Hippocampal Neuron Changes During Trace Eyeblink Conditioning in the Rabbit

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The hippocampus is thought to be required for the storage of most new cognitive memories in the human. Dramatic evidence for this came from the initial description of the profound amnesia exhibited by the patient HM after bilateral neurosurgical removal of most of the hippocampal formation, amygdala, and associated temporal cortex (Scoville and Milner, 1957). An important feature of temporal lobe amnesia is that patients (such as HM) suffering from it have intact recall of information that was learned and consolidated prior to the trauma to their temporal lobes. In addition, if allowed without distraction to rehearse material containing a limited number of bits of new information, patients can demonstrate recall successfully (Squire, 1987). Their difficulty is in transferring newly learned information from the immediate- or short-term store to long-term storage sites. Further important support for the role of the hippocampus in forming long-term memories came with the description of patient RB, who suffered neuropathologically confirmed bilateral destruction of only his hippocampal CA1 pyramidal neuron region after an ischemic episode (Zola-Morgan, Squire, and Amaral, 1986). RB showed the same type of amnesia as that shown by HM, although it was somewhat less severe, apparently owing to the fact that the neural damage was more limited.

There has been significant effort during the past 40 years (extensively reviewed elsewhere: Cohen and Eichenbaum, 1993; Squire, 1987) to use animal models to explore the mechanisms by which the hippocampal system stores new information in the mammalian brain. The literature covering this research is not only extensive but complicated, as many apparently contradictory results have been reported, but from it a few points may be distilled relevant to the material we will discuss here. First, the lesion method often has been used to determine whether the hippocampus is required for the learning of a particular task (i.e., if the task is hippocampally dependent). This method has the acknowledged limitation that a demonstration of the effect of a lesion on the ability of an animal to learn a task merely indicates that the region under study is a necessary component of the brain circuitry that mediates learning the task. A positive effect of a lesion cannot be interpreted necessarily as indicating that a brain region such as the hippocampus shows
localized changes during learning. Second, the kinds of learning tasks sensi-
tive to hippocampal lesions in animal studies are higher-order tasks requiring
the animal to integrate spatial information (O'Keefe & Nadel, 1978), to use
previously learned information in a flexible fashion (Sutherland and Rudy,
1989; Cohen and Eichenbaum, 1993), or to integrate information temporally
(Solomon, 1979; Meck, Church, and Olton, 1984; Moyer, Deyo, and Dister-
hoft, 1990). Third, in several situations, the hippocampus has shown well-
defined neurophysiological or neurochemical changes during learning, even in
tasks that do not require the hippocampus for successful acquisition (Olds,
Anderson, McPhie, Staten, and Alkon, 1989; Segal, 1973; Berger, Alger, and
Thompson, 1976). This could reflect the fact that the hippocampus may store
information as a parallel processor, even when it is not required for successful
acquisition of a learned task being adequately handled by neural circuits in
other brain regions.

Our laboratory has chosen eyeblink or nictitating membrane conditioning
in the rabbit as a behavioral model system (Thompson, Berger, Cegavske,
Patterson, Roemer, Teyler, and Young, 1976; Disterhoft, Kwan, and Lo, 1977)
for exploring the cellular mechanisms by which hippocampal neurons become
engaged during the learning process. The eyeblink conditioning model has
several advantages for a program such as ours. Rabbits accept restraint readi-
ly, facilitating conditioned (CS) and unconditioned stimulus (US) application
and measurement of behavioral responses (Akase, Thompson, and Disterhoft,
1994; Thompson et al., 1994a), intravenous or intraventricular drug applica-
tion (Deyo, Straube, and Disterhoft, 1989; Thompson, Moskal, and Dister-
hoft, 1992), and single-neuron recording during and after conditioning
(Kraus and Disterhoft, 1982; Disterhoft, Quinn, Weiss, and Shipley, 1985;
Weiss, Kronforst, Thompson, and Disterhoft, 1993; Weiss, Kronforst, and
Disterhoft, 1994). The behavioral parameters of eyeblink conditioning in the
rabbit are low in variance and have been well characterized (Gormezano,
Prokasy, and Thompson, 1987). Eyeblink conditioning was originally designed
by experimental psychologists as a well-defined task for investigating the
laws of learning in humans and was subsequently adapted for the rabbit
(Gormezano, 1966). In recent years, we and other groups have begun to
experiment with human subjects to evaluate hypotheses generated from the
animal work (Woodruff-Pak, 1988; Solomon, Beal, and Pendlebury, 1988).
Thus, we have considerable confidence that insights into the neurobiological
substrates of learning possibly gained from experiments done in rabbits may
be generalized to the human (e.g., McGlinchey-Berroth, Cermak, Carrillo,
Armfield, Gabrieli, and Disterhoft, 1995; Gabrieli, McGlinchey-Berroth,
Carrillo, Gluck, Cermak, and Disterhoft, 1995).

An issue of some relevance for us is our rationale for using the eyeblink
conditioning paradigm to study the involvement of the hippocampus in
associative learning. The crux of the issue is that, although hippocampal
multiple and single neurons have been shown definitively to alter their activ-
ity in a conditioning-specific fashion during delay eyeblink conditioning (tone CS precedes and overlaps the air puff US: Berger, Alger, and Thompson, 1976; Berger, Rinaldi, Weisz, and Thompson, 1983), hippocampectomized rabbits can readily acquire this version of the task (Schmalz and Theios, 1972; Akase, Alkon, and Disterhoft, 1989). In addition, lesions of the cerebellar dentate-interpositus will eliminate ipsilaterally conditioned eyeblink responses that have been acquired previously and make it impossible to acquire new eyeblink conditioned responses (CRs) on that side (Thompson, 1990; Steinmetz, Lavond, Ivkovich, Logan, and Thompson, 1992; however, see Welsh and Harvey, 1989, for a differing view). This has led some neuroscientists to view eyeblink conditioning as a cerebellar or brainstem-mediated task and not an appropriate behavioral model to use for investigating the involvement of forebrain structures such as the hippocampus in learning.

Our strategy for dealing with these issues has been to use a version of the eyeblink task that adds a temporal requirement to the behavioral association. A short tone CS is paired, after a 500-ms interval, with the air puff. Conceptually, the animal must retain a "stimulus trace" that the CS has occurred at a specific point so as to blink its eye at a time that minimizes the impact of the air puff on the cornea. This variant of eyeblink conditioning is termed trace conditioning, after the terminology of Pavlov (1927). Trace eyeblink conditioning has been demonstrated to be dependent on the hippocampus for acquisition in the rabbit with a 500-ms trace interval (Solomon, Vander Schaaf, Thompson, and Weisz, 1986; Moyer, Dyo and Disterhoft, 1990). Our paradigm with a short CS (100 ms) is especially sensitive to hippocampal damage. We found that hippocampectomized rabbits did not acquire the trace eyeblink response even when trained for 25 successive 80-trial sessions (Moyer et al., 1990). These data indicate empirically that the addition of the long trace period (especially after a short CS, which makes it more difficult to time the CR successfully), makes the contribution of the hippocampus obligatory to form the association. This is consistent with the general concept that the hippocampus is required when animals are learning higher-order tasks that necessitate using information in a novel fashion or to form temporal maps of the environment (Solomon, 1979; Cohen and Eichenbaum, 1993).

In this chapter, we will review our recent experiments using the hippocampally dependent trace eyeblink conditioning task in the albino rabbit. These studies have focused on exploring how single hippocampal neurons change their firing patterns during and after trace conditioning with in vivo recording of single neurons in the behaving animal. They also have focused on determining the involvement of different isoforms of protein kinase C in neuronal changes during learning by using immunocytochemical and biochemical approaches. Finally, they have focused on characterizing the fashion in which postburst afterhyperpolarization (AHP) and spike-frequency accommodation both change in CA1 and CA3 pyramidal neurons during the time at which the trace eyeblink CR is being consolidated (Kim, Clark, and Thompson, 1995).
SINGLE-NEURON RECORDING STUDIES

A well-known series of multiple- and single-unit recording studies were done by Berger, Thompson, and their colleagues during and after delay eyeblink conditioning (Berger and Thompson, 1978; Thompson, Berger, Berry, Hoehler, Kettner, and Weisz, 1980; Berger and Weisz, 1987). They first demonstrated that multiple units in the hippocampus showed enhanced firing very early in the trial sequence, well before rabbits began to show eyeblink CRs (Berger et al., 1976). The altered firing occurred first during the unconditioned response (UR) period of the trial, then moved forward in time and began to occur as the CR was being elicited. In many multiple-unit recordings from CA1 and CA3 hippocampus, the overall shapes of the poststimulus time histogram and of the averaged behavioral response were very similar. Berger and Thompson demonstrated that this temporal modeling was especially prominent in the CA1 and CA3 output regions of the hippocampus. In a subsequent single-neuron study, this group demonstrated that the neural models in hippocampus occurred in pyramidal neurons in the CA1 and CA3 (Berger et al., 1983). They also reported that 83 percent of the hippocampal pyramidal neurons recorded after delay eyeblink conditioning demonstrated altered firing rates at some point within the trial period (i.e., during the CS or US periods).

Permeating the literature from the important series of studies by Berger and Thompson is an overall impression that the majority of neurons in the hippocampus change during eyeblink conditioning, even though this structure is not required for successful acquisition of this version of the task (Schmaltz and Theios, 1972; Berger and Orr, 1983; Akase et al., 1989). Because the "neural modeling" figures have been so widely reprinted, another impression exists: that a large number of neurons demonstrate such a dramatic response during eyeblink conditioning. We sought to reevaluate the percentage of single neurons showing this phenomenon. We were intrigued by the fact that almost all the alterations in pyramidal neurons reported by Berger and Thompson were excitatory, with a relatively small number of changes in the inhibitory direction reported. We also wanted to determine the pattern of single-neuron activity alterations when recordings were done during trace eyeblink conditioning, a hippocampally dependent version of the task. Finally, we set out to sample hippocampal single-neuron activity pattern changes with indwelling electrode arrays, which might record from a broader range of neurons than the sharpened tungsten electrodes inserted on the recording day by Berger et al. (1983) in their single-neuron recording study. This is particularly important given the high percentage of "silent" and slowly firing neurons which have been reported for the hippocampus (Berger et al., 1983; Thompson and Best, 1989).

Therefore, we undertook a series of studies using a moveable array of microwires for recording (Kubie, 1984). The DataWave (formerly BrainWave) computerized waveform separation system was used to isolate single-
neuron activity from the wires. The conditioning task involved pairing a brief tone CS (6 kHz, 100 ms, 85 dB, 5-ms rise and fall time) with an air puff US (150 ms, 3 psi), after a 500-ms stimulus-free trace period. The CS and US presentations were controlled, and the behavioral data was taken with a computerized data acquisition system (Akase et al., 1994; Thompson et al., 1994a). Conditioned rabbits were trained with paired presentations of tones and air puffs. Control rabbits were presented with randomly presented, unpaired tones and air puffs to ensure that the changes were associative and not related to pseudoconditioning or sensitization. The data we will summarize here were gathered during and after behavioral learning had occurred.

Our observations suggest that a smaller percentage of neurons are related to CRs during trace than during delay conditioning. We observed many well-isolated but slow firing “silent cells” that were engaged during trace eyelink conditioning and that would have been difficult to study with previously used recording techniques. The same number of neurons exhibited decreased as exhibited increased firing rates during various temporal epochs of the conditioning trial. The most frequent response that we observed during the posttrial period (2-s window after a 500-ms UR period) was inhibition. This response appeared to be nonspecific, because it was equally likely to occur during conditioning as during pseudoconditioning. Finally, both excitatory and inhibitory neuron response changes during the trial period occurred.

We have recently completed recordings from 220 neurons in 11 conditioned rabbits (Weiss, Kronforst, Thompson, and Disterhoft, 1993, Weiss, Kronforst, and Disterhoft, 1994; 93 of those neurons were identified as CA1 pyramidal cells and 4 (plus 3 probable) based on the spike rate and width criteria accepted for use in these studies (Ranck, 1973; Berger et al., 1983). Surprisingly few theta cells were recorded in this sample, in agreement with Jung, Wiener, and McNaughton (1994) who recorded from rat hippocampus with similar techniques. Statistical criteria were used to define individual neurons as demonstrating excitation or inhibition during a set of trials (40 or 80 trials) as compared to neuronal firing rate during a comparable background period just before the time of CS onset. An analysis of the CA1 pyramidal cells indicated that 58 percent of the cells recorded from rabbits showing at least 60 percent CRs had significant changes in firing rate during the trial. Another 13 percent of the cells also had a significant change in their firing rate, but only during the posttrial period (2 s after a 500-ms UR period). Significant changes during the trial period occurred for all three periods (CS, trace, and UR). During the tone CS, 23 percent of the CA1 pyramidal neurons changed their firing rate. Excitation predominated, but inhibition did occur (14 percent versus 9 percent). During the trace period, 28 percent of the cells had a significant change in firing rate with both excitation and inhibition noted (11 percent versus 17 percent). During the UR period, excitation and inhibition were noted equally, with 47 percent of the cells showing a significant change in firing rate. Approximately
40 percent of the cells had a baseline firing rate of 0.5 Hz or less. Many of these silent CA1 pyramidal cells (N = 34) responded in 5 or fewer of the 20 bins within each analysis period. Those cells were analyzed with the nonparametric binomial test. During the trial period, 12 percent of those slow firing cells had a significant change in firing rate and another 8 percent changed their firing rate during the posttrial period. An approximately equal number of neurons responded both in the 2-s posttrial time period during conditioning and pseudoconditioning trials. This indicates that the posttrial responses, most of which were inhibitory, were not specific for learning the association between tone and air puff.

We did not see the pattern of responses we had anticipated based on the previously published data from delay conditioning (Thompson et al., 1980; Berger et al., 1983). As mentioned, the total number of neurons that changed during the trial period was 58 percent; this number increases to 85 percent if the silent cells are omitted from the analysis. Regardless of whether the silent cells are included, as many of the changes were excitatory as were inhibitory. Figure 7.1 shows as good an example of a "neural model" of the CR as we observed in our recordings during trace conditioning. Because these responses were relatively infrequent, we trained some rabbits in the short-delay paradigm, using a CS which overlapped and coterminated with the US after a 250-ms interstimulus interval as Berger et al. did (1983). As can be seen in figure 7.2, we observed robust neural modeling of the CRs and URs by CA1 pyramidal neurons similar to that which had been reported previously by Berger, Thompson, et al. in both multiple-unit and single-unit recording studies.

Our recordings were made from rabbits that showed at least 60 percent CRs during trace eyeblink conditioning sessions. When we compared activity patterns of the same neurons on trials in which rabbits exhibited CRs and on those where no CR was given, interesting differences were seen in the overall patterns of responses (figure 7.3). Pyramidal neurons simultaneously recorded from nearby electrodes showed a burst of activity during the trace interval and a second burst in correlation with the UR. These responses were distributed more evenly and were considerably larger (on average) across the trial period on CR trials than on interspersed trials in which no CR was present. It was clear that hippocampal neuron activity patterns differentiated CR from non-CR behavioral responses.

One other interesting response pattern might be mentioned. Some presumably inhibitory interneurons showed a temporal model of the CRs and URs sculpted from a high baseline firing rate (figure 7.4). These were termed inhibitory theta cells by Berger et al. (1983). The neuron illustrated had a relatively high background firing rate, then reduced its firing rate sharply at or shortly before the beginning of the CR and the UR.

In summary, comparisons of single-unit recordings from rabbits trained in a delay or trace eyeblink conditioning paradigm indicate that neurons recorded in the trace paradigm exhibit less robust but more diverse responses than
...less. Many of the CRs, however, involved fewer of the conditioning prepotent behaviors with the conditioning behavior than did those slow to evident CRs. Thus, approximately 8 percent of the CRs had a prepotent period during which the conditioning behavior was evident. The posttrial period was marked by the same pattern for learning the task...

This study was conducted based on the methodology described by Maren et al., 1980; in which the animals were trained to respond to an US that changed from a tone to a shock. The animals were trained to 85 percent if the tone was not changed. The silent periods after the US were inhibitory. The difference score between the CR as we measured it and the URs of these responses before the training was performed on a short-delay paradigm. The USs were presented after the US after a variable interstimulus interval. The USs can be seen in the figure as the shaded areas and the shaded URs by CA1 CA1 hippocampal area neurons. The previously by CA1 and CA3 hippocampal area neurons were unit recording before and during training.

The data from at least 60 percent of the neurons showed increased activity for the CRs compared to the URs. The increased CRs and URs were evident in the overall activity of the neurons. The previously recorded activity of the neurons during the trace interval was typically low, and the activity was distributed across the trial period. During the US presentation, the activity was present. It is interesting to note that prepotent CRs and URs were detected in some neurons. These neurons were termed prepotent because the CR occurred more rapidly and was stronger than the URs.

Some presumptive information about the prepotent CRs and URs was obtained. For example, the animals trained in a water maze were able to perform on the memory test of the prepotent behavior more quickly than on the US-alone test of the prepotent behavior.
Figure 7.2 The top panel shows a raster display of activity from two single neurons recorded from the same microwire during a delay conditioning session with 97 percent of CRs. A plot of the average behavioral data is shown by the top line in this panel. The rasters for the two cells are separated by a horizontal line. (Note that the first cell responds during very few trials.) Histograms of the activity for each of the two cells are shown below the rasters with an inset which portrays the shape of the average action potential (± 1 standard deviation). The timing and duration of the tone and air puff are indicated at the bottom of the figure.
Figure 7.3 Example of activity from the same set of five neurons during a trace conditioning session with 70% of CRs. The data are separated into trials with and without CRs. Histograms from two of the five neurons are presented. The firing rate per 10-ms bin is indicated to equate for the difference in the number of trials: 20 CRs, 11 nonCRs. (Note the responses to the air puff during non-CR trials.) The responses have greater "signal to noise" during CR trials, especially during the trace period. Time of tone CS and air puff US presentation are indicated at the bottom of the figure.

Two single neurons were recorded at 97% of CRs. The traces are shown in the upper panel. The rasters show the neuronal activity, and the firing rate per 10-ms bin is shown below the rasters. The mean and standard deviation (±1 standard deviation) are shown at the bottom of the figure.
Figure 7.4 Data from an inhibitory interneuron recorded from CA1 during a trace conditioning session with 100 percent of CRs. The top panel shows several superimposed waveforms that characterize this cell. The second panel indicates a raster of the activity recorded from this cell in relation to the tone and air puff. A histogram of the summed activity in the raster is shown in the third panel just above the time-line for the tone CS and air puff US. Tick marks are every 200 ms, and time is measured from tone onset. The bottom panel shows the average behavioral response for this rabbit during the session. (Note the dramatic decrease in firing rate after tone onset with excitation superimposed on the inhibition.) The inhibition persists for approximately 500 ms after US onset.
those recorded in the delay paradigm. This may reflect more complex processing by specific neurons in hippocampally dependent trace conditioning as compared to a generalized activation of most neurons in the delay paradigm. The prolonged changes in the posttrial period may represent consolidation processes that occur during the intertrial interval or nonspecific changes, because similar changes were seen after both paired and unpaired trials. It will be necessary to record from single neurons over the course of trace conditioning to determine if the changes during and after the trial period occur as early in the trial sequence as they have been reported to occur in delay conditioning. It also will be of interest to determine if the percentage of neurons which are significantly related to different portions of the CR increases or decreases during training.

ALTERATIONS IN PROTEIN KINASE Cγ

The protein kinase C (PKC) family consists of at least 10 different isoforms, of which 4 (α, βI, βII, and γ) are calcium-dependent (Nishizuka, 1988). PKC is a cellular second messenger regulated protein involved in neuronal signal transduction pathways by which neurons increase their excitability in response to external inputs (Nishizuka, 1986). PKC activation may serve as a critical step in the chain of biologic events leading to memory formation. PKC activity has been shown to be translocated from the cytosol to the membrane 1 hr after the induction of long-term potentiation (Akers, Lovinger, Colley, Linden, and Routtenberg, 1986), but the data regarding translocation of total PKC from the cytosolic to the membrane fraction in hippocampus 24 hr after delay eyeblink conditioning are inconsistent (Bank, DeWeer, Kuzirian, Rasmussen, and Alkon, 1988; Sunayashiki, Lester, Schreurs, and Alkon, 1993). Evidence for a role of PKC in associative learning has been observed in the rabbit hippocampus after delay eyeblink conditioning (Olds et al., 1989) and in the rat hippocampus after swimming-maze learning (Olds, Golskie, McPhie, Olton, Mishkin, and Alkon, 1990). Increased immunoreactivity for PKCγ has been found in mouse hippocampus after learning a spatial food retrieval task (Van der Zee, Compaa, de Boer, and Luiten, 1992). It is known that application of phorbol esters onto hippocampal slices, which activates PKC, also increases excitability of hippocampal pyramidal neurons by reducing the postburst AHP (Baraban, Snyder, and Alger, 1985). Phorbol ester stimulation of brain slices mimics the increased immunoreactivity for PKCγ as observed after learning (Van der Zee, Strossberg, Bohus, and Luiten, 1993). As will be shown in a following section, hippocampal pyramidal neurons show increased excitability, as evidenced by reduced AHPs, after trace eyeblink conditioning. We sought to determine whether hippocampally dependent 500-ms trace eyeblink conditioning induced changes in the immunoreactivity for calcium-dependent PKC isoforms (Van der Zee, Kronforst, and Disterhoft, 1994). Such altered immunoreactivity could reflect cellular changes that modulate neuronal activity in the hippocampus during learning and memory processes.
Figure 7.5 Photomicrographs depicting the changes in PKCβ immunoreactivity (regulatory domain, A–D; catalytic domain, E–H) in the hippocampal CA1 pyramidal cells of naive (A, E), pseudoconditioned (B, F), and trace-conditioned (C, G) rabbits. A significant increase in staining intensity in the cell bodies and apical and basal (small arrows in C, G) dendrites was found only after trace conditioning, although a few cells showed enhanced staining for PKCβ after pseudoc conditioning (large arrows in B, F). Fluorescence double-labeling demonstrated that the changes for both antibodies raised against different parts of the PKCβ protein occurred within single cells at the same cellular compartments (arrows in D, H). Scale bar in A–C and E–G = 20 μm; in D and H = 30 μm.

Young adult rabbits were trained to a criterion of 80 percent of CRs in an 80-trial training session in the hippocampally dependent 500-ms trace eyeblink conditioning task as described above. The rabbits were sacrificed 24 hr after reaching behavioral criterion. Brain sections were prepared and immunostained with polyclonal antibodies raised against the catalytic subdomain of the calcium-dependent isoforms α, βI, βII, and γ, and a monoclonal antibody raised against the regulatory subdomain of PKCβ. The occurrence of translocation, splicing of PKC protein into the single catalytic and regulatory domain, and the total amount of PKC were determined by Western blotting. Hippocampal slices were prepared and stimulated with phorbol ester so as to study the relations among activated PKC, the degree of immunostaining, and Western blot results.

The immunoreactivity for PKCβ was markedly enhanced in the hippocampus after conditioning for both the catalytic and regulatory subdomain as compared to pseudoconditioning (unpaired presentations of the tone CS and air puff US) and naive control rabbits (see figure 7.5). Fluorescence double-labeling (figure 7.5 D–H) showed that the increase was found in individual neurons, supporting the assumption that the increase for both antibodies reflects changes in single PKCβ molecules, both at the level of catalytic and
PKCgamma

optical density

region of interest

- naive
- pseudo
- trace

DG CA3 CA1 Sub Hyp

Figure 7.6 Optical density measures for PKCgamma immunoreactivity in the principle cells of the dentate gyrus (DG), CA3, CA1, subiculum (Sub), and hypothalamus (Hyp) of naive (n = 7), pseudoconditioned (n = 7), and trace-conditioned (n = 7) rabbits. A significant increase (p < .05) in optical density was found in the DG, CA3, and CA1 in trace-conditioned as compared to naive and pseudoconditioned animals. No changes were found in the subiculum or hypothalamus.

regulatory subdomain. No gross changes were observed in immunoreactivity for PKC\(\gamma\), \(\beta I\), or \(\beta II\) in the three conditions. The optical density of the immunoreactivity for all PKC isoforms was determined, and conditioned animals revealed an approximate 2.5- and 1.8-fold increase in staining intensity in CA1 pyramidal cells for both PKC\(\gamma\) antibodies over naive and pseudoconditioned animals, respectively (figure 7.6). The change for PKC\(\gamma\) was found in all hippocampal subregions but the subiculum (figure 7.6). The hypothalamus, serving as a control region, showed no changes for any PKC isoform. Western blotting indicated that the total amount of PKC\(\gamma\) (both catalytic and regulatory subdomain) did not change after conditioning; nor was there translocation or splicing of PKC\(\gamma\) after conditioning as compared to the control conditions. An identical increase in PKC\(\gamma\) immunoreactivity was seen in the phorbol ester-stimulated hippocampal slices as compared to nonstimulated slices. Western blots of such slices revealed translocation from the cytosol to the membrane for PKC\(\gamma\), whereas no translocation occurred for PKC\(\gamma\).

Our data suggest that PKC\(\gamma\) is the crucial calcium-dependent PKC isoform involved in the signal transduction pathways which alter cellular excitability...
in the hippocampus during learning. Translocation of PKC, seems not to be a crucial aspect of PKC, functioning, a finding in agreement with the literature (Oda, Shearman, and Nishizuka, 1991). Because the total amount of PKC, is unaltered and there is no detectable translocation or splicing of this PKC isoform, we conclude that a change in protein-protein interaction or a conformational change within the PKC, protein determines the degree of immuno-reactivity which becomes evident after conditioning. This change must affect both subdomains of the PKC, molecule but leaves it intact from splicing. A corollary to this assumption is that this change in the protein could well be the critical cellular change that occurs during learning and contributes to the altered excitability which we observe both in vivo and in vitro after hippocampally dependent trace eyeblink conditioning.

POSTSYNAPTIC EXCITABILITY CHANGES AFTER LEARNING

Recordings of multiple and single neurons in the hippocampus during and after the eyeblink conditioning process show dramatic alterations in firing rate during and after learning. We have reviewed some of the experiments which demonstrate this in delay eyeblink conditioning (Berger et al., 1976, 1983), and we have demonstrated some of our own observations showing large firing rate changes in single CA1 pyramidal neurons after trace eyeblink conditioning (Weiss et al., 1993, 1994). During and after learning, hippocampal neurons increase their firing rate during the trial period (i.e., to the tone presentation) during the CS-US interstimulus interval, or after the air puff US presentation. In both delay and trace eyeblink conditioning, these firing rate alterations can be shown to be conditioning-specific, as they occur only occasionally to the tone CS or the air puff US when they are presented in a random, unpaired fashion during pseudoconditioning. Examination of the histograms shown in figures 7.1 to 7.4 indicate that a large percentage of hippocampal neurons exhibit substantial increases in excitability (evidenced by increased firing rates) during and after conditioning.

Our challenge is to begin to offer an explanation at the cellular or membrane level for these dramatic changes in firing rate which we can record with extracellular recording electrodes. A problem inherent in interpreting firing rate changes recorded in vivo, no matter how dramatic, is to localize the change to a specific set of cells. The interconnections between neurons in the brain are known to be substantial. In fact, there is such extensive connectivity within the brain that it is said that any one point is connected to all other points in the brain by some pathway or combination of pathways. Thus, it becomes extremely difficult to determine if an alternation in firing rate recorded from a CA1 pyramidal neuron is localized to that neuron (i.e., it is hard to know if the change in firing rate represents an alternation in the firing characteristics of the neuron under study or of the strength of synaptic weights onto that neuron). Serious consideration must be given to the possibility that the alternation in firing rate recorded from a single neuron in vivo
may rather be projected from one of many other places in the brain and expressed secondarily by the CA1 neuron under study.

Localization of learning effects has been approached within in vivo recording situations with a detailed analysis of the timing of alterations between successive sites in a portion of the circuitry involved in the conditioned association (Olds, Disterhoft, Segal, Kornblith, and Hirsch, 1972; Woody, 1974). Our own approach has been somewhat different. We decided to train rabbits, then remove the hippocampus for in vitro analysis. We reasoned that any conditioning-specific alterations detected in this situation cannot be a secondary consequence of changes projected from elsewhere in the brain. Because all normal afferent and efferent connections are severed in the preparation of hippocampal slices, the alterations which are present must be a reflection of changes present within the hippocampus.

The most prominent alteration detected using this approach to date has been a postsynaptic excitability change within CA1 pyramidal neurons (i.e., a reduction in the postburst AHP (Disterhoft, Coulter, and Alkon, 1986). The reduction in the postburst AHP after learning has been shown to be conditioning-specific, to be dependent upon acquisition of the conditioned eyeblink response, and to be present in CA1 neurons even when sodium-dependent synaptic transmission was blocked by tetrodotoxin (TTX) and tetraethylammonium (TEA) (Disterhoft, Golden, Read, Coulter, and Alkon, 1988; Coulter, LoTurco, Kubota, Disterhoft, Moore, and Alkon, 1989; de Jonge Black, Deyo, and Disterhoft, 1990). The AHP has been well-characterized by others and is known to be a reflection of an outward potassium current which is activated by the influx of calcium into the neuron during the burst of action potentials (Hotson and Prince, 1980; Lancaster and Adams, 1986). The AHP is one postsynaptic mechanism to control a neuron’s readiness to fire action potentials. A reduction in the AHP brings the membrane potential to a relative state of depolarization sooner after a burst of action potentials, thus allowing more activity to follow. We have suggested that a reduction in the AHP may be one mechanism contributing to the tendency for neurons to be more excitable (i.e., to fire more action potentials) during conditioning trials (Disterhoft et al., 1986).

The experiments we will summarize in this chapter have extended our earlier experiments in several ways. We have used the hippocampally dependent trace eyeblink conditioning paradigm, rather than delay conditioning, to train the rabbits. We have examined the time-course of retention of the excitability changes after rabbits reach a learning criterion, so as to determine how long-lasting the excitability changes are and to detect any potential relation of the excitability changes to the consolidation of the learned behavioral response that is occurring during the period after acquisition occurs. We have demonstrated an alteration in spike frequency accommodation, another measure of cellular excitability related to the postburst AHP, after conditioning. We have examined CA3 pyramidal neurons to determine if they are altered after conditioning in a fashion similar to that of changes observed in CA1 neurons.
Rabbits were trained in the 500-ms trace eyeblink conditioning paradigm or served as pseudoconditioning controls (Moyer et al., 1990). Hippocampal slices were prepared at various intervals after learning to a criterion of 80 percent of CRs in a training session (Moyer, Thompson, Black, and Disterhoft, 1992). Intracellular current-clamp recordings were performed on more than 200 CA1 and 190 CA3 pyramidal neurons in slices taken from rabbits at time intervals ranging from 1 hr to 14 days after behavioral acquisition to criterion, so as to identify cellular changes occurring during memory consolidation. Strict neuronal stability criteria were used, including resting membrane potentials less than −60 mV and input resistance greater than 30 MΩ. Recordings were made at resting membrane potentials between −65 and −70 mV to control for voltage-dependent effects. Cells were injected with sufficient depolarizing current to elicit a burst of four action potentials for the purpose of studying hippocampal excitability. The amplitude, integrated area, and duration of the postburst AHP were measured.

Postburst AHPs were significantly reduced in both CA1 and CA3 neurons after acquisition of hippocampally dependent trace eyeblink conditioning (figures 7.7 and 7.8). The AHPs were significantly reduced in cells from slices prepared as early as 1 hr after learning (earliest interval tested), were maximally reduced by 24 hr after learning, and slowly decayed over a 1-week time period (figures 7.7 and 7.9). The effect was substantial, with more than 75

Figure 7.7 Acquisition of hippocampally dependent trace eyeblink conditioning transiently reduced the postburst AHP. A. Voltage trace shows an overlay of recordings of the postburst AHPs in CA1 neurons from a naive rabbit (Naive) and from trace-conditioned rabbits studied 24 hr after initial learning (Trace 24 hr) or 24 hr after receiving an additional training session given 14 days after initial learning (Retention). B. Learning-related reductions of the AHP amplitude were transient, lasting about 1 week in slices prepared at various times after learning (1 hr, 0 days; 1 day, 3 days, 5 days, 7 days, 14 days). Numbers in parentheses indicate the ratio of cells with reduced AHPs to number of cells studied (an AHP was considered reduced if its amplitude was more than 1 standard deviation less than the mean for all naive control CA1 cells). Similar numbers of cells were obtained per rabbit in both control and trace-conditioned rabbits, which were counterbalanced to minimize cohort effects. Asterisks indicate data significantly different from all three control groups. N, naive; P, pseudoconditioned; S, slow learners; R, retention; *, indicates p < .001. Slow learners were defined as rabbits that did not reach criterion within 15 training sessions and exhibited less than 30% conditioned responses on the last training session. Retention rabbits received an additional training session on the fourteenth day after initial learning. C. After learning, rabbits maintained the learned association. The left panel (acquisition) shows the normalized learning curves for trace-conditioned as compared with pseudoconditioned and slow-learning rabbits. Trace-conditioned rabbits (O, n = 46) required an average of 7.1 ± 0.6 sessions to learn the task. Neither the pseudoconditioned (Δ, n = 11) nor the slow-learning rabbits (□, n = 3) showed significant improvement across sessions. Thus, the pseudoconditioned and slow-learning rabbits served as excellent controls for nonspecific effects of training unrelated to associative learning. The right panel (retention) shows the percent of CRs elicited during 20 paired CS-US trials delivered at various intervals after acquisition. (Note that when retention rabbits (●, n = 10) received 20 paired CS-US conditioning trials at the indicated times after learning, they maintained their criterion performance.)
Figure 7.8 Intracellular postsynaptic measures from CA3 pyramidal neurons in hippocampal slices prepared at varying intervals after acquisition of hippocampally dependent 500-ms trace eyeblink conditioning. Evoked postburst AHPs, one measure of postsynaptic excitability, were markedly but transiently reduced after learning. Similarly, spike-frequency adaptation or accommodation was transiently reduced after learning. Other measures, including input resistance, were unaffected by conditioning. Calibration bars for accommodation and current-voltage (I/V) relationships: 20 mV, 100 ms.

percent of cells studied 24 hr after learning showing reduced AHPs (i.e., an AHP amplitude 1 standard deviation below the mean for all naive control cells). The AHPs remained reduced in cells studied up to 5 days after learning but by 14 days after learning, postburst AHPs were indistinguishable from controls, and reduced AHPs were seen in less than 15 percent of the cells studied. The results were the same when data from individual cells were pooled to obtain a mean CA1 or CA3 AHP amplitude value for each rabbit. Learning-related effects on the postburst AHP were not confined to reductions in the peak amplitude. Both the duration and the integrated area of the AHP also were significantly reduced in cells from conditioned rabbits. The reduced AHPs seen after learning were not due to differences in injected current, as the current required to fire a burst of four action potentials did not differ significantly between the groups. No statistically significant differences in resting membrane potential, apparent input resistance, action potential amplitude or action potential width were observed in cells from the trace-conditioned or control rabbits, indicating that the reduced AHPs seen after learning did not result from other voltage-dependent differences in CA1 or CA3.
The postburst AHPs of CA3 pyramidal neurons were significantly reduced soon after successful acquisition of trace eyeblink conditioning. Neurons from pseudoconditioned or from unsuccessful (slow) learners showed no such change in postsynaptic excitability. The enhanced excitability was transient, sustained maximally for only 24 hr after learning. Over a 2-week time-course appropriate for consolidation of the new association, excitability was enhanced but slowly decayed to basal levels, taking approximately 7 days to return to normal. The enhancement was not retention-specific, as the excitability of neurons from rabbits exhibiting asymptotic behavioral retention 2 weeks after acquisition showed basal levels of excitability, quite different from that observed for similarly performing rabbits soon after acquisition.

Spike frequency adaptation or accommodation, another index of excitability, was also transiently reduced after associative learning (figures 7.8 and 7.10). Hippocampal CA1 and CA3 pyramidal neurons from naive, pseudoconditioned, or slow-learning rabbits showed robust accommodation, which limited their within-burst firing frequencies. Reduced accommodation was apparent as early as 1 hr after learning but decayed quite rapidly (see figure 7.10A). The effect was still significant in CA1 neurons 3 days after learning but by 5 days after learning, accommodation had returned nearly to normal in both regions. Decreased accommodation suggests that hippocampal neurons would be more likely to fire action potentials to excitatory afferent inputs. This effect on accommodation was not previously observed after delay conditioning (Disterhoft, et al., 1986; Coulter et al., 1989), which is not hippocampally dependent. Decreased accommodation in pyramidal neurons from trace-conditioned rabbits may reflect the added demand for hippocampal processing required for the acquisition or consolidation of the hippocampally
Figure 7.10  Trace eyeblink conditioning resulted in decreased spike frequency adaptation (accommodation) in CA1 neurons. A. The time-course of learning-related changes in accommodation of CA1 neurons was evaluated in the same cells as in figure 7.7, with the same depolarizing current intensity used to study the AHP. Neurons were depolarized for 800 ms and the number of action potentials (APs) was noted. Cells from slow-learning (S) or pseudo-conditioned (P) control rabbits showed no changes, as did cells from retention (R) rabbits that received an additional training session 14 days later. The ratio of cells with reduced accommodation versus the number of cells studied for each group is indicated in parentheses. A cell was classified as having reduced accommodation if the number of APs elicited was at least one standard deviation more than the mean for all naive (N) control cells. Asterisk indicates significant difference from all three control groups: * p < .001. B. Examples of typical accommodation responses in CA1 pyramidal cells from rabbits after pseudoconditioning (Pseudo), 24 hr after learning (Trace 24 hr) and after receiving an additional training session 14 days after initial learning (Retention). (Note that although the cell from the trace-conditioned rabbit fired more action potentials, accommodation was certainly not abolished—as evidenced by the increase in interspike interval with time—but rather was significantly and transiently reduced after learning.)
dependent form of the eyeblink conditioning task (Kim and Faneslow, 1992; Kim, Clark, and Thompson, 1995). Alterations in accommodation may be present in the time period after acquisition of the trace conditioning task during which the hippocampus serves an important function for consolidation of this learned behavior.

Previous multiple- and single-neuron recording studies in delay conditioning (as well as our own single-neuron recording studies with trace conditioning) indicate that CA1 and CA3 pyramidal neurons demonstrate functionally similar changes when recording in vivo in the behaving rabbit. We sought to determine what would occur with our brain-slice approach. The cellular excitability changes in CA3 neurons showed a qualitatively similar size and time-course as did those in CA1 (see figures 7.8 and 7.9). However, the baseline size of the postburst AHP and of the spike frequency accommodation were considerably larger in CA3 than in CA1 pyramidal neurons. This apparently reflects the fact that somatic calcium currents are much larger in CA3 neurons, resulting in larger AHPs and more spike frequency accommodation. The time-course of decay of the reduced spike frequency accommodation was somewhat more rapid than that for the reduced AHP. This suggests that the alterations in accommodation were determined by factors other than just the AHP.

The decay rate of learning-related changes in CA1 and CA3 neurons after trace conditioning was not an artifact related to differences in behavioral acquisition, as there was no difference in learning rate between the trace-conditioned rabbits studied at different intervals after learning. Alternatively, the decay rate could reflect a decrease in behavioral performance over time, as might occur with normal forgetting. To test this hypothesis, rabbits were trace-conditioned to a behavioral criterion of 80 percent of CRs, and CR retention (i.e., consistent performance of the CR) was tested using 20 paired CS-US conditioning trials presented at various intervals ranging from 1 to 128 days after initial acquisition. Their retention performance remained nearly asymptotic at all time intervals tested, evidence that the learned association was maintained or remembered (figure 7.7C). This suggests that the electrophysiological changes observed after learning were not directly related to performance or retention of the CR, as the physiological changes returned to baseline within 7 days, whereas animals maintained behavioral performance above criterion for months. Thus, increased excitability of CA1 and CA3 neurons was required transiently for long-term retention of the learned association.

One more manipulation was done to study more directly the time-dependent dissociation between behavioral retention and in vitro excitability changes. An additional group of rabbits was trained to criterion as described previously and returned to their cages for 14 days without additional training. On the fourteenth day, these rabbits received one 80-trial session of trace eyeblink conditioning. Hippocampal slices were prepared 24 hr later. No excitability increases were observed in CA1 or CA3 cells from this group (see

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figures 7.7B, 7.8A, and 7.10A), even though these rabbits retained the learned association near the behavioral criterion of 80 percent of CRs. In fact, rabbits that received an 80-trial trace conditioning session 14 days after initial learning performed in a manner indistinguishable from that seen on the day they reached criterion. This direct test, combined with the behavioral retention data cited, led to our conclusion that increased excitability of CA1 pyramidal neurons is related to consolidation (and possibly acquisition), but not to performance or to long-term memory of the learned association.

It is currently of great interest to determine how plastic changes are established during learning. Membrane conductance changes may represent one conserved mechanism used for learning. Such changes have been observed in both vertebrates (Disterhoft et al., 1986; Coulter et al., 1989; Woody et al., 1991) and invertebrates (Alkon, 1984; Carew and Sahley, 1986). Cholinergic blockers such as scopolamine have been shown to impair classical conditioning in rabbits, but only when the hippocampus is intact (Solomon, Solomon, Vander Schaaf, and Perry, 1983), and acetylcholine application, like conditioning itself, decreases the AHP and blocks accommodation in hippocampal neurons in vitro (Madison and Nicoll, 1984). These data suggest involvement of the cholinergic system and activity of hippocampal neurons with associative learning. Varied other neurotransmitters modulate hippocampal membrane conductances (such as the slow AHP and spike frequency adaptation; Haas and Konnerth, 1983; Colino and Halliwell, 1987). Thus, changes in the release or uptake of neurotransmitters in vivo during learning may set up the excitability changes we observed in vitro after trace eyeblink conditioning. The prominent conductance underlying the postburst AHP is a calcium-activated potassium current, $I_{\text{AHP}}$ (Lancaster and Adams, 1986; Storm, 1990). This current regulates excitability by clamping the membrane potential at hyperpolarized levels following a burst of action potentials. With smaller AHPs of shorter duration, CA1 and CA3 neurons can respond more readily to excitatory afferents. Such increased responsiveness could facilitate propagation of information through the hippocampal circuit to other cortical or subcortical regions involved in the trace-conditioned reflex arc (Thompson, 1990; Berger and Bassett, 1992).

The increased excitability that we observed after learning may be maintained by changes in second-messenger systems (e.g., kinases and phosphatases) which regulate the phosphorylation state of ion channels underlying the postburst AHP. Calcium imaging studies in hippocampal neurons have shown that acetylcholine and norepinephrine reduce the calcium-activated slow AHP directly, without reducing calcium influx (Knöpfel, Vranescic, Gähwiller, and Brown, 1990; Müller, Petrozzini, Griffith, Danho, and Connor, 1992). Muscarinic block of the AHP is mediated by increased activity of calcium-calmodulin-dependent protein kinase II (CaMKII; Müller et al., 1992), whereas norepinephrine and other monoamines modulate the AHP via increased activity of protein kinase A (PKA) (Pedrazzani and Storm, 1993). Similarly, phorbol esters that directly activate PKC also reduce the AHP.
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response, and to determine whether or not these findings are a generalized feature
common to other kinds of learning tasks.

The time-course of changes in hippocampal excitability which we observed are
convergent with recent behavioral experiments from other laboratories. Rabbits
receiving hippocampal lesions 1 day after acquisition of trace eyelink conditioning
did not retain the CR and could not relearn it, yet when lesions were performed
30 days after learning, retention of the CR was not affected (Kim et al., 1995). These
data support our observations and suggest that the hippocampus is not the
long-term storage site for this behavioral task. Rather, they indicate that the
hippocampus is required temporarily to store information about the CS-US
association during acquisition and during consolidation of the learned response, as
neither occur if the hippocampus is removed before or shortly after training
(Solomon et al., 1986; Moyer et al., 1990; Kim et al., 1995). Similar observations
also have been noted in other hippocampally dependent learning tasks in other species. For
example, contextual fear memory was abolished in rats that received hippocampal lesions
1 day after conditioned fear training, but not in rats that received lesions 7 to 28
days after training (Kim and Faneslow, 1992). Also, object discrimination
learning revealed that monkeys with bilateral hippocampal lesions were
severely impaired in their ability to remember recently learned objects, yet they
showed normal memory for remotely learned objects (Zola-Morgan and
Squire, 1990). The generalizability of these findings suggests that time-dependent
memory consolidation is a conserved feature important for long-term storage of
certain kinds of memories.

Finally, we should address the issue of the postsynaptic excitability changes we have
described in CA1 and CA3 pyramidal neurons. Changes occur in a large number of pyramidal neurons in both hippocampal areas. In this
particular set of data, by using a criterion of an AHP or accommodation

Baraban et al., 1985; Malenka, Madison, Andrade, and Nicoll, 1986), and
altered PKC was reported in the CA1 region from classically conditioned
rabbits (Bank et al., 1988; Olds et al., 1989; Sunayashiki-Kusuzuki et al., 1993).
Immunoreactivity of PKC, and of muscarinic receptor subunits is enhanced in
hippocampal pyramidal cells after trace conditioning (Van der Zee et al.,
1994), suggesting that phosphorylation participates in the regulation of
hippocampal excitability and that acetylcholine may be pivotal in regulating
the AHP during trace eyelink conditioning. Because the slow AHP is generated
by current through a calcium- and voltage-dependent potassium channel,
phosphorylation of this channel during learning would decrease potassium
efflux through the channel (and increase excitability), whereas dephosphoryla-
tion would leave the channel under the control of membrane potential and
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change more than 1 standard deviation smaller than the mean of the naive group of neurons, we estimate that 70 to 80 percent of CA1 and CA3 neurons show excitability changes. The percentage of neurons showing an excitability change is much larger than one would expect to occur at random in a large population of neurons in the hippocampus. One might ask how such a postsynaptic excitability change, which would tend to alter the overall responsivity of a large number of neurons, could contribute to the specific patterns of synaptic change that might be anticipated to occur in a structure storing information during the process of associative learning. Conversely, if one considers the pattern of alterations in single-neuron firing rates described earlier in the chapter, how can a rather generalized enhancement in cellular excitability be congruent with the variable pattern of single-neuron firing rates which we have observed with in vivo recording techniques? This is especially paradoxical if one considers that a fairly large number of hippocampal pyramidal neurons (which we have recorded in vivo) are relatively silent or show inhibitory changes in response as compared to baseline firing levels.

Our answer to this paradox is a preliminary one at present. We would point out that a generalized postsynaptic alteration in excitability could be one component of the cellular mechanisms involved in setting the stage for more specific changes in synaptic excitability levels. A generalized excitability increase might make the neuron more able or more likely to store a pattern of inputs which were active during the time when the excitability change was being expressed (the period of consolidation of the memory?). Thus, the specificity in the information stored in hippocampal neurons during associative learning would be imposed on the system by the pattern of afferents activated during the learning trials. It seems theoretically possible for a generalized excitability change in the soma and proximal dendrites to contribute to the formation of a rather precise pattern of changes at specific synapses, as defined in a Hebbian fashion, by which afferents are activated by the learning situation. We should stress that while the AHP contributes to the overall tendency of neurons to fire, this one potential does not define totally the overall output firing of the neuron. Therefore, it seems reasonable to posit the theory that the AHP and accommodation changes are members of a family of cellular changes which occur during the learning process. The hippocampal neuron integrates all these changes in its information processing function and produces an individual pattern of firing by that neuron which contributes to the learned behavior.

CONCLUSIONS

We have reviewed recently gathered data with three approaches which describe different levels of neuronal change that occur in the hippocampus during learning and consolidation of an associative task. We assume that the alterations in PKCγ, postsynaptic excitability, and firing patterns of neurons
must be interrelated aspects of changes within the hippocampal system that contribute to the acquisition and performance of the eyeblink conditioned response. Because the variant of eyeblink conditioning that we have used is dependent upon the hippocampus for its acquisition, at least in the rabbit (Solomon et al., 1986; Moyer et al., 1990), we are confident that the conditioning-specific changes we have observed form an integral part of the neural alterations underlying the conditioned response.

The cellular integration of the immunocytochemical, biophysical, and neurophysiological changes we have seen is interesting, although the changes do not necessarily reflect successive steps in a cellular pathway of change. As mentioned in the section on the protein kinase C work, we examined the hippocampus for evidence of alteration in the calcium-dependent PKC isoforms. Our findings indicate that PKC\(_\gamma\) is the isofrom most involved in the cellular mechanisms of change during eyeblink conditioning. This finding is consistent with suggestions of those studying PKC involvement in long-term potentiation and in other learning tasks (Akers et al., 1986; Olds et al., 1990; Van der Zee et al., 1992). It is known that phorbol ester activation of PKC pathways leads to reduction in the postburst AHP (Baraban et al., 1985; Malenka et al., 1986). Thus it is reasonable to find reductions in the AHP in the same hippocampal pyramidal cell populations in which PKC\(_\gamma\) is changed. Because the amount of spike frequency accommodation is dependent in large part on the size of the calcium-mediated potassium current underlying the AHP in hippocampal pyramidal neurons, it is reasonable that the two postsynaptic excitability changes we observed occurred together and in concert with PKC\(_\gamma\) changes. It should also be pointed out that the PKC\(_\gamma\), the AHP, and the accommodation changes all involve calcium-activated cellular responses in an important fashion.

The alterations in single-neuron firing rate that we have observed in vivo are certainly dependent on or related to some important fashion to the immunocytochemical and membrane conductance changes during learning, which we have described in the hippocampus. However, the relationship is likely to be at a higher level. Both the alterations in PKC\(_\gamma\), and those in postsynaptic excitability occur in a large percentage (in fact, in the large majority) of hippocampal pyramidal neurons after learning. Yet the pattern of in vivo firing rate changes is much more complex—for example, there are as many inhibitory as excitatory changes observed, a large number of cells show no firing rate changes and, during the intertrial interval, there are a large number of inhibitory changes that are not conditioning-specific. We do not possess a complete understanding of the relationship between these rather generalized and much more specific patterns of change observed when different techniques are employed for observation. However, our working hypothesis (alluded to in discussing the brain-slice biophysical experiments) is that the generalized second messenger and membrane excitability changes act as facilitators or mediators of cellular change in a general fashion. The more specific pattern of neuronal changes, as defined by which synapses are
altered, is defined by the particular pattern of inputs activated during a particular associative task. Thought of in another way, the cells are set up to change by the generalized mechanism. The pattern of change that actually occurs (and allows considerably more specificity of information storage during learning) depends on the population of active inputs as defined in a Hebbian fashion (Hebb, 1949). This conceptual approach is very reminiscent of that proposed by Woody in discussing the pattern of changes observed following eyeblink conditioning in the cat precurrite motor cortex (Woody, 1974).

Finally, we should touch briefly on the relation between alterations that occur in the hippocampus during eyeblink conditioning and other brain structures. The cerebellum is the other brain structure about which we know the most relating to rabbit eyeblink or nictitating membrane conditioning. Several groups agree that unilateral damage to the dentate-interpositus output nuclei leads to the elimination of delay eyeblink responses already acquired and make it impossible to form new CRs (Yeo, Hardiman, and Glickstein, 1984; Thompson, 1990; Steinmetz et al., 1993). In an interesting study combining multiple-unit recording and the lesion approaches, neural engrams in the hippocampus were shown to disappear when delay eyeblink CRs were eliminated by cerebellar deep nuclear damage (Clark, McCormick, Lavond, and Thompson, 1984; Sears and Steinmetz, 1990). Perrett, Ruiz, and Mauk (1993) have subsequently shown that lesions to the anterior cerebellar cortex cause interesting changes in the timing of eyeblink CRs very similar to those resulting from bilateral hippocampectomy. These studies indicated that there is an important relationship between alterations in the hippocampus and in the cerebellum during eyeblink conditioning.

The question of how the hippocampus and cerebellum may be interconnected must then be addressed. Berger and Bassett (1992) described an important multisynaptic pathway by which the hippocampus can affect the cerebellar sensorimotor program running from subiculum, to retrosplenial cortex, to dorsolateral pontine nuclei, and hence to the cerebellar cortex via mossy fibers. Our working assumption is that hippocampal neuron output, altered during conditioning in the ways that we have described, may have a profound effect on cerebellar output via this hippocampal-cerebellar pathway which traverses the retrosplenial cortex as it travels toward the brainstem. A reciprocal multisynaptic cerebellar-hippocampal pathway—from the cerebellar deep nuclei via the ventroanterior thalamus or directly to the frontal cortex and then back to hippocampus—is becoming better appreciated and could play an important role in mediating feedback from the cerebellum to the hippocampus (Larsell and Jansen, 1972; Goldman-Rakic, 1987; Kim, Ungerbl, and Strick, 1994; Middleton and Strick, 1994). In conclusion, the alterations in hippocampal output that we have observed have the potential to impact on the cerebellar circuitry, and thus the developing CR at the sensorimotor control level, via reciprocal pathways that are becoming better understood but remain relatively understudied, given their potential importance.
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