

The medial perforant path was stimulated in our electrophysiological experiment to evoke neural responses in the dorsal CA3 as well as in other subregions. However, we could not assess which NMDAR distribution (for example, NMDARs in different layers of CA3 such as stratum moleculare and stratum radiatum) was affected mostly by APV. This was mainly due to the fact that the cannula-coupled electrode used for CA3 recording did not allow sophisticated recording from an electrode attached to a guide cannula, as the guide cannula (22G) itself, while being lowered into the CA3 region produced some damage in the fiber system of CA3 at the site of recording, although pyramidal cells were largely intact. Further studies are suggested to investigate differential contribution from different NMDAR populations within CA3 in acquiring spatial working memory, using more sophisticated recording setups.

The injection of APV (0.5 μl/side, 30 mM) into CA1 produced a selective impairment in the LTP-induction in CA1, whereas it minimally affected LTP induction in other subregions (unpublished observations). However, injecting APV (0.5 μl/side, 30 mM) into DG resulted in a large decrease in the amount of LTP produced in CA1 as well as in DG presumably due to an upward spread, whereas the CA3 region was minimally affected from the APV injection into DG (unpublished observation). Based on these electrophysiological observations, in addition to the lack of difference between the CA1-APV group and the DG-APV group in our behavioral data, it is suggested that the deficit in performance demonstrated in the DG-APV group in the variable delay (10-second versus 5-minute) paradigm (**Fig. 3a**) might be produced by the spread of APV into CA1.

The possibility of regional heterogeneity in cognitive function within the hippocampus has been raised from lesion studies[33–35], a chronic electrophysiological study[36] and computational models[11–15]. The CA3 network has an ideal architecture to serve as a representational space for specific spatial environments because, anatomically, CA3 receives the richest inputs among the hippocampal subregions. Such property of CA3 has encouraged computational models[11,37] to label CA3 as an autoassociative network critically involved in memory formation. The involvement of CA3 in the representational reorganization in response to novel mnemonic demands may be an emergent property of the network in the process of associating external inputs (such as perforant path and mossy fiber input) as well as internal inputs (such as recurrent collateral and commissural input). Our behavioral data encourage a computational approach to consider the CA1 and/or the DG as gateways or interfaces between the hippocampus and extrahippocampal regions in acquiring and retrieving spatial memory that needs to be maintained possibly in the extrahippocampal areas for an amount of time exceeding a short-term period. The NMDARs in those subregions, based on our data, may be critical in establishing reliable interfaces between the hippocampus and the extrahippocampal regions.

Methods

Cannula-implant surgery. Guide cannulas (22G) coupled with stylets (28G) protruding 1 mm from the tips of guide cannulas were bilaterally implanted in different subregions of the hippocampus ($n = 15$ for CA3, $n = 10$ for CA1 and $n = 10$ for DG) after behavioral pretraining (see below). All protocols conformed to the NIH Guide for the Care and Use of Laboratory Animals and the Institutional Animal Care and Use Committee at the University of Utah.

Intrahippocampal microinjection. The drug-injection protocol used for the behavioral testing was as follows. APV was dissolved in phosphate buffered saline (PBS; pH 7.4) in final concentration of 30 mM. Either APV or PBS was injected bilaterally via an injection needle (28G) 10 min before each day's behavioral experiment. The injection quantity was 0.5 μl/side and the injection rate was 0.1 μl/min. The injection needle was left in place for 1 min after the injection. The rat was then returned to its home cage and any abnormality in movement from the drug injection was carefully examined for 10 min before the rat was placed on the maze.

Behavioral task. Male Long-Evans rats ($n = 35$) were trained on a radial 8-arm maze with a delayed-nonmatching-to-place (DNMP) protocol. After visiting an arm (the study arm) for a reward, each rat was confined in a bucket on the center platform for a 10-s delay period during which doors for an adjacent arm (the choice arm) and the study arm were opened. The rat had to choose the choice arm to receive a reward. On each trial, a study arm was randomly selected and a choice arm was always an adjacent arm on either side (randomly chosen) of the study arm. The purpose of this design was to make two arms (study and test arms) equally available at the time of the choice phase by preventing the situation where the rat might easily avoid choosing a study arm by remembering the direction of the study arm rather than spatial cues. The time spent on the study arm (that is, the study-arm duration) was recorded. The latency to obtain the reward at the end of the choice arm (the choice latency) was also measured. Eight trials were given with an intertrial interval of 20 s for 12 days. For each 20-s intertrial interval period, the rats were left on the center platform with the bucket raised and all 8 doors of the maze closed. After the pretraining to a criterion (>95% correct choices), bilateral cannulas were implanted in different subregions (CA1, CA3 and DG) of the dorsal hippocampus (see above) and a week of recovery period was given to the animals.

After the recovery period, the rats were tested in the familiar room used for the pretraining, with 2 days of PBS injection for a total of 16 trials followed by 2 days of APV injection. Then, the animals in each cannula-group were divided into a PBS group and an APV group and were transferred to a novel room with different spatial cues. PBS or APV was injected for 8 consecutive days in each group with the same DNMP protocol. All the animals were retested in the familiar room afterward with APV injection for 2 days. Finally, the animals were tested in the familiar room with either PBS or APV for 8 days using a variable delay DNMP paradigm where new 5-min delays (4 trials) were randomly intermixed with familiar 10-s delays (4 trials) in a given day's experiment. Histological verification of cannula positions was done after all the behavioral experiment, using a cresyl violet stain.

Electrophysiological recording. Six male Long-Evans rats were used only for the electrophysiological experiments. A twisted bipolar recording electrode (125 mm, 1 mm vertical tip distance between the two poles, 3.6 mm posterior to bregma and ±2.0 mm lateral to midline), a guide cannula (22G, 3.6 mm posterior to bregma and ±3.7 mm lateral from midline) attached with an electrode (200 mm, 1 mm projection from the tip of the guide cannula) and a twisted bipolar stimulating electrode (125 mm, 1 mm tip distance, 8.0 mm posterior from bregma and ±4.4 mm lateral from midline) were implanted in hippocampal subregions by monitoring evoked responses from each subregion (**Fig. 4a**), while stimulating the medial perforant path at low frequency (0.05 Hz). The ventral coordinates of electrodes were adjusted so that the electrode tips were located in the vicinity of the cell layers of the dorsal hippocampal subregions by monitoring positively going field EPSPs superimposed by maximal population spikes maintained for at least 20 min in response to the perforant path stimulation. Histological verification confirmed the placement of electrode tips in cell layers in each subregion.

For the LTP induction experiment after the recovery period, low-frequency test pulses (100–1,200 mA, 100 ms, 0.05 Hz) were given through the stimulating electrode and the evoked response from each subregion

was monitored simultaneously to obtain stable evoked responses. Then, the vehicle solution (PBS) was injected (0.5 µl, 0.1 µl/min, 30°C) and evoked responses were monitored during the next 10-min period (baseline) that was used as a drug-diffusion period in our behavioral protocol. The 10-min baseline period was immediately followed by strong tetanic stimulation (4 trains of 8 pulses at 400 Hz, 20-s intervals) through the stimulating electrode to induce LTP. Immediately following LTP induction, the low-frequency stimulation period (0.05 Hz) resumed and evoked field EPSPs and population spikes were monitored in each subregion for 1 h. The percent change in the population spike amplitude relative to the one recorded during the baseline period was calculated in each subregion. As the twisted bipolar electrodes with the pre-fixed tip-distance were aimed at the granule cell layer in DG and the pyramidal cell layer in CA1 at the same time during the implant procedure, it was technically difficult to achieve equally maximal responses in both subregions. Therefore, an effort was made to produce maximal population spikes in DG following perforant path stimulation, which also resulted in robust detection of population spikes in CA1.

At least two weeks after the pre-LTP induction baseline was established, the same procedure was repeated as described above except that APV (30 mM, 0.5 µl) was injected using an injection cannula (28G) before the LTP-induction instead of PBS. Data were presented as mean ± s.e.m. After completion of the experiments, cathodal current (300 µA, 1.5 s) was applied to each implanted electrode to verify the placement of electrodes.

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Competing interests statement

The authors declare that they have no competing financial interests.

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