Neuron, Vol. 31, 631–638, August 30, 2001, Copyright ©2001 by Cell Press

NMDA Receptor Antagonism Blocks Experience-Dependent Expansion of Hippocampal "Place Fields"

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Summary

In agreement with theories of sequence learning, hippocampal place representations expand asymmetrically during repeated route following. This behaviorally induced, experience-dependent expression of neuronal plasticity was blocked by the $NMDA_R$ antagonist CPP, suggesting that it may result from the temporal asymmetry and associative properties of LTP. $NMDA_R$ antagonism, however, had no effect on the range of the progressive shift of firing phase of hippocampal cells, relative to the theta rhythm, as the rat traverses the cell's "place field." Thus, when place fields normally expand with experience, the relationship between firing phase and position is altered, as predicted by models that account for "phase precession" on the basis of asymmetry of synaptic connection strengths. These effects of CPP mimic changes that occur during normal aging, suggesting mechanisms by which sequence learning deficits may arise in aged animals.

Introduction

O'Keefe and Dostrovsky (1971) reported that hippocampal neurons increased their firing rates in specific regions of space and defined these regions as "place fields." This spatial selectivity is dependent upon behavioral context and other variables, and a rich and complex experimental literature has accumulated as a result of the attempt to characterize the determinants of hippocampal neuronal activity. It has been proposed that the hippocampus may store route or nonspatial sequence information (i.e., episodes) through asymmetric Hebbian strengthening of synapses among cells with overlapping activity along a given route or sequence. Neural network models that derive from Hebb's "phase sequence" concept (Hebb, 1949) provide a reasonable framework in which to understand how such sequence learning occurs. The theory of sequential association predicts that in a sequence of neural representations A, B, C the forward connections will be modified as a consequence of the asymmetric characteristic of the Hebbian plasticity mechanism (i.e., presynaptic activity must precede postsynaptic activity; Levy and Steward, 1983). The result would be that a network with such properties would begin to predict the next element in the sequence before it actually occurred (Abbott and Blum, 1996; August, 1997; Blum and Abbott, 1996; Jensen and Lisman, 1996; McNaughton and Morris, 1987; Muller et al., 1991; Prespicius and Levy, 1994; Tsodkys et al., 1996; Wallenstein and Hasselmo, 1997). Applied to the spatial selectivity of hippocampal cells, these theoretical considerations predict that place fields should show an asymmetric expansion over repeated traversals of a route—i.e., fields should elongate and shift in a direction opposite to the direction of motion of the animal. Mehta et al. (1997, 2000) verified this hypothesis. When rats repeatedly traversed a track in one direction, the place fields expanded backward relative to the rat's direction of motion. The expansion occurred rapidly, over the first several laps around the track. Its asymmetric nature argues strongly against a nonspecific (i.e., nonsynaptic) mechanism. Moreover, when cells whose fields had expanded in one environment expressed a field in a second environment shortly thereafter, the expansion did not carry over between environments (Mehta et al., 1997), suggesting synapse selectivity.

The 6–10 Hz theta oscillation in the hippocampal electroencephalogram (EEG; Green and Arduni, 1954) is tightly coupled to spatial, exploratory behaviors such as rearing and walking (Vanderwolf et al., 1975; MacFarland et al., 1975) and may play a role in determining the time over which optimal LTP plasticity can occur (Pavlides et al., 1988; Huerta and Lisman, 1995). O'Keefe and Recce (1993) first noted that, as an animal moves through the cell's place field, the phase relationship between the theta rhythm and the cell's firing changes systematically. Spikes at the entry point of a place field occur late in the theta cycle, whereas spikes at the exit of the place field occur near the beginning of the theta cycle. As pointed out by O'Keefe and Recce (1993), this phenomenon, known as phase precession, provides a possible additional source of location information during route-following behaviors, because phase varies systematically with position. For this and other reasons, it of interest to know how the phase precession phenomenon is changed when place fields expand. There are, in principle, two a priori possibilities. First, the range of phase shift could expand as the place field expanded. This would increase the phase-related spatial information provided that the initial range of phase shift was less than 360° and that the expansion resulted in a net shift of not more than 360°. Alternatively, the range of phase shift might remain constant, in which case, there would actually be a disruption of the initial relationship between firing phase and location. These two possible outcomes would also have different implications concerning the possible mechanism of phase precession (see Discussion).

The foregoing suggests that the experience-dependent place field expansion effect may involve an associative (McNaughton et al., 1978), asymmetric (Levy and Steward, 1983; Bi and Poo, 1998), LTP-like process, possibly mediated by the NMDA receptor ($NMDA_R$). In-

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terestingly, old rats (Barnes, 1979; Gage et al., 1989; Gallagher et al., 1993; Deupree et al., 1991; Moore et al., 1993; Rosenzweig et al., 1997; Barnes et al., 1997) and rats with $NMDA_R$ blockade (Collingridge et al., 1983; Morris et al., 1986; Kentros et al., 1998) exhibit impaired LTP and impaired performance in spatial navigation tasks and have deficits maintaining a consistent hippocampal neuronal representation of space across repeated episodes in a given environment. Old rats also exhibit deficient experience-dependent place field expansion (Shen et al., 1997). The present experiments were therefore designed specifically to test the hypothesis that place field expansion depends on $NMDA_R$ activation and to examine the consequences of blockade of expansion on phase precession. Portions of these results have been previously published in abstract form (Ekstrom et al., 1999, Soc. Neurosci., abstract; Rosenzweig et al., 2000, Soc. Neurosci., abstract).

Results

The activity of 863 pyramidal cells in the CA1 region of 6 rats was recorded using multiple tetrodes. On different days, the same rats were injected with saline or 3.5 mg/kg d-CPP (r-[−]-3-[2-carboxypiperazin-4-yl]propane-phosphonic acid; Sigma, St. Louis, MO) at a dose that was determined in behavioral pretesting to affect spatial memory but not to impair locomotion (see Experimental Procedures). A total of 16 drug and 16 control sessions were obtained. After injection, rats were allowed to rest on a towel-lined flowerpot for 30 min. Fifteen min of baseline data were then recorded, followed by a variable period of maze running (control: 12.0 ± 0.7 min; drug 12.5 ± 0.8 min), and then 30 min of postbehavior rest and/or sleep. Three of the six rats ran on a rectangular track (95 × 44 cm), while the other three rats ran on a circular track (144 cm in diameter). The minimum number of laps run in a given session was 19 and, therefore, the analysis was restricted to the first 19 laps for all sessions. An average of 26 pyramidal cells was recorded per session under saline and 29 pyramidal cells per session under the drug. A total of 158 control place fields and 152 drug place fields were analyzed.

To compare qualitatively the characteristics of place fields under saline and CPP, the average place field over all laps was computed by aligning every place field on its center of mass and normalizing by its mean firing rate; averages were computed separately for control and drug conditions (Figure 1; see Experimental Procedures). Because of differences in place field size and firing rate, larger fields may contribute disproportionately to the average. Nevertheless, an ANOVA showed a significant difference between the two average place fields ($F_{[1,308]} = 6.8$, $p < 0.01$; a Fischer-PLSD revealed no significant difference in peak-firing rate). The composite fields recorded under saline were larger and slightly elongated in the direction opposite to the rat's direction of motion compared to place fields recorded under CPP, which were more symmetric.

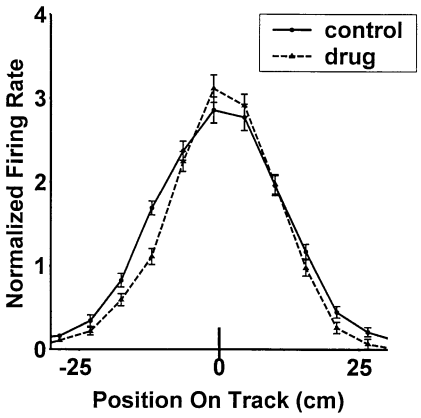

Figure 1. Effects of CPP (Dashed Line) and Saline (Solid Line) on Median Place Field Shape and Size

Normalized place field firing rate distributions were aligned on their centers of mass, and then the median values were computed. The composite place field has a larger area and is elongated to the left in the control condition, compared to during $NMDA_R$ antagonism by CPP. (Error bars represent standard error of the mean in this and subsequent figures).

Under CPP, the rats ran significantly faster on average (Table 1), and their running speed also increased significantly over the first ten laps ($F_{[1,304]} = 4.0$, $p < 0.05$; linear regression slope of 0.60 (cm/s)/lap; Figure 2A). This effect of CPP is consistent with earlier reports that $NMDA_R$ antagonists can produce ataxia and elevated levels of locomotion (Tricklebank et al., 1989; Jevtovic-Todorovic et al., 1998).

Table 1. Effect of CPP on Single Unit Firing Characteristics, Theta Rhythm, and Running Speed

	Control	Drug	p Value
Mean In-Field Firing Rate (Hz)	9.3 ± 0.6	10.1 ± 0.7	$t_{308} = 0.81$, $p < 0.42$
Mean Firing Rate for All Behavior (Hz)	0.87 ± 0.03	0.84 ± 0.03	$t_{861} = 1.4$, $p = 0.15$
Maximum In-Field Firing Rate (Hz)	20.0 ± 1.5	20.7 ± 1.5	$t_{308} = 0.35$, $p < 0.73$
Mean Theta Frequency (Hz)	7.93 ± 0.06	8.04 ± 0.03	$t_{24} = 2.4$, $p < 0.05$
Mean Theta Power (Arbitrary Units)	13653 ± 3540	19216 ± 4195	$t_{24} = 2.2$, $p < 0.05$
Mean Place Field Size (cm)	42.5 ± 2.0	36.0 ± 1.7	$t_{308} = 2.5$, $p < 0.01$
Mean Running Speed (cm/s)	16.2 ± 0.6	21.2 ± 0.7	$F_{[18,30]} = 4.7$, $p < 0.05$

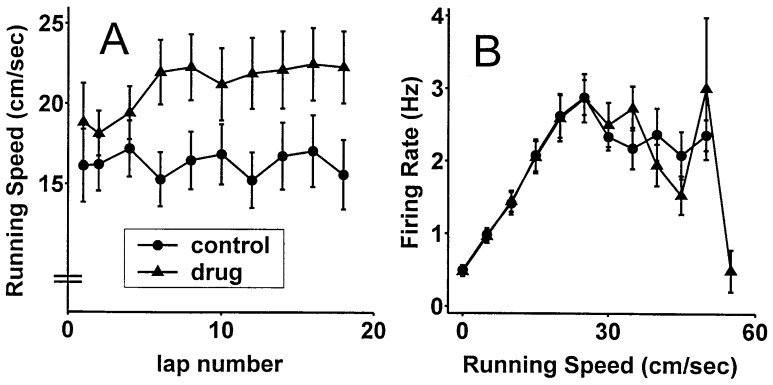

Figure 2. Effects of CPP on Running Speed and CA1 Pyramidal Cell Firing Rate

(A) Rats ran faster under the influence of CPP (triangles) than following saline injection (circles). There was also a significant acceleration in running speed over the first ten laps as a result of treatment with CPP but not saline.

(B) The effect of running speed on firing rate was computed by adding up the total number of spikes within each speed interval and dividing this by the total time spent by the animal within each interval. Firing rate increased with running speed (except at the highest speeds, which occurred only rarely). CPP did not affect the relationship between running speed and firing rate.

Because the firing rate of hippocampal pyramidal neurons has been shown to be proportionally related to an animal's running speed within certain ranges (Czurko et al., 1999; McNaughton et al., 1983), the relationship between running speed on the track and the firing rate of hippocampal pyramidal cells was investigated (Figure 2B). The spike counts at each velocity interval were divided by the time spent at the corresponding velocity (see Experimental Procedures). There was an overall effect of running speed on the firing rate averaged over sessions in both conditions ($F_{[9,30]} = 4.1$, $p < 0.05$), but there was no effect of treatment on the two distributions ($F_{[1,30]} = 0.82$, $p > 0.05$), nor an interaction between the two. Although the firing rate tended to fall with increases in running speed after around 35 cm/s, as has been reported in earlier studies (Czurko et al., 1999), running speed only rarely fell outside the linear range of Figure 2B.

In order to assess changes in place field characteristics, regions of high firing rate were identified by locating areas on the track where the firing rate exceeded 1 Hz for at least nine contiguous pixels (see Experimental Procedures; Muller et al., 1987). Place fields near reward sites were excluded because of possible contamination with spikes that occurred while the EEG exhibited non-theta activity (i.e., large irregular activity [LIA]); place fields located on any of the corners of the rectangular track were also excluded. The place field intensity on a given lap was computed by taking the total number of spikes within place field boundaries. This measure of place field change was selected in addition to other measures such as the integral of firing rate (Mehta et al., 1997) because it is less dependent on running speed, due to the relationship between speed and firing rate, which, as can be deduced from Figure 2B, tends to keep the number of spikes in the place field constant when speed changes. By the last lap in the control condition, the number of spikes increased 66% from the first lap (paired t test, $t_{157} = 6.4$, $p < 0.001$) while the number of spikes in the last lap of the drug condition did not differ significantly from the first lap (paired t test, $t_{151} = 0.09$, $p = 0.93$, Figure 3A). Control and drug place field sizes did not differ significantly on the first lap (t test, $t_{308} = 1.8$, $p = 0.08$). When compared over all laps, there was a significant effect of drug treatment on the number of spikes (ANOVA, $F_{[1,308]} = 50$, $p < 0.001$). As indicated by a linear regression (slope: 0.50 ± 0.21 spikes/lap, $F_{[1,3002]} = 23$, $p < 0.0001$), there was a significant increase in spikes over the first 19 laps, which is consistent with previous studies (Mehta et al., 1997). There was no similar increase in the drug condition (slope: 0.08 ± 0.18 spikes/lap, $F_{[1,2888]} = 0.72$, $p = 0.40$). Firing rate, when the increase in running speed due to CPP injection was taken into account (see Experimental Procedures), increased significantly in the control condition over laps with a slope of .34 Hz/lap $\pm$ 0.19 (linear regression: $F_{[1,3002]} = 12$, $p < 0.0001$) but not in the drug condition ($F_{[1,2888]} =$

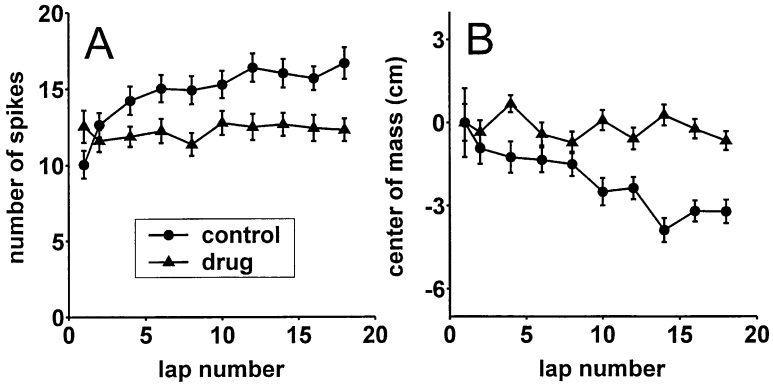

Figure 3. Effects of CPP and Saline on Place Field Intensity and Center of Mass

Effects of CPP and saline on place field intensity as measured by the number of spikes in the field, and center of mass as measured by the weighted average of location and firing rate, within place field boundaries. Under saline injection ([A], closed circles), the mean place field intensity increased significantly by 66% over the first 19 laps, as was indicated by a significant linear regression, and the center of mass shifted backward significantly after the first lap ([B], closed circles). Under CPP injection ([A], closed triangles), however, place field intensity did not significantly expand across laps, nor did the center of mass shift backward ([B], closed triangles). There were also significant effects of CPP on the mean place field size and firing rate integrals (see text).

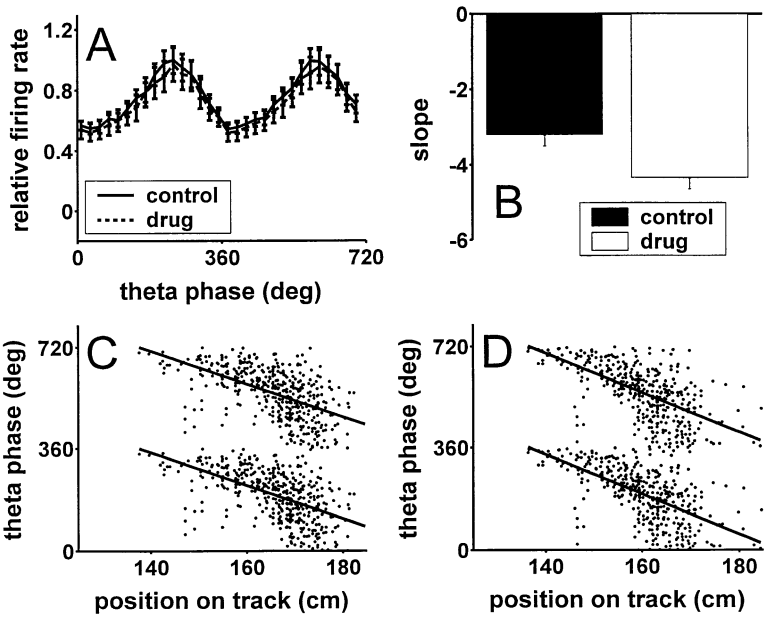

Figure 4. Effects of CPP and Saline on Theta Rhythm and Phase Precession

(A) Modulation of pyramidal cell firing probability as a function of theta phase was not affected directly by CPP. In both drug and control conditions, the average relative firing probability was modulated by about 50% by the theta rhythm. The lack of any effect of CPP on the mean firing phase distribution means that there was no change in the range of phase shift (precession) over the place field after expansion. (B) There was, however, a differential effect of saline (closed bar) and CPP (open bar) on the mean slope of the linear regression of firing phase relative to position (see Experimental Procedures). This is illustrated by examples of representative place fields whose slopes of phase versus position were close to the mean slopes recorded under saline (C) and CPP (D) injections. Thus, place field expansion results in a slowing of phase precession. Note that, although place fields are smaller (on average, ~5 cm) under CPP (D) than saline (C), the phase shift nevertheless spans one complete cycle in both cases. The two examples are taken from the same tetrode in one rat on consecutive saline and CPP days. For clarity, two cycles of phase are plotted, in order to illustrate the restriction of the in-field firing to a single cycle of phase shift.

0.02, $p = 0.90$). Similarly, the lap-specific place field integral (see Experimental Procedures) increased over laps in the control condition with a slope of 19 ± 7 cm*Hz/lap ($F_{[1,3002]}$, $p < 0.0001$) but did not change significantly in the drug condition (linear regression, $F_{[1,2888]} = 0.80$, $p = 0.37$). Consistent with the finding that place field size increased in place fields recorded under saline but not in place fields recorded under CPP, there was also a significant decrease in mean place field size due to the drug (42.5 ± 2.0 cm; drug: 36.0 ± 1.7 cm; Table 1).

In addition to expanding in size, the center of mass of the place field distribution also shifts backward over laps (Mehta et al., 1997). The center of mass is the weighted average of location and firing rate within the overall place field boundary. In order to assess how the place field location shifted over laps, the center of mass of the field on a given lap was subtracted from its overall center of mass. The center of mass value on the first lap in both conditions was then set to zero. The mean center of mass was shifted backward by 3.2 cm in the control condition when first lap values were compared with last lap values (paired t test: $t_{119} = 2.3$, $p < 0.02$) but did not move significantly backward in the drug condition (paired t test: $t_{116} = 1.4$, $p = 0.17$; Figure 3B). There was a significant effect of treatment on the center of mass (ANOVA, $F_{[1,305]} = 94$, $p < 0.0001$). A linear regression performed over laps showed that the center of mass moved backward in the control condition (slope: −0.36 ± 0.11 cm/lap, $F_{[1,2496]} = 40$, $p < 0.0001$) but not in the drug condition (slope: −0.04 ± 0.09 cm/lap, $F_{[1,2379]} = 0.83$, $p = 0.36$).

Theta activity recorded from the EEG electrodes near the hippocampal fissure of four rats and near the cell body layer of one rat was assessed by filtering between 6 and 10 Hz. After removing points recorded at low running speeds in order to eliminate contamination by LIA near the reward sites, both theta power and theta frequency (reciprocal of the mean period as assessed by a thresholded peak detection algorithm; see Experimental Procedures) were found to be slightly but significantly higher in the CPP condition compared to saline (Table 1). Both of these results are consistent with previous findings, which have shown a positive correlation between running speed and theta frequency and theta power (MacFarland et al., 1975; Recce, 1994; Shen et al., 1997). The average depth of firing rate modulation in the population was determined by averaging firing rate as a function of theta phase (2.8° per bin; Figure 4A). The depth of theta modulation of the ensemble firing rate was about 50% in both control and drug conditions, which is comparable to other studies (Skaggs and McNaughton, 1996), and when estimated on a cell by cell basis, there was no significant difference in modulation depth between treatment groups ($t_{231} = 0.98$, $p = 0.32$). Note, however, that the phase precession phenomenon obscures the true depth of theta modulation at the single cell level, which can approach 100% on a cycle by cycle analysis (Skaggs and McNaughton, 1996). The fact that the phase distribution profiles for pyramidal cell firing was not altered by the drug treatment means that the range of phase in the two conditions did not change precession (see below).

Under both drug and control conditions, there was a systematic shift of firing phase of CA1 pyramidal cells (Figures 4C and 4D) as the rat ran through the corresponding place fields (i.e., phase precession). Representative examples of pyramidal cell firing phase as a function of position under saline and under CPP are shown in Figures 4C and 4D, respectively. These examples were taken from the same tetrode on consecutive control and drug days and illustrate the effect of CPP on place field size and the related effect on the slope of the regression of firing phase versus location. The latter is steeper under CPP because the field is smaller.

To assess this phenomenon quantitatively, over all five rats with useable theta records, in all place fields included in the foregoing expansion analysis, the slopes of the firing phase versus position functions for each cell were computed separately. After excluding place fields that had fewer than 100 spikes, a total of 93 place fields in the control condition and 75 place fields in the drug condition were analyzed. The means of these phase versus position slopes (see Experimental Procedures) were significantly more negative under CPP than under saline (-4.3 ± 0.4 deg/cm versus -3.2 ± 0.5 deg/cm; t test, $t_{93} = 2.2$, $p < 0.03$; Figure 4B).

Discussion

At a dosage that impairs spatial memory in rats but does not severely affect their locomotor performance, the $NMDA_R$ antagonist CPP prevents the dynamic expansion of CA1 pyramidal cell firing fields during repetitive, route-following behavior. This conclusion is derived from three independent analyses. First, during repeated traversals of the same region of the environment, saline-injected rats showed an increase in place field size and intensity while the same rats, when injected with the $NMDA_R$ antagonist CPP, showed no significant change in place field characteristics. Second, on successive laps, the center of mass of the place fields shifted backward relative to the first lap following saline injection, but not after CPP injection. Third, $NMDA_R$ blockade revealed that place field expansion alters the relationship between the phase of the theta rhythm at which pyramidal cells fire and the animal's position within the corresponding place field. The relationship was different because the place fields were on average larger in the absence of CPP, due to the experience-dependent expansion, whereas the range of the theta phase shift over which firing occurred remained constant at about 360°. These differences between the treatment condition and controls resemble the previously reported differences between aged and young rats (Shen et al., 1997) in the absence of any drug, suggesting that the lack of expansion in aged rats may be due to a deficiency in $NMDA_R$-dependent LTP. Such age differences have been reported for artificially induced LTP (Deupree et al., 1991; Moore et al., 1993; Rosenzweig et al., 1997).

Systemic injection of NMDA antagonists is known to have a variety of "nonspecific" effects. For example, Clineschmidt et al. (1982) observed a sympathomimetic effect of MK-801 and a corresponding increase in rotational activity in rodents with unilateral striatal lesions, which was blocked by catecholamine depletion. This effect probably accounts for the elevation in running speed observed in the present study; however, although mean running speed was higher under CPP and tended to increase over laps, the firing rate of place cells measured at a given running speed did not differ over the range of running speeds observed. Furthermore, although theta frequency and theta power were slightly, but significantly elevated in CPP-injected animals, this elevation was completely accounted for by the increased running speed. The overall depth of modulation of the pyramidal cell population activity by the theta rhythm did not differ significantly between drug and control conditions, nor did $NMDA_R$ blockade prevent phase precession in a familiar environment. Thus, $NMDA_R$ blockade appears to have little if any direct effect on pyramidal cell firing dynamics apart from those related to experience-dependent plasticity.

It has been proposed that an asymmetrically associative, LTP-like, synaptic strengthening mechanism could underlie the learning of sequences (Hebb, 1949). The present results show that the place field expansion effect is LTP-like in its NMDA dependence, supporting the hypothesis that such plasticity mechanisms are engaged in the awake, freely behaving animal in the normal course of spatial exploration. From the present results, this appears to be the case even when animals return to a familiar environment after a period of absence, when one might expect that no new learning (at the behavioral level) would be required, particularly given the simple task employed in these studies. The fact that a physiological mechanism might continue to be expressed even though no longer required to support a behavioral adaptation, however, does not negate the hypothesis that it supports the initial learning. Some behavioral studies indeed suggest that the role of $NMDA_R$-dependent LTP may be limited to novel environments (Bannerman et al., 1995). Because the relationship between the strength of a memory trace and behavioral performance is likely to be nonlinear, however, these studies do not rule out the likelihood that some loss of strength of the memory trace does occur during the period since the last episode in the environment. The present results suggest that even in a familiar context, $NMDA_R$-dependent LTP may still play an important role in the dynamic tuning of hippocampal activity, compensating for any loss of trace strength that may have occurred since the previous episode. It is also possible that the synaptic connections underlying the expression of place fields in a given environment may be regulated by multiple plasticity mechanisms with different persistence characteristics, i.e., both short- and long-term traces may exist. The presumed associative synaptic changes underlying the relatively transient place field expansion may provide a means of focusing the network on the current context and may facilitate both predictive activity within this context and short-term memory for it.

Following place field expansion, the rate of change of firing phase with position is reduced, while the net change in phase remains at nearly 360°. These observations have further implications for the possible mechanisms underlying the phase precession phenomenon. For example, Tsodyks et al. (1996) developed a hypothesis that accounted for phase precession on the basis of asymmetry in the synaptic connectivity matrix. The basic idea was that, as global excitability rises at the beginning of each theta cycle, the first cells to fire are those with strong external inputs. The asymmetric connections from these cells to the next ones in the sequence of place representations cause the latter cells to begin firing before the rat actually reaches the point where they would be triggered by external inputs; hence, firing begins late in the theta cycle, and this firing reflects the associative recall of the next elements in the sequence. At the end of the cycle, network excitability falls, thus allowing the representation to revert to the rat's current position in the sequence. The same in-

crease in the asymmetry parameter of the synaptic connections that predicts place field expansion also predicts that rate of change of firing phase with position should be slowed, as observed here. This supports the idea that asymmetry may contribute to the mechanism of phase precession in the same manner that it could contribute to place field expansion. Previous results (Rosenzweig et al., 2000, Soc. Neurosci., abstract), however, suggest that phase precession is present on the first experience of a novel route. Thus, either some intrinsic connection asymmetry must be present in the network prior to the initial experience of the route, or a more complex mechanism would be required to explain phase precession in its entirety (e.g., Burgess et al., 1994; Kamondi et al., 1998; Yamaguchi and McNaughton, 1998).

The results of the present study also have implications for understanding memory retrieval deficits that accompany normal aging. The dynamics of phase precession are altered in old rats (Shen et al., 1997), in a manner similar to the CPP-injected rats in the present study. Old rats also exhibit deficits in both place field expansion and in the maintenance of stable distributions of place fields even in highly familiar environments that have been experienced a short time previously (Barnes et al., 1997). A similar instability of place field distributions occurs in CPP-injected rats (Kentros et al., 1998).

Together with the present data, these results support the conclusion that deficits in $NMDA_R$-dependent LTP could be responsible for the frequent failure of old rats to retrieve the place field pattern previously allocated to a given environmental and behavioral context, a phenomenon that might contribute to poorer spatial and episodic memory in aged animals, including humans.

Experimental Procedures

Experiments

Six Fischer-344 rats (age: 13–16 months) were pretrained to run for food reinforcement at a fixed location on a rectangular or circular track. Populations of hippocampal CA1 pyramidal cells were simultaneously recorded using a "hyperdrive" consisting of 14 independently moving probes, 12 of which were "tetrodes" used for unit recording, one served as a reference, and one was used for EEG recording (see Gothard et al., 1996, for details). The positions of two head-mounted diode arrays were tracked at 60 Hz by a video camera. Each recording session included 15 min of prebehavior sleep or quiet waking (in a towel-lined pot located next to the track), followed by a minimum of 19 unidirectional laps with one or two food rewards allowed at consistent locations on the track. The rat was then immediately placed back into the pot for a final 30 min sleep/quiet rest session. For both control (saline) and drug (3.5 mg/kg d-CPP) recording sessions, the injection was given 30 min prior to the initial 15 min "sleep" session; the rat remained in the pot for that time. The dose of CPP was selected on the basis of previous behavioral testing in two rats as the amount that produced clear memory impairment without obvious motor impairment. The rats were tested on a reference memory version of the radial eight-arm maze in which only one arm was baited on any given day, and this arm was chosen randomly at the start of each day. The objective of the task was to locate this one arm to obtain food reward, and the criterion of success was three correct choices in a row. Lower doses of CPP (5.0 mg/kg d,l CPP = 2.5 mg/kg D-CPP) than that used here have been shown to abolish (Morimoto et al., 1991) or greatly attenuate (Wayner et al., 2000) LTP in vivo.

Analysis

In order to identify significant place-specific cell firing, the criteria of Muller, Kubie, and Ranck (1987) were used. Significant place fields were identified when the average firing rate of a given pyramidal cell was above 1 Hz for more than nine contiguous pixels over the track (which was divided into 64 bins). After Gaussian smoothing, the two-dimensional tracker coordinates were then projected onto one dimension corresponding to the center of the track. The two-dimensional firing distributions were used to define place fields (Muller et al., 1987), which were then analyzed one-dimensionally. The one-dimensional field sizes were defined as the area bounded by the points where firing rates fell to less than 10% of the peak rate for at least 10 cm. Running speed was determined from the two-dimensional data. The relationship between speed and firing rate was assessed by computing the number of spikes at a given speed (5 cm/s bins) divided by the time spent at that speed. Overall firing rate was determined by the total number of spikes fired in an epoch divided by the epoch length. Cells that had >5 Hz firing rate were assumed to be interneurons and were not included in this analysis.

The qualitative place fields in Figure 1 were computed by centering all place fields on their center of mass and dividing each place field by its mean firing rate within the field, so that place fields with higher firing rates would not dominate in the analysis. The median firing rate at each location was then computed separately for drug and control conditions, as is shown in Figure 1. Median rather than mean was used to preserve skewedness in the place fields more accurately by minimizing the dominance of large fields.

Changes in place field characteristics over laps were assessed as follows. Fields with low spike counts (<1 Hz) were first excluded from the analysis (Mehta et al., 1997). Place field intensity was defined operationally as the total number of spikes recorded within place field boundaries on a given lap, which is simply the number of spikes that occurred in the lap-specific place field. The center of mass was determined by taking the weighted average of firing rate and spatial location. In order to define a "center" properly, only cells with two or more spikes on a lap occurring on two or more laps were included in the position analysis. Firing rate on a lap-by-lap basis was determined by dividing the firing rate on a given lap by the mean relative running speed (which is the mean running speed divided by the running speed on the first lap) in each condition. The lap-specific place field integral was calculated by summing the lap-specific firing rates within place field boundaries (see Mehta et al., 1997).

For the theta modulation and phase precession analysis, one rat was excluded because its EEG electrode showed no noticeable theta modulation and was therefore presumably not located appropriately. For another rat, the EEG electrode exhibited similar low-amplitude theta; however, an adequate theta signal was obtained from a tetrode near the cell body layer. Because this electrode's signal was offset $\sim 60^\circ$ relative to the peak firing rate for theta modulation recorded in the hippocampal fissure, the theta phase for this electrode was corrected by 60° to be aligned with that from the other rats. Theta phase was defined in terms of the fraction of the period between two successive above-threshold peaks of the 6–10 Hz band pass filtered data. Intervals less than 75 ms and greater than 225 ms were excluded. Theta power was defined as the square of the amplitude of the filtered EEG and frequency as the reciprocal of the periods as defined above. Data for which running speeds on the track were less than 10 cm/s were excluded from the analysis in order to prevent contamination with non-theta activity (LIA).

Acknowledgments

We thank Laleh Quinn for conducting the initial dose-response behavioral experiments, A.D. Redish and Peter Lipa for useful discussions and assistance with MatLab analysis routines, and F. Battaglia for helpful comments on the manuscript. This work was supported by AG12609, MH01565, NS20331, and an IGERT training grant from NSF.

Received April 25, 2001; revised August 8, 2001.

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