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Spike timing in CA3 pyramidal cells during behavior: implications for synaptic transmission

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Abstract

Spike timing is thought to be an important mechanism for transmitting information in the CNS. Recent studies have emphasized millisecond precision in spike timing, to allow temporal summation of rapid synaptic signals. However, spike timing over slower timescales could also be important, through mechanisms including activity-dependent synaptic plasticity, or temporal summation of slow PSPs such as those mediated by kainate receptors. To determine the extent to which these slower mechanisms contribute to information processing, it is first necessary to understand the properties of behaviorally relevant spike timing over this slow timescale. In this study, we examine the activity of CA3 pyramidal cells during the performance of a complex behavioral task in rats. Sustained firing rates vary over a wide range, and the firing rate of a cell is poorly correlated with the behavioral cues to which the cell responds. Non-random interactions between successive spikes can last for several seconds, but the non-random distribution of interspike intervals (ISIs) can account for the majority of nonrandom multi-spike patterns. During a stimulus, cellular responses are temporally complex, causing a shift in spike timing that favors intermediate ISIs over short and long ISIs. Response discrimination between related stimuli occurs through changes in both response time-course and response intensity. Precise synchrony between cells is limited, but loosely correlated firing between cells is common. This study indicates that spike timing is regulated over long timescales, and suggests that slow synaptic mechanisms could play a substantial role in information processing in the CNS.

Introduction

Spike timing is widely thought to be a mechanism for transmission of information in the CNS. Spike timing is thought to be important mainly because of synchronization of inputs and temporal summation of rapid synaptic signals, which both require precise timing of spikes on a millisecond timescale. There has been considerable interest in the level of precision that is achieved (Mainen and Sejnowski, 1995; de Ruyter van Steveninck et al., 1997) and the mechanisms that underlie it (Stevens and Zador, 1998; Pouille and Scanziani, 2001; Mehta et al., 2002; Harris et al., 2002).

However, it is clear that there are also slower synaptic mechanisms that can relate spike timing to signal transmission over a much slower timescale. First, not all synaptic signals are rapid; even in glutamatergic transmission, kainate receptor activation can generate EPSPs at resting

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potential that last for hundreds of milliseconds, leading to a much longer time window for synaptic integration (Frerking and Ohliger-Frerking, 2002). Second, multiple forms of activity-dependent synaptic plasticity can combine during complex activity patterns to alter the synaptic response, depending on the temporal structure of prior activity; these synaptic dynamics can cause synaptic output to depend on spike timing over several seconds (Tsodyks and Markram, 1997; Dobrunz and Stevens, 1997; Zador and Dobrunz, 1997; Varela et al., 1998; Ohliger-Frerking et al., 2003).

One difficulty in evaluating the degree to which slower synaptic mechanisms contribute to information processing is that doing so requires an understanding of how these mechanisms are engaged during physiologically relevant patterns of activity. To date, few studies have used such patterns to characterize synaptic function. This is not because of a paucity of data; indeed, single unit recordings are made throughout the CNS. Rather, the spike patterns observed during behavioral tasks are frequently presented in a form that does not translate into useful experimental paradigms for synaptic physiology, because this is not a focus of *in vivo* studies.

In this study, we examined spike patterns in CA3 pyramidal cells, the presynaptic source of Schaffer collateral synapses, from awake, behaving rats during the performance of a complex behavioral task, in which correct task performance is correlated with changes in the ensemble activity of hippocampal neurons (Deadwyler et al., 1996; Wiebe and Stäubli, 1999). We will address several questions: what is the level of overall activity during a complex task, and how consistent is that activity among different cells? How closely does a spike train during a complex behavior match random firing, and what are the sources of non-random firing? How do environmental cues change afferent activity, and how are different choices within the same general class of cue encoded? How prevalent is synchronous firing between cells, and what is the time window over which synchrony occurs? The answers to these questions will be used to construct a model for producing artificial spike trains, and to make some generalizations about synaptic transmission during physiologically relevant patterns of activity.

Materials and Methods

Single unit recordings were made in CA3 pyramidal cells in awake behaving rats (for details, see Wiebe and Stäubli, 1999) during performance of an olfactory delayed match-to-sample task. The sequence of events involved in the task are outlined in Fig. 1A. Briefly, the task was initiated by a nose-poke on a button in the sample arm. After a 2 second delay, an odor (the sample odor) was presented in the sample arm of a Y-maze for 10 seconds, followed by a variable delay. After the delay, two different odors were presented, one in each of the two test arms of the maze (the test odors). One of these test odors was the same as the sample odor, and the other was a novel odor. The rat could indicate which test arm contained the novel odor by entering a test arm and pressing a button located at the end of that arm. If the rat was correct, it was rewarded with water; if not, it was punished with a brief flash of light.

Although much study has been dedicated to the role of the hippocampus in processing spatial information (reviewed in Best et al., 2001), it has been shown that many hippocampal pyramidal neurons respond to both spatial and non-spatial cues during a complex task (Wood et al., 1999; Stäubli and Wiebe, 1999; Hampson et al., 1999). In this study, cells could generate responses (defined as a change in the firing rate) to various cues during this task, for example exposure to the sample odor, entry into a test arm, or presentation of the reward/punishment. There were also parameters within the task that differed between individual trials of the task, so that cellular responses during a cue could discriminate between different options of that parameter. There were 3 parameters monitored: which of two odors the rat was exposed to in the sample phase (odor-discrimination), which test arm the rat entered (location-discrimination), and whether the rat was rewarded or punished (performance-discrimination).

Previous study has indicated that during this task, most cells generate responses that discriminate multiple parameters, although some cells discriminate only one parameter, or respond to cues in a non-discriminatory fashion, or do not respond to any of the monitored cues at all (Wiebe and Stäubli, 1999).

Adult Long Evans rats were trained over a ~1 month period to perform the task, and were then surgically implanted with a microwire electrode array consisting of two rows of 8 microwires, cemented in place with bone wax and dental cement. An uninsulated reference wire was used to eliminate low-frequency movement artifacts. After a 6–7 day recovery period, the animals were retrained and recordings were performed during the task. At the end of the recordings, each rat was euthanized with sodium pentobarbital (100 mg/kg) and 40 μ A of current was passed through the recording electrodes. The brain was fixed, removed, and sectioned to localize the electrode tips.

Recordings were made using a multichannel neuronal acquisition processor system (Plexon Inc., Dallas, TX; www.plexoninc.com) that performed on-line multichannel spike sorting. Neural activity and behavioral responses were digitized and time-stamped for subsequent computer processing. Spikes were isolated using time-amplitude window discrimination and waveform template matching; up to 4 single units could be isolated per microwire. Single units were isolated and separated into pyramidal cells and interneurons based on a linear cut-off in the relationship between mean firing rate and spike width, as described (Wiebe and Stäubli, 1999).

An initial characterization of this data set has been published (Wiebe and Stäubli, 1999). In the present study, the data was further analyzed using a quantitative analysis of spike timing. Spike trains for individual cells were separated and analyzed using Neuroexplorer (Plexon Inc.) and user-written programs in SigmaPlot (Jandel Scientific). All data are presented as mean $\pm$ SEM. Significance was assessed at $P < 0.05$.

Results

General Firing Properties of CA3 pyramidal cells

We will first consider several general properties of CA3 pyramidal cell activity during a behavioral task (outlined in Figure 1A; for details of the task, see Materials and Methods). We will address several questions. What is the overall level of activity, and how does it correlate with cell function? Which ISIs are observed during the task, and how consistent is the ISI distribution across different cells? How does the ISI distribution change as a function of the mean firing rate? The answers to these questions will allow us to relate cellular activity *in vivo* with the stimulation protocols used *in vitro* to monitor synaptic function.

What are the firing rates of CA3 pyramidal cells during a behavioral task?—It has been reported since the earliest studies of hippocampal pyramidal cells in awake, unrestrained rats that essentially all hippocampal pyramidal cells fire in irregular patterns with sustained firing rates below 10 Hz, and that there is a qualitatively large variability in overall firing rate for different neurons (Ranck, 1973). Consistent with this finding, we observed that different CA3 pyramidal cells had highly variable mean levels of activity even during a single trial of the task, as shown for several cells recorded simultaneously in Fig. 1B. The mean firing rate for all 766 CA3 pyramidal cells available in recordings from 18 animals was 0.97 ± 0.05 Hz, considerably higher than that typically used in studies of synaptic transmission (0.05–0.2 Hz, typically 0.1 Hz). However, because cross-cell variability in the firing rate extended over several orders of magnitude, the mean has limited value in describing the population. To provide a more complete quantitative description of the population of CA3 pyramidal cells, we measured the distribution of firing rates from the population of CA3 pyramidal cells

recorded in each animal, and averaged this distribution for all animals that had a large enough population of CA3 pyramidal cells to accurately measure that distribution ($n=13$; Fig. 1C). This average distribution reveals that firing around 1 Hz is most common, and most (~75%) cells fire at rates >0.2 Hz.

Does the function of a cell predict its firing rate?—An obvious mechanism for explaining the large variability in firing rate would be that different cells encode different numbers of functional parameters during performance of the task, and are therefore active to different degrees. To evaluate this possibility, we considered the impact of functional responses during the task on cellular activity. We first considered the possibility that cells that generated discriminatory responses based on different parameters might have different mean firing rates. However, we did not observe any difference in mean firing rate between cells that selectively discriminated between odor, location, or performance (data not shown).

However, it remains possible that cells might have higher firing rates if they respond to more parameters during the task. For those cells with sufficient data to do so, we separated the cells into 5 functional categories: cells that did not respond to (or discriminate based upon) any of the cues that were monitored ($n=86$); cells that responded to one or more cues but that did not discriminate based on odor, location, or performance (0 parameters; $n=211$); cells that responded in a discriminatory fashion based on only one of the three parameters (1 parameter; $n=52$); cells that responded in a discriminatory fashion based on two of the three parameters (2 parameters; $n=89$); and cells that responded in a discriminatory fashion based on all three parameters monitored (3 parameters; $n=276$). We found that non-responsive cells had lower mean firing rates than did responsive cells, and cells that discriminated all three parameters had higher mean firing rates than did cells discriminating fewer parameters; the intermediate three categories had indistinguishable mean firing rates when compared to each other (Fig. 2A). However, the mean firing rates of these different categories differed by roughly an order of magnitude, while the total variability in mean firing rate encompassed a much larger range, over three orders of magnitude. As a result, mean firing rates were poor predictors of cell function (Figure 2B). These results indicate that the mean firing rate of a cell does depend weakly on its function, but the majority of the variability in the mean firing rate is determined by some other factor; moreover, the overall firing rate of a cell during the task is independent of the specific identity of the parameter(s) that it encodes.

What ISIs are observed during task performance?—The mean firing rate provides an overall view of the general activity level of the cell but does not address the variability in firing around that mean. To determine specific patterns of spiking with higher resolution, we examined the interspike interval distributions of CA3 pyramidal cells. As with mean firing rates, individual cells had highly variable ISI distributions, as shown by 9 representative cells in Fig. 3. Many studies, including our own, have approximated the firing properties of CNS neurons with random firing patterns governed by Poisson statistics; however, we observed that most ISI distributions were clearly indicative of a non-random firing pattern (see Fig. 4A, grey lines, for ISI distributions expected based on homogeneous Poisson statistics), frequently having obvious structure within the distribution or strongly preferred ISIs; this observation was consistent even among cells that did not respond to any of the cues that were monitored (for example, cell 1). Although occasionally cells had qualitatively similar ISI distributions, such as cells 4 and 8 in Figure 3, major subpopulations of cells with similar ISI distributions were not observed (data not shown).

The results in Figure 3 indicate that cross-cell variability in the ISI distribution is considerable. One variable on which the ISI distribution must depend, at least to some degree, is the mean firing rate. To examine how the ISI distribution varies as a function of mean firing rate, we arbitrarily separated cells into different groups based on the logarithm of the mean firing rate

and averaged together all ISI distributions within a single group. Average ISI distributions for groups from 0.1 Hz to 10 Hz are shown in Fig. 4A. Two features are evident from this data. The first is that, on average, as the mean firing rate increases, the longest ISIs become less prominent (Fig. 4B, grey triangles), an effect accounted for almost entirely by the increased representation of intermediate ISIs (Fig. 4B, open squares). The relative frequency of the shortest ISIs (<10 msec) was largely insensitive to the mean firing rate (Fig. 4B, filled circles). At mean firing rates above 0.1 Hz, there was also a modest peak of ISIs corresponding to oscillations in the theta frequency range (100–200 msec).

The second feature that is clear from the data in Figure 4A is that, even when averaged across cells that are matched to similar mean firing rates, the ISI distributions are not well approximated by random firing over most of the firing rates observed. For illustrative purposes, the ISI distributions expected from random firing at the appropriate frequency are shown in Figure 4A (grey lines). The observed ISI distributions from 0.1 to 3 Hz were strongly overdispersed compared to what would be expected from random firing, with overrepresentation of both the longest and shortest ISIs. Overdispersion was modest in cells firing around 10 Hz (~1% of the total population), but was still apparent on closer inspection. These results suggest that random firing patterns will cover a much smaller range of ISIs than are observed *in vivo*.

Sources of non-random activity in CA3 pyramidal cells

Up to this point, we have noted a number of ways in which the ISI distributions of CA3 pyramidal cells do not meet the expectations of random firing patterns. We therefore turned our attention to the issue of how prevalent non-random patterns of activity are. We considered three issues to be most important: first, over what time scale does non-random activity persist? Second, to what extent can long, non-random activity patterns be described solely by taking into account the non-random ISI distribution? And finally, to what extent do cellular responses to environmental cues contribute to non-random activity? We will address the first two questions here; the third is sufficiently complex that we will address it in a separate dedicated subsection.

How long does the occurrence of a spike affect the probability of firing?—To

assess the timescale over which non-random patterns of activity persist, we used an autocorrelogram analysis. The autocorrelogram indicates the probability of observing a spike as a function of time, given that a spike occurred at the zero time-point. The point at which the probability of spiking becomes independent of time indicates the length of time over which non-random interactions between spikes occur. One common use of autocorrelograms is to detect theta oscillations, which are widely observed in the hippocampus (reviewed in Kahana et al., 2001; Buzsaki, 2002). Theta oscillations are prominent in recordings from CA1 pyramidal cells during performance of tasks requiring odor- and spatial-discrimination (Otto et al., 1991). In the CA3 pyramidal cells examined in the present study, theta oscillations were somewhat less pronounced, with only a minority of analyzed cells (21 cells out of 86 analyzed) showing a damped oscillation in the theta frequency range (5–10 Hz, average=7.4±0.4 Hz), that was resolvable for between 2 and 25 cycles (average=4.2±0.9 cycles; Fig. 5A). These theta oscillations will contribute to long-lasting non-random activity in the minority of cells that have them.

We also noted that a small minority of cells (6 cells out of 86) had autocorrelograms in which non-random interactions between spikes decayed rapidly, lasting for 50–500 msec (Fig. 5B). However, in most cells, we found that the autocorrelogram showed two phases of non-random interactions between spikes: a fast phase that decayed rapidly or contained damped oscillations, and a slow phase that persisted over very long periods of time (between 1 and 10 seconds),

indicating the existence of long-range interactions between spikes. The slow phase was sufficiently prolonged that it was often not apparent for autocorrelograms with a time range restricted to less than a second (Fig. 5C₁), although it was immediately apparent in autocorrelograms with longer time ranges (Fig. 5C₂). The decay of the slow phase was frequently well fit by an exponential, and the average time constant of decay was 2.7 ± 0.5 sec ($n=86$). The slow phase was small, but because of its long-lasting decay, it accounted for $70 \pm 3\%$ of all non-random spiking.

This slow phase indicates that there is a long-range interaction between spikes that can last for several seconds. It remains unclear whether the long-range interactions observed are due to long-range interactions between intrinsic spiking mechanisms or due to long-range interactions between environmental cues that drive spiking, although we note that similar long-range interactions can be generated by sustained firing at a constant frequency (see Figure 11D, below). However, regardless of the mechanism that underlies them, these long-range interactions will affect the activity pattern transmitted to the synapse.

How prevalent are non-random sequences of several spikes?—Given that non-random spiking occurs over such a long timescale, we wondered whether this indicates that there are long sequences of many nonrandom spikes that fall into a distinct pattern. To address this issue, we reasoned that nonrandom spike sequences would be manifested as a deviation from repeated random samples from the ISI distribution. We therefore examined whether the joint distribution of ISIs during spike triplets could be explained as the random superposition of two spike doublets drawn from the ISI distribution. The ISI distribution of a representative cell is shown in Fig. 6A, and the joint distribution of two ISIs in succession during a spike triplet is shown on a color-scale in Fig. 6B for all triplets in the train. The expected distribution if each of the two ISIs in the triplet were randomly drawn from the ISI distribution is also shown (Fig. 6C).

The expected and observed distributions are obviously similar, but the difference between the expected distribution and the observed distribution (shown in Fig. 6D) reveals a tendency in this cell for the second ISI in a triplet to be similar to the first ISI in the triplet. A similar distribution of non-random triplets with similar ISIs was seen for 12 out of 21 cells; of the remaining 9 cells, 5 showed a strong tendency for non-random triplets with both ISIs in the high-frequency range corresponding to complex spike bursts (brief, >100 Hz trains of 2–6 spikes; Kandel and Spencer, 1961; Ranck, 1973), and the remaining 4 showed a more complex or diffuse pattern of non-random triplets.

As a control to ensure that these non-random triplets are not artifacts due to undersampling or overbinning, we shuffled the sequence of ISIs in the original data set to remove any non-random triplets (data not shown) and then subtracted the expected distribution from the new shuffled data set (Fig. 6E). In a total population of 21 cells, the difference between the expected distribution and the shuffled distribution was clearly smaller ($P < 10^{-5}$) than that seen using unshuffled data, and there is no structure to the difference function after shuffling the data. The fraction of spikes that are genuinely involved in nonrandom spiking, as opposed to sampling or binning errors, was calculated by subtracting the fraction of non-random triplets in the shuffled data set from the fraction of non-random triplets in the actual data set. These non-random patterns that were unaccounted for by random sampling from the ISI distribution were a small minority of the total spiking ($<10\%$), indicating that the vast majority of spiking ($>90\%$) could be predicted by random sampling from the ISI distribution.

To determine if the non-random patterns are associated with responses to environmental cues, we compared the non-random triplets in responsive cells to the triplets in non-responsive cells (Fig. 6F). We found that non-random triplets were significantly more frequent in cells that

responded to a monitored environmental cue during the task ($n=14$) than in those that did not ($n=7$). We conclude that most spiking in CA3 pyramidal cells ($>90\%$) can be described as random sampling from a non-random ISI distribution; however, a small minority of spikes are generated by patterned sequences that cannot be readily predicted by the overall ISI distribution, and these patterned sequences are more frequent in cells that respond to environmental cues, suggesting that they may be associated with cellular responses to environmental cues.

Responses to environmental cues

Up to this point, spike trains have mainly been considered without regard to whether or where responses occur, and what the properties of those responses are. We now turn our attention to characterizing cellular responses to environmental cues, with a focus on three questions: first, what is the time-course over which cells respond to a cue, and how complex is that response as a function of time? Second, how does the presence or absence of a cellular response affect the ISI distribution? Finally, how do cellular responses differ during discrimination of cues that are functionally similar?

How do CA3 pyramidal cells respond to environmental cues?—To determine the time course of cellular responses to environmental cues, we examined two types of responses in detail: responses to the onset of the sample odor (Fig. 7A), and responses to leaving the maze sample arm (Fig 7B). For the present analysis, we only examined cells that did not discriminate in their responses based on any of the parameters monitored. For these cells, responses were fairly stereotypical. Raster plots from representative cells that responded to these phases of the task are shown in Figs. 7A₁ and 7B₁, respectively, and average responses from those representative cells are shown in Figs. 7A₂ and 7B₂. The average responses for all cells that responded to these cues are shown in Figs. 7A₃ ($n=109$) and 7B₃ ($n=92$). The time courses of the cellular responses were complex in both cases. It is not clear from this data whether such temporally complex responses during maze performance are due to a simple relationship between firing and complex stimuli, or to a complex relationship between firing and simple stimuli; however, the conclusion that cellular responses to behavioral cues during a task are temporally complex is independent of the mechanism underlying that complexity.

How does the ISI distribution change during cellular responses?—To more quantitatively consider the firing patterns involved in responses, we examined whether specific ISIs were associated with cellular responses. As an initial attempt to ascertain this information, we compared the total ISI distributions of cells that did not show a response to any of the cues during the task ($n=86$; Fig 8A₁) with those that did ($n=628$; Fig 8A₂). The difference between these distributions shows ISIs that were more common among responsive cells as positive values, and ISIs that were more common among non-responsive cells as negative values (Fig 8A₃). Cells that had responses clearly showed a prominent shift toward intermediate frequencies, with both short and long ISIs having a larger representation in cells that did not respond.

The difference function in Fig 8A₃ is a useful first approximation, but it does not directly reflect which ISIs are selectively associated with responses, because the magnitude of the difference for any given ISI is determined not only by the difference between responsive cells and non-responsive cells but also by the relative frequency of that ISI compared to other ISIs in the same population of cells. To provide a linear difference index of which ISIs are selective for responsive cells or non-responsive cells, we used the parameter $(PDF_{\text{responsive}} - PDF_{\text{nonresponsive}}) / (PDF_{\text{responsive}} + PDF_{\text{nonresponsive}})$, in which a value of 1 indicates an ISI that is only seen in responsive cells, and a value of -1 indicates an ISI that is only seen in

nonresponsive cells. This correction did not change the fundamental observation that intermediate ISIs were a more prominent feature of responsive cells (Fig. 8A₄).

The analysis in Figure 8A provides an estimate of how cellular responses change the ISI distribution, but does so by comparing data from different cells. To provide a within-cell comparison of responses and baseline periods, we selected cells that only responded to entry into the test arms during the task ($n=34$). The ISI distribution of a representative cell during the entire trial is shown in Fig. 8B₁, and during the task prior to test-arm entry in Fig. 8B₂. These two distributions are very similar, indicating that the majority of spiking occurs during the non-response phase of the task. However, the ISI distribution following task arm entry (Fig. 8B₃) showed a prominent increase in intermediate ISIs and a prominent decrease in the longest ISIs, consistent with results from cross-cell comparisons. This increase in intermediate ISIs was more obvious when the linear difference index was calculated, comparing ISIs before and during the test phase (Fig. 8B₄). There was qualitative agreement between the cross-cell (Fig. 8A) and within-cell (Fig. 8B) comparisons, in that both showed a shift during responses from long and short ISIs to intermediate ISIs, with a maximal increase in ISIs of a few hundred milliseconds during the responses. Quantitative differences between the two methods are possibly due to the fact that all within-cell comparisons were only during test-arm entry, while the cross-cell comparisons were used for all cells that responded to any of several different cues, potentially with different temporal statistics.

How do cells discriminate between different options of the same cue?—Many CA3 pyramidal cells can discriminate between different options of a behaviorally relevant parameter. There are two different mechanisms by which cellular responses could discriminate between two options: first, the cellular responses to the two options might have similar time courses, but differences in response amplitude; alternatively, responses to each option might have distinct time courses. To differentiate between these two possibilities, we examined the time courses of responses to either of two choices in cells that showed discriminatory responses to environmental cues. We found that results were variable; there were some cells that showed discriminatory responses that were different in their time course (Fig. 9A), but others that showed discriminatory responses that were similar in time course but differed in magnitude (Fig. 9B).

To quantify this effect, we plotted the firing rate for each choice at corresponding times during the response; the firing rates during responses to each option should be highly correlated if discrimination is expressed as a difference in response amplitude but not time course, but poorly correlated if discrimination is expressed as a difference in response time course. As expected, the cell in Fig. 9A showed no correlation in firing between the two choices (Fig. 9C₁), while the cell in Fig. 9B showed a strong correlation (Fig. 9C₂). The strength of the correlation can be quantified by the r^2 value of the correlation. In 53 comparisons of different choices during discriminatory responses, we found that most cells encoded the discrimination as a difference in response time course, but a minority of cells encoded the discrimination at least in part as a difference in response magnitude (Fig. 9D).

Synchrony between cells

Up to this point, we have considered the firing of individual neurons in isolation. This is because most forms of short-term, activity-dependent synaptic plasticity that have been described are independent of the number of fibers activated. However, it is clear that the timing of spikes in one cell relative to another is a critical factor in temporal summation, a major mechanism for population coding that is also a factor underlying the associative properties of many forms of long-term synaptic plasticity. We therefore consider two major questions regarding the correlated firing of different CA3 pyramidal cells: first, how common is tightly-coupled firing

between cells? Second, how precise is correlated firing, and over what time-scale does correlated firing persist?

Precisely correlated firing between cells is rare—To determine the extent to which cells are precisely correlated in their firing, we examined 202 crosscorrelations between 43 CA3 pyramidal cells in 8 separate clusters. Representative crosscorrelograms are shown in Figure 10A. In many crosscorrelograms (90 out of 202), there was little if any resolvable correlated firing between cells (Figure 10A, top left). However, in a minority of crosscorrelograms, there was a strong and precise correlation in firing between cells (37 out of 202; Figure 10A, top right). Precisely correlated firing could be due to synchrony, in which two cells fire at the same time, or to the existence of a “synfire” chain, in which cells in a network are strongly connected so that firing in one cell leads to subsequent firing in another cell with a highly precise latency (Yuste et al., 2004). We did not observe precisely correlated firing with a nonzero latency, indicating that precisely correlated firing was invariably due to synchrony. This argues against synfire chains as a common mechanism of correlated firing between CA3 pyramidal cells. We note that even in the cases where synchrony was clearly evident in the cross-correlogram, the probability that any given spike in one cell was synchronous with a spike in the other cell was still modest (<0.2 for most cases).

Loosely correlated firing between cells is common—While precise cross-correlations were infrequent, many cells had correlated firing that was imprecise. Some of these correlations (25 out of 202) were asymmetrical, with a strong tendency for one cell to fire before the other (Figure 10A, middle left); however, other correlations (46 out of 202) were symmetrical, indicating that overall, the cells fired together but with imprecise timing (Figure 10A, middle right). Some correlations (22 out of 202) were also observed with oscillations in the theta frequency range (Figure 10A, bottom left), although the amplitude of these oscillations was invariably very weak. The categories defined here were not mutually exclusive, and cells could express both precise and loose coupling simultaneously (Figure 10A, bottom right).

To quantify the prevalence of correlated cell firing, we examined the probability of synchronous firing as function of the time window used to define synchrony. Only non-random synchrony, defined as cross-correlations exceeding the baseline of the crosscorrelogram (established at ± 1 second), was analyzed. When the window of synchrony was narrow (± 10 msec), most cell pairs (76%) had a modest (<3%) probability of synchronous firing, and 97% of the cell pairs had a probability of synchronous firing of <20% (Figure 10B, top graph). However, when the window of synchrony was broadened to 100 msec, the fraction of cell pairs with a modest probability of synchronous firing dropped to 54%, and the remaining cell pairs had a probability of synchronous firing that was much higher, even exceeding 1 in some cases (indicating that firing of a spike in one cell was often accompanied by firing of multiple spikes in the other) (Figure 10B, bottom graph). When the average probability of synchronous firing plotted as a function of the time window of synchrony for all cell pairs, the probability of synchronous firing was strongly dependent on the precision of the window used to define synchrony, with loosely coupled spiking occurring over hundreds of milliseconds (Figure 10C).

These results indicate that precisely correlated firing in CA3 pyramidal cells is uncommon, although loosely correlated firing is widespread. Loosely correlated firing could be due to weak network activity or uncorrelated responses of multiple cells to the same complex environmental cues. The timing of this loose coupling is sufficiently imprecise that temporal summation of rapid PSPs, like those mediated by AMPA receptors, is unlikely to be affected much by it. However, imprecisely correlated firing could have a strong effect on temporal summation of slow PSPs, like those mediated by kainate receptors.

An empirical model for generating artificial spike trains

A major purpose of this study has been to highlight features of *in vivo* spike trains that could impact synaptic function, so that these features can then be incorporated into studies of synaptic physiology. Obviously, the simplest way to ensure that afferent activation *in vitro* closely mimics *in vivo* activity is to use *in vivo* spike trains as externally-imposed stimuli. This approach has been used in the past (Dobrunz and Stevens, 1997; Froemke and Dan, 2002; Hampson et al., 2003; Ohliger-Frerking et al., 2003), but the heterogeneity of activity patterns seen in the present data set raise the concern that data from an individual cell may not be representative of the activity patterns seen in the overall population. Here we compile the features of the available data that we consider to be most relevant, and propose a simple method to generate artificial spike trains that incorporate those features.

In our view, artificial spike trains that generally mimic *in vivo* spiking should be designed according to the following general guidelines: 1) The population of cells is so heterogeneous that the model should be based on averaged data from many cells. 2) The average ISI distributions are overdispersed when compared to random ISI distributions, and the model should include this overdispersion. 3) Increased mean firing frequency, including during cellular responses, is predominantly due to an increased representation of intermediate ISIs, and the model should recapitulate this feature. 4) The non-random ISI distribution can explain more than 90% of spiking, so a model that uses only the ISI distribution without regard to higher-order interactions is sufficiently accurate to mimic firing patterns at this early stage of investigation.

Based on these constraints, we have constructed a simple empirical model from the available data, using the family of ISI distributions shown in Figure 4. These distributions were organized on a third axis, mean firing rate, and interpolated to generate a continuous 3-dimensional function in which the probability of a given ISI for a given overall firing rate was calculated (Figure 11A; see Supplemental Materials). This function was then sampled at different mean firing frequencies to construct firing patterns that mimic *in vivo* firing at those frequencies, and those patterns were then spliced together appropriately to construct artificial spike trains that incorporate changes in firing rate (Figure 11B, raster plots). This method can be used to construct trains that successfully reproduce rapid, sharp changes in firing frequency between steady states (Figure 11B) or cellular response waveforms from experimental recordings (Figure 11C; response waveform from Figure 9A). At a constant frequency of 1Hz, the model produced an autocorrelogram that indicated non-random interactions between spikes over a long period, with a slow exponential phase of decay ($\tau = 2$ sec), similar to that seen with real spike trains (Figure 11D).

To what extent does the model successfully reproduce the features of the data discussed above? The model will by definition be effective over sustained firing rates ranging approximately between 0.1 Hz and 10 Hz, encompassing the vast majority of cells. Moreover, because the model is empirical, it will reproduce the ISI distributions in Figure 4. By definition, increased firing (eg. during simulated responses to environmental cues) will be associated with an increased representation of intermediate ISIs. The model also reproduces the long-lasting, non-random interactions between spikes that are observed in the autocorrelogram, and can be used to mimic simulated response waveforms of arbitrary temporal complexity.

However, we caution that there are some features of the data that will not be reproduced by the model. The model cannot describe sustained firing at frequencies that are outside the limits of the distribution in Figure 11A. The model also will not mimic the heterogeneity in the ISI distributions of different cells, because it is based on averaged ISI distributions. The model will not reproduce the small fraction of non-random sequences of multiple spikes, because it is based on random sampling from the ISI distribution; however, extending the model to

incorporate a given non-random sequence into the train would be trivial. Finally, the model in its current form mimics activity for a single afferent fiber, precluding the generation of cross-correlations between fibers. However, cross-correlations between afferents are typically weak and loose, making them irrelevant for many experimental aims, and extending the model to include a specific pattern of cross-correlations for those scenarios in which it must be taken into account would be relatively simple. We conclude that the model reproduces many properties of cell firing that we have observed, and can be extended to incorporate weak cross-correlations or non-random sequences, according to the specific experimental parameters for which the spike trains are being used.

Discussion

We have characterized the activity of CA3 pyramidal cells during a behavioral task, with an emphasis on the features that have implications for synaptic transmission driven by that activity. We now consider how these findings relate to studies of synaptic transmission.

CA3 cells have variable firing properties, but generally fire at around 1 Hz—

Different CA3 pyramidal cells have different firing properties, complicating description of these firing properties in general terms. Variability in the number or identity of parameters to which a cell responds accounts for only a minor fraction of the total variability in firing. ISI distributions are also highly variable, indicating that large intrinsic variability in firing within a broad population may be the most productive way of describing firing patterns in CA3 pyramidal cells.

Mean firing rates of around 1 Hz are most commonly observed in CA3 pyramidal cells. This firing rate is considerably higher than is typically used in most synaptic physiology protocols, and it remains to be seen whether the forms of short- or long-term plasticity that are generated at this level of activity are similar to those that have been widely characterized during lower frequencies of stimulation.

The observation that sustained firing at 1–10 Hz is common for CA3 pyramidal cells is surprising, because long-term depression (LTD) is reliably induced by constant frequency trains in this range *in vitro* (Dudek and Bear, 1992; Mulkey and Malenka, 1992). LTD at these synapses is difficult to induce in awake adult rats (Staubli and Scafidi, 1997), for unknown reasons. One possible explanation raised by our data is that the activity patterns experienced *in vivo* normally cause a tonic induction of LTD that occludes externally-imposed induction protocols. However, since complex spike trains differ from constant-frequency trains in their efficacy at inducing long-term synaptic plasticity (Dobrunz and Stevens, 1997; Perrett et al., 2001; Sjostrom et al., 2001), the patterns seen *in vivo* may not induce LTD as effectively as constant frequency stimulation. It will be of interest to assess the induction of long-term plasticity in response to physiologically relevant patterns of activity.

Firing patterns of CA3 pyramidal cells are non-random—Firing is non-random in these cells, with ISI distributions showing a broader range than predicted by random firing. Others have noted non-random activity patterns in hippocampal pyramidal cells in a variety of contexts (Fenton and Muller, 1998; Lansky and Vaillant, 2000; Barbieri et al., 2001; Kahana et al., 2001; Buzsaki, 2002). The majority of non-random firing in our study is due to non-random structure in the ISI distribution. Random sampling from the ISI distribution predicts spike triplet sequences with >90% accuracy, suggesting that the ISI distribution of a cell can generate a fairly accurate artificial spike train by itself. However, the residual sequences that cannot be explained in these terms may be important for information processing, and further characterization of these sequences will be of interest.

Obviously, some non-random firing is generated by responses to environmental cues embedded in the overall activity. However, since the ISI distributions of non-responsive cells were non-random (Fig. 8A₁) and the ISI distribution of responsive cells are relatively unaffected by removal of responses (compare Fig. 8B₁ and 8B₂), environmental cues are likely to be a minor source of non-random firing. We cannot exclude the possibility that some non-random firing is generated by responses to unidentified cues. However, because the population of non-responsive cells could be clearly separated from the population of responsive cells by mean firing rate, ISI distribution, and prevalence of non-random spike sequences, it seems unlikely that much cellular activity is dictated by environmental cues that are outside our control.

Intermediate ISIs are selectively promoted during increased activity—A third conclusion is that increased firing is represented as an increase in intermediate ISIs, for both different cells firing at different firing rates, and for increased firing associated with responses to environmental cues. The shortest ISIs, which correspond to frequencies seen during complex spike bursting, are largely insensitive to overall firing rate and are less prominent during cellular responses. This argues against complex spike bursts as a major mechanism for encoding environmentally relevant cues, consistent with the observations of Harris et al. (2001).

The range of intermediate ISIs is similar to the range in which short-term plasticity is maximal, as assessed by paradigms such as paired-pulse facilitation (Creager et al., 1980). These ISIs are also a range in which kainate receptor-mediated EPSPs will undergo temporal summation, while AMPA receptor-mediated EPSPs will not (Frerking and Ohliger-Frerking, 2002). The importance of slow synaptic mechanisms may therefore be increased in cells with a higher overall firing rate, or during responses to environmental cues.

Cellular responses to environmental cues are temporally complex—During realistic behaviors, cellular responses to environmental cues are complex. Discrimination between different options within a cue is also complex, expressed mainly as differences in the time course of the response. It will be of interest to identify features of cellular responses that are enhanced or suppressed by synaptic transmission, to determine whether synaptic transmission enhances or prevents transmission of parameter discrimination from CA3 to CA1. In this context, it is noteworthy that CA3 pyramidal cells are more specific in the cues that activate them than CA1 pyramidal cells (Barnes et al., 1990; Wiebe and Stäubli, 1999).

Above a limiting frequency of afferent firing, cortical synapses transmit transitions between firing frequencies but provide the same mean depolarization at steady-state (Abbott et al., 1997; Tsodyks and Markram, 1997). This does not appear to be relevant at Schaffer collateral synapses, for two reasons. First, the cellular responses that we have observed are temporally complex, without simple transitions from one steady-state firing frequency to another. Second, the limiting frequencies at cortical synapses are 10–20 Hz (Abbott et al., 1997; Tsodyks and Markram, 1997), well above the sustained firing rates seen here. The limiting frequency at Schaffer collateral synapses has not been determined but is probably higher than at cortical synapses, because the synaptic depression that sets the limiting frequency is modest at Schaffer collateral synapses (Selig et al., 1999).

Precise synchrony between CA3 pyramidal cells is rare, but loosely-coupled firing is common—CA3 pyramidal cells have little precisely correlated firing. The lack of precisely correlated firing indicates that extracellular stimulation of multiple afferent fibers, used in the vast majority of studies of synaptic transmission, causes afferent synchronization to a degree that does not occur *in vivo*. However, this method of stimulation retains its usefulness, with two caveats. First, the synaptic behavior elicited by this technique is averaged over several synapses, and cross-synapse heterogeneity will not be reflected. Second, the absolute threshold for activation of associative synaptic processes, including long-term

plasticity and glutamate spillover, may be more sensitive in experiments using multifiber stimulation than it is *in vivo*, and the prevalence of these processes may therefore be overestimated.

Loosely correlated firing, however, is common. This correlation is sufficiently imprecise that it is unlikely to promote temporal summation of AMPA receptor-mediated synaptic transmission, although its effects on kainate receptor-mediated transmission will likely be substantial.

Loosely correlated spike timing may also be important in the induction of spike-timing dependent long-term plasticity (Bell et al., 1997; Markram et al., 1997). Correlated spike trains based on *in vivo* recordings can induce long-term plasticity in visual cortex (Froemke and Dan, 2002), and behavioral manipulations can alter the correlated firing, thereby altering the induction of LTP or LTD, in barrel cortex (Celikel et al., 2004). Asymmetrical cross-correlations between CA3 pyramidal cells could, at least in principle, strongly engage spike timing-dependent plasticity between CA3 pyramidal cells. However, the importance of spike timing-dependent plasticity in the hippocampus is unclear, as pairing of individual presynaptic and postsynaptic spikes does not reliably induce long-term plasticity in the hippocampus (Thomas et al., 1998; Pike et al., 1999; but see Nishiyama et al., 2000).

A model for artificial spike trains mimics spike trains observed *in vivo*—We have developed an empirical model for artificial spike trains that mimic those seen *in vivo*. This model is described at length in the Results section, but we note here that the model can generate spike trains to mimic sustained firing over a range of frequencies, and can also be used to mimic complex changes in frequency such as those seen in response to environmental cues.

We envision several experimental contexts in which this model will be useful. The model will aid in characterizing synaptic dynamics, by ensuring that the appropriate frequencies and ISIs are examined. It will also allow elucidation of whether physiologically relevant patterns of activity favor or suppress the induction of long-term plasticity, relative to constant frequency trains that have been used so far. Finally, it will assist studies to define the contexts in which fast or slow EPSPs dominate cellular excitability. These studies will be helpful in defining the contribution of slow synaptic mechanisms to information processing in the CNS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

CA3 pyramidal cells are highly heterogeneous in their overall activity levels during the performance of a complex task. (A) A summary of the delayed non-match to sample task is shown. (B) Raster plots are shown from 12 CA3 pyramidal cells recorded simultaneously during a trial of the task. There is considerable heterogeneity in the overall activity level between cells. (C) The distribution of mean firing rates during performance of the task is shown, averaged across 13 animals.

Figure 2.

Overall activity is only weakly dependent of cellular function during the task. (A) The mean firing rates of cells, separated by the number of parameters that they discriminate (see text), are shown. There are three significantly different levels of firing: cells that are non-responsive (black), cells that respond but do not discriminate based on all 3 monitored parameters (grey) and cells that respond and discriminate based on every parameter monitored (white). (B) The distributions of mean firing rates are shown for the three groups defined in (A) (solid line, non-responsive cells; dashed line, responsive cells that do not discriminate all 3 parameters; dotted line, responsive cells that discriminate all 3 parameters). Within each group, there is still large variability in firing rate.

Figure 3. ISI distributions of CA3 pyramidal cells are highly heterogeneous. ISI distributions from 9 representative cells are shown as probability density functions (PDFs). There is considerable cross-cell variability in the structure of the ISI distribution.

Figure 4.

As the overall firing rate increases, intermediate ISIs are enhanced. (A) Average ISI distributions for cells grouped together according to the logarithm of the overall firing rate (0.1 Hz, $n=148$; 0.3 Hz, $n=176$; 1 Hz, $n=188$; 3 Hz, $n=119$; 10 Hz, $n=14$). As the firing rate increases, the proportion of very long ISIs falls off dramatically, but the shift in the distribution mainly enhances the intermediate ISIs; the proportion of very short ISIs is largely unchanged. ISI distributions expected based on random firing are also shown (grey lines). (B) Quantification of the change in proportion for very short ISIs (1–10 msec), intermediate ISIs (100 msec–1 sec), and very long ISIs (10–100 sec). The longest ISIs were dramatically reduced as firing rates increased, while intermediate ISIs became correspondingly more prevalent. Very short ISIs were largely insensitive to firing rate.

Figure 5.

Cells show long-range non-random interactions. (A) An autocorrelogram from a representative cell shows a damped oscillation in the theta range. (B) An autocorrelogram from a different cell shows no oscillations, and a rapid decay of non-random interactions between spikes. (C) An autocorrelogram from a third cell shows a rapid decay when examined with high temporal resolution over a short time scale (C₁), but examination of a longer time scale reveals that non-random interactions between spikes that persist for several seconds (C₂). Inset in C₂ shows the slow decay of the autocorrelogram at an expanded scale. The relative contributions of the fast and slow phases of decay could be estimated by fitting the autocorrelogram to multiple exponentials and calculating the integral of each component; in the cell shown, the slow phase of decay accounts for 62% of all non-random spiking.

Figure 6.

The non-random ISI distribution is the main factor generating nonrandom spike sequences. (A) The ISI distribution from a representative cell is shown. (B) The joint distribution of ISIs within each spike triplet from the cell in (A) is shown, with the PDF displayed on a pseudocolor scale (range: dark blue=0; red=0.015) (C) The expected joint distribution of ISIs, presuming random sampling from the ISI distribution in (A), is shown on the same PDF scale as in (B). (D) The difference between the observed joint distribution in (B) and the expected joint distribution in (C) is shown. There is a clear tendency for the spike pairs during a triplet to have matched ISIs, and a clear tendency against mismatched ISIs. (range: dark blue=- were shuffled to remove any non-random spike sequences (not shown), and the difference between the joint distribution of shuffled ISIs and the expected joint distribution in (C) is shown on the same PDF scale as in (D). Shuffling the data strongly reduces the total fraction of non-random spike triplets, and the residual difference (presumably caused by binning and sampling artifacts) shows no obvious pattern. (F) A summary of the total fraction of non-random triplets from cells which did not respond to any of the cues monitored, and those that did. Cells that were responsive during the task had more non-random triplets, but even in this case more than 90% of spike triplets were accurately predicted by random sampling from the ISI distribution.

Figure 7.

Responses to environmental cues are temporally complex. (A) Cells that responded to the onset of the sample odor were analyzed. Raster plots from several repeated exposures to the sample odor are shown for a representative cell (A₁; odor onset at time=0), and the average response for the cell shown in A₁ is shown in A₂ (error bars indicate variability between trials for a single cell). A₃ shows the average change in firing rate during the response for all cells analyzed (error bars indicate variability between average responses for different cells). The response shows distinct phases, with a rapid initial response that decays completely followed by a slow increase in firing. (B) A similar analysis to that in (A) was performed, but for cells that responded to leaving the sample arm (sample arm exit at time=0). The response is biphasic, with a rapid increase in firing followed by a suppression.

Figure 8.

Cellular responses to environmental cues are accompanied by an increased proportion of intermediate ISIs. (A) The ISI distributions of nonresponsive cells (A₁) were compared to the ISI distributions of responsive cells (A₂). Responsive cells had a larger proportion of intermediate ISIs and a smaller proportion of very short and very long ISIs, as seen by the difference between A₁ and A₂ (shown in A₃). To determine the relative prevalence of specific ISIs in responsive or non-responsive cells, the data was re-plotted using a linear difference index on the y-axis, in which a value of 1 indicated an ISI that was seen exclusively in responsive cells, a value of -1 indicated an ISI that was seen exclusively in non-responsive cells, and a value of 0 indicated equal representation in responsive and non-responsive cells (see text; shown in A₄). (B) The ISI distribution from a representative cell that responded during the test phase of the task is shown, during the entire task (B₁), before the test phase (B₂), and during the test phase (B₃). The linear difference index was used to assess which ISIs were more frequent during the test phase (positive values), or before the test phase (negative values) (B₄).

Figure 9.

Discrimination between two options within a parameter can be expressed as a difference in the time-course of the response, or the scaling of the response. (A) A cell that had different responses depending on whether the rat turned into the right test arm (top) or the left test arm (bottom) is shown. The responses differ in their time-course; both responses showed a gradual increase in firing leading up to test arm entry (time=0), but the increase was interrupted by a transient peak of firing preceding right arm entry, and a transient suppression of firing preceding left arm entry. The firing rate also increased after right arm entry, but decreased after left arm entry. (B) A cell that gave different responses just prior to exit of the sample arm (time=0), depending on the identity of the sample odor, is shown. The responses differ in their magnitudes, but have essentially identical time courses. (C) The similarity in time course between responses to each option within a parameter was quantified by measuring the correlation between firing rates seen during different options, at corresponding times during the response. For the cell shown in (A), the correlation is poor (C₁); for the cell shown in (B), the correlation is strong but the slope is obviously less than one (C₂). (D) A summary of 53 cells in which the correlation in firing was assessed during different options of a parameter. The sign of r was preserved in the r^2 calculation, to assess possible anti-correlations in firing (which were not observed). Most (but not all) cells showed little correlated firing during different options, indicating that discrimination between different options of a parameter are generally encoded as differences in response time-course.

Figure 10.

Tightly correlated firing between CA3 pyramidal cells is modest, but loosely correlated firing is common. (A) Six representative cross-correlograms are shown, indicating cell pairs with little correlated firing at all (top left), a peak of synchronous firing (top right), asymmetrically correlated firing (middle left), loosely correlated firing with a slow decay (middle right), weak correlated theta oscillations (bottom left), and a combination of precisely and loosely correlated firing (bottom right). (B) The frequency of non-random synchronous firing is shown for a 10 msec window of synchrony (top) or a 100 msec window of synchrony (bottom). As the time window used to define synchrony is increased, there is increased non-random synchronous firing, allowing quantification of the loose correlation in firing between cell pairs. (C) The average probability of non-random synchronous firing is plotted against the time window used to define synchrony. The loosely correlated firing takes place over a timescale of hundreds of milliseconds.

Figure 11.

An empirical model of CA3 spiking based on the ISI distribution can be used to generate artificial spike trains. (A) The distribution of ISIs as a function of mean firing frequency is shown, with PDF displayed on a pseudocolor scale (range: blue=0; red=0.075). The distribution was based on the ISI distributions in Figure 4 and interpolated, and the mean frequency of each ISI distribution within the total range was measured empirically by random sampling from the ISI distribution. (B) The distribution in (A) was used to model a simulated response in which firing started at 1 Hz, then increased to 5 Hz, then decreased to 2 Hz (top trace, line). The distribution was randomly sampled at 1 Hz, 2 Hz, and 5 Hz to generate raster plots of firing at those frequencies, and then spliced together to mimic the response waveform (raster traces). Although individual simulated responses were variable, as expected, the average of 500 repeated trials of the simulation reproduced the response waveform well (symbols, top trace). (C) Complex cellular responses to environmental cues (top trace, line) could also be simulated in individual trials (raster plots). The average of 500 repeated trials of the simulation demonstrated that the model successfully reproduced the complex changes in mean firing frequency during the response (top trace, symbols). (D) An autocorrelogram of an artificial spike train of 20,000 spikes at 1 Hz mean frequency showed a slow component of decay, similar to that observed in autocorrelograms from CA3 pyramidal cell recordings. Inset shows the slow decay on an expanded scale.