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Electrophysiological Characteristics of Hippocampal Complex-spike Cells and Theta Cells*

S.E. Fox and J.B. Ranck, Jr.

Department of Physiology, Downstate Medical Center, State University of New York, 450 Clarkson Avenue, Brooklyn, NY 11203, USA

Summary. Stimulating electrodes were chronically implanted in the ventral hippocampal commissure and the entorhinal cortex or angular bundle of rats. Moveable metal microelectrodes which could be passed through the hippocampus were implanted. All hippocampal units were classified as complex-spike cells or theta cells on the basis of the form of their action potentials and their rates of firing in various behaviors. Field potentials and unit firing evoked from the stimulating electrodes were recorded during slow wave sleep.

Complex-spike cells (1) could often be antidromically activated in CA3 (it was not attempted in CA1); (2) could only be induced to fire one or two action potentials in response to a single stimulus; (3) had action potentials at the same time as the local population-spike and, in condition-test studies, were depressed when the population-spike was depressed. (The population-spike is presumably the summed synchronous action potentials of pyramidal cells.)

Theta cells: (1) were antidromically activated in only one out of 25 cases; (2) usually could fire long bursts of action potentials in response to a sufficiently intense single stimulus; (3) this firing occurred before, during, and after the local orthodromic population-spike.

Most complex-spike cells in Ammon's horn must be pyramidal cells (projection cells), and vice versa. The case for theta cells is more difficult. Some are non-pyramidal cells with locally ramifying axons, but at least some are projection cells. The data is consistent with most of them being inhibitory interneurons, but this is not established.

Key words: Hippocampal electrophysiology – Complex-spike cells – Theta cells – Projection cells – Interneurons

There are two major classes of units in Ammon's horn of freely-moving rats as defined by their behavioral correlates and firing repertoires, which we have called complex-spike cells and theta cells (Ranck 1973b). The object of this study was to determine their electrophysiological characteristics.

Theta cells are defined as cells which increase (approx. double) their rate of firing if, and only if, there is a slow wave theta rhythm in hippocampus (Ranck 1973b). The defining characteristic of a *complex-spike cell* is that it sometimes has a spontaneously occurring burst of about 2–10 action potentials of decreasing amplitude and increasing duration recorded extracellularly, with very short ($\leq$ 5 ms) interspike intervals. We call this burst a complex-spike. However, a complex-spike may be a rare event in such a cell. These cells also have single isolated spikes of constant amplitude, which are the same amplitude as the first spike of the complex-spike. Some of these cells never fire complex-spikes except in slow wave sleep.

Because of their relative proportions and locations we have previously suggested that the class of projection cells and the class of complex-spike cells have a very large overlap and the class of interneurons and the class of theta cells have a very large overlap (Fox and Ranck 1975). We were interested in electrophysiological evidence for or against this suggestion. We were also interested in determining whether the electrophysiological characteristics of

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Offprint requests to: Dr. S.E. Fox (address see above)

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theta cells were like the electrophysiological characteristics of neurons which Andersen and his colleagues recording in acute preparations have called "basket" cells (Andersen et al. 1964b).

Methods

The methods are described in greater detail elsewhere (Fox and Ranck 1979).

Surgery and Electrodes

Fifteen male Long-Evans (hooded) rats weighing 350–450 g were anesthetized with pentobarbital (40 mg/kg, i.p.). Electrodes were implanted stereotaxically under electrophysiological control using aseptic techniques. Microelectrodes were etched tungsten or stainless steel insulated with Epoxylite to within 10 μm of the tip. They were held in moveable carriers modified after Ranck (1973a). Stimulating electrodes were pairs of 150 μm nichrome wires cut off square and cemented together with their tips separated by 0.7–1.0 mm longitudinally. These were placed in *ventral hippocampal commissure* (VHC) contralateral to unit recording sites and in angular bundle or *entorhinal cortex* (EC-AB) on the same side as the unit recording sites. Hippocampal macroelectrodes (150 μm nichrome), neck muscle EMG electrodes (150 μm nicrome), neocortical EEG electrodes (stainless steel screws inserted through frontal or temporal bones) and an animal ground wire (silver) were also implanted. Animals were allowed to recover 5–7 days prior to electrophysiological studies.

Recording Equipment

The output of each recording electrode passed through an FET headstage in source-follower configuraion mounted on the rat's head. All microelectrode recording was with respect to one of the EEG screws. A mercury commutator carried both the outputs of the headstages and the incoming electrical stimuli. Amplifiers were Grass 7P511's (–6 dB/octave below 0.1 Hz and above 10 KHz) ("wide band"). For unit recording their outputs were passed through active filters (–12 dB/octave below 500 Hz) ("narrow band"). Modified Tektronix PG505 pulse generators drove Grass PSIU6 constant-current stimulus isolation units, capacitatively coupled (0.47 μF) to the stimulating electrodes.

Data Collection and Analysis

The EC-AB and VHC stimulating sites were stimulated in rotation at 0.1 Hz, which is too slow for frequency potentiation to occur (Douglas and Goddard 1975). Stimulus intensity was usually ≤ 1 mA. Chronaxies for stimulation of VHC and EC-AB were 0.2–0.3 ms. Therefore, durations were always 0.1–0.2 ms for VHC and EC-AB stimulation; in all cases intensities were kept below threshold for an overt behavioral response.

Data was either analyzed on-line with a storage oscilloscope and Polaroid photographs, or recorded on a 7 channel FM tape recorder for later analysis. A PDP 11/45 computer was used to average field potential data and to generate peri-stimulus time histograms for unit data. Since Winson and Abzug (1978) have shown that hippocampal evoked responses vary as a function of behavioral state, all of the data were collected during slow wave sleep.

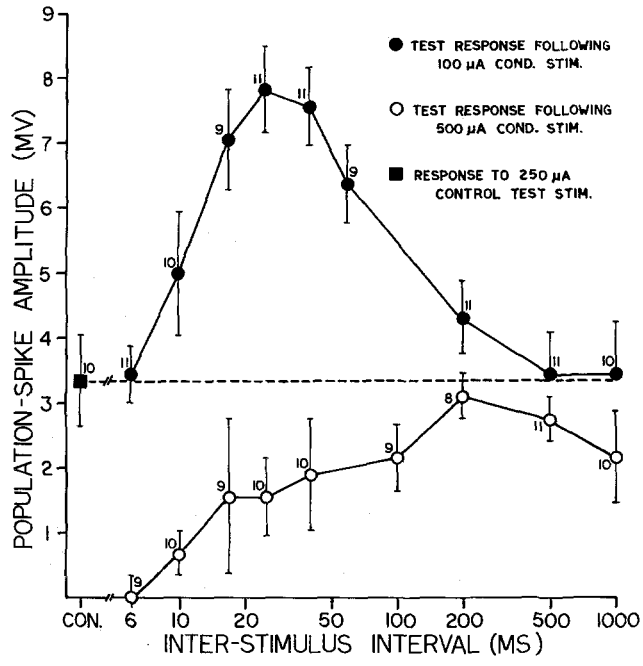

Fig. 1. Amplitude of CA1 pyramidal cell population-spike in response to a test stimulus to ventral hippocampal commissure at different intervals following a conditioning stimulus. The test stimulus intensity is 250 μA in all cases. Filled circles represent response to the test stimulus following a 100 μA conditioning stimulus. Open circles represent response to the test stimulus following a 500 μA conditioning stimulus. All points are averages of about ten responses. Exact number is indicated near each point. Bars indicate 95% confidence limits (±2 times standard error of the mean). All stimuli 200 μs in duration. Note that the time scale is logarithmic

Field Potential Studies

Field potential components evoked by electrical stimulation of major monosynaptic inputs to hippocampal formation have been studied extensively (Andersen et al. 1966, 1971). Briefly, in the layer of cell bodies of projection cells the most common pattern is a positive wave followed by a negative spike, which is followed, in turn, by a relatively long duration positive wave. The initial positive wave is presumed to represent, at least in part, the summed extracellular currents due to EPSPs generated in the distal dendrites (Andersen et al. 1966). The negative spike ("population-spike") has been shown to represent synchronous activation of large numbers of projection cells (Andersen et al. 1971). The subsequent positive wave is interpreted as having a large contribution from summed extracellular currents due to IPSP's in the projection cells (Andersen et al. 1964a). A negative spike, which arises from the baseline, replaces the initial positive wave in the CA3–4 pyramidal layer in response to ventral hippocampal commissure stimulation. This spike presumably reflects synchronous antidromic activation of pyramidal cells (Andersen et al. 1971) which project to the opposite hippocampal formation. Field potential studies were done because they tell us what charateristics to expect in "most" of the neurons in Ammon's horn or dentate gyrus.

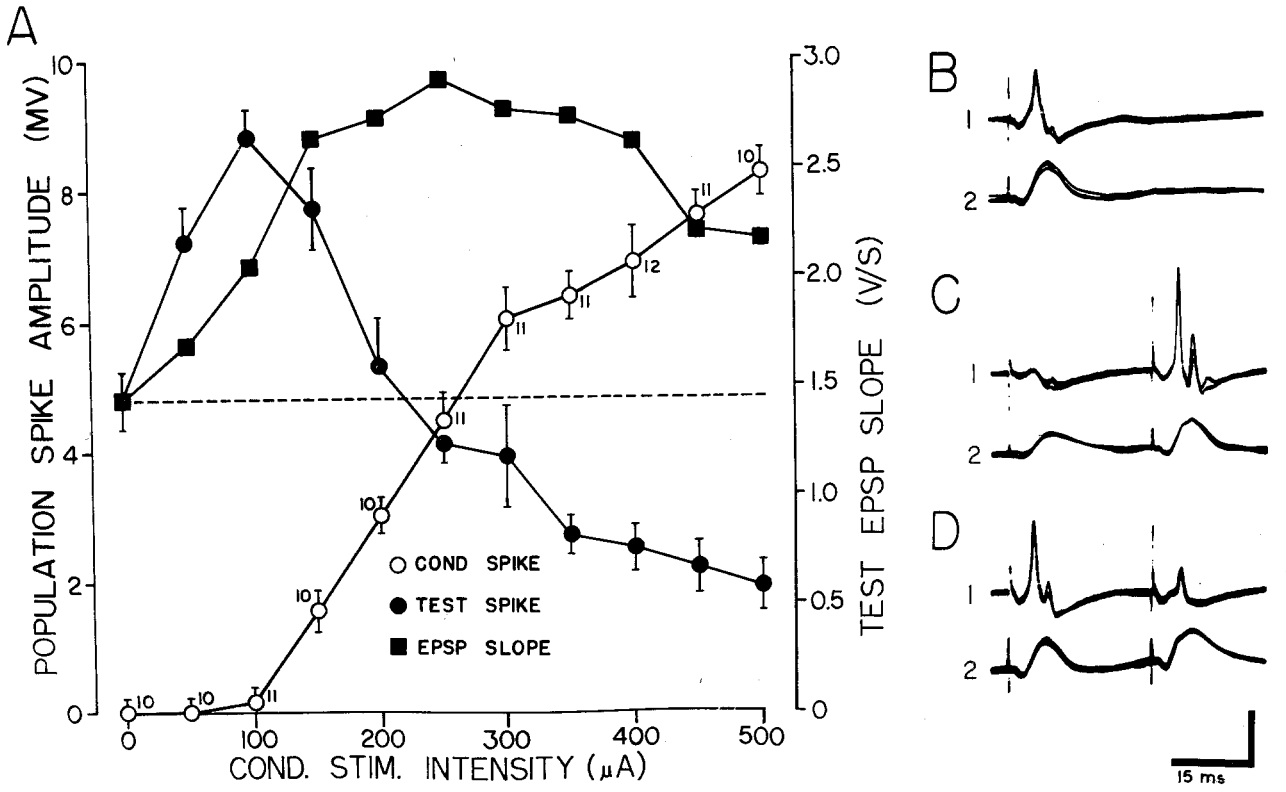

Fig. 2A–D. Facilitation and depression of field potential components in CA1 demonstrated by conditioning and test stimuli to ventral hippocampal commissure. **A** Graphic representation. The left ordinate shows amplitude of population-spike in the pyramidal cell layer of CA1; the right shows rate of rise of the negativity in the apical dendritic layers (representing summed extracellular potentials due to EPSPs) at 4.0 ms after a test stimulus. Otherwise, same as Fig. 1. **B** Response to a 250 μA test stimulus. Trace 1: CA1 pyramidal cell layer. Trace 2: apical dendritic layers. **C** Response to 100 μA conditioning stimulus and 250 μA test stimulus. Traces 1 and 2 same as in **B.** Condition-test interval: 27 ms. **D** Response to 400 μA conditioning stimulus and 250 μA test stimulus. Otherwise same as **C.** There is a second small population-spike after the first in **C1** and **D1.** This sometimes occurs with higher intensity stimulation. The amplitude of the first and larger population-spike is the one which is reported. Calibrations for **B–D;** 15 ms, trace 1: 6 mV, trace 2: 12 mV. All stimuli 200 μs in duration. Negative is up in this and all other figures showing traces

Unit Recording Protocol

After recording microelectrodes had been lowered through neocortex and sub-cortical white matter, the amplitude and duration of spike, number of turns below surface, and presumed localization (based on form of field potentials) were noted for each isolated hippocampal unit with a signal to noise ratio greater than 3:1. In addition, each such unit was classified according to the criteria discussed in the Introduction as a complex-spike cell or a theta cell. If the unit was not driven (or inhibited) at short latency (≤ 20 ms) by stimulation of any of the sites at moderate intensities, it was abandoned, and the microelectrode was advanced until another unit was isolated. In occasional cases multi-unit complex-spike cell data was used to provide an indication of the properties of that group as a whole. Only those single units for which there was histological confirmation of localization were included for analysis.

For all units driven from one or more of the stimulation sites, stimulus intensity at threshold, latency and latency jitter at threshold, and relation to local field potential components were recorded during slow wave sleep. Units which were driven at very short latency and/or showed ≤ 200 μs latency jitter at threshold were tested for antidromic activation by measuring refractory period for dual pulses, and attempting collision by triggering the stimulator with a spontaneous action potential.

Histology

Rats were perfused with formal-saline, and frozen sections were stained with cresyl violet. The recording sites were identified by distance from lesions made with tungsten electrodes or with spots made the Prussian blue reaction after passing current from stainless steel electrodes.

Results

Field Potential Responses to Dual Stimuli

Dual pulse field potential studies were carried out to describe the recovery cycle "most" neurons in an area to monosynaptic inputs so that unit data could

be compared to it. Dual pulse studies of monosynaptic excitation of CA1 by neurons of CA3 and CA4 from stimulation of ventral hippocampal commissure (activating commissural fibers and/or Schaffer collaterals) have not been previously reported, although they may be related to some observations in hippocampal slices (Alger and Teyler 1977). About ten responses were recorded for each pair of stimuli during slow wave sleep. Average evoked potentials were computed and various measures were plotted for each combination of conditioning and test stimuli.

After a small conditioning stimulus (Fig. 1) there is a facilitation of the test population-spike starting after 6 ms and returning to control levels at 500 ms. After a large conditioning stimulus there is a depression of the test population-spike lasting for at least 100 ms. In other animals there was sometimes a small facilitation after 100 ms. If the conditioning-test interval is constant at 27.0 ms and the intensity of the conditioning stimulus increased (Fig. 2), the test population-spike is facilitated with weak conditioning stimuli, and depressed with stronger ones, the facilitation becoming less or being replaced by depression with conditioning stimuli which produce a population-spike. The test EPSP is facilitated at all strengths of conditioning stimuli. The pattern shown in Figs. 1 and 2 was seen in all five rats tested. Negativity is up in all figures.

Dual pulse stimulation of perforant path while recording field potentials in dentate was performed in three rats. The data was about the same as reported previously in anesthetized (Lømo 1971) and unanesthetized (Bliss and Gardner-Medwin 1973) rabbits and anesthetized rats (Steward et al. 1976). It was also similar to that shown for the CA3 to CA1 dual pulse studies except that there was a depression of test stimuli at interstimulus intervals less than about 15 ms for weak stimuli.

Units Responding to Ventral Hippocampal Commissure Stimulation

Complex Spike Cells. Seven of eight complex-spike cells in CA3 and CA4 were apparently driven antidromically (by either the "soft" criteria of latency and latency jitter ($\leq$ 0.2 ms) or the "hard" criterion of a positive collision test). Since the VHC electrode was contralateral to the recording electrodes one would not expect antidromic activation of CA1 pyramidal cells. Indeed, there was never even suggestive evidence of CA1 complex-spike cells being activated antidromically from VHC. No attempt was made to antidromically activate CA1 complex spike cells from sites which would have been appropriate.

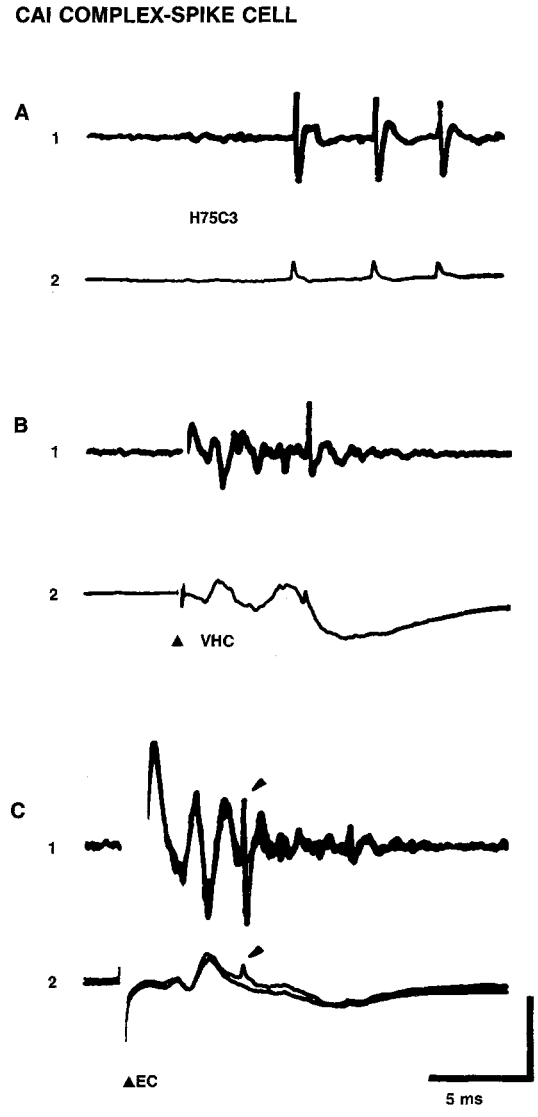

Fig. 3A–C. Relation of evoked action potentials of CA1 complex-spike cell to field potentials. Trace 1: narrow band. Trace 2: wide band. **A** Spontaneous complex-spike. **B** Response to 200 μA stimulus (at triangle) to ventral hippocampal commissure. Note that unit response occurs on falling phase of small population-spike. **C** Response to a 1.7 mA stimulus to entorhinal cortex. There is no CA1 population-spike. Action potential occurs at small arrow in one of the sweeps. All stimuli 200 μs in duration. Calibration: trace 1: 800 μV, trace 2: 5 mV

The seven apparently antidromically driven cells fired at short latency (2–3 ms) on or near the "antidromic" population-spike component of the field potential. Three of these were also activated orthodromically, so that two action potentials occurred, one at constant short latency and a second at a longer more variable latency. The antidromic population-spike component of the field potential was often very large (up to 15 mV) in comparison to the single units (0.1–1. mV), and had a somewhat

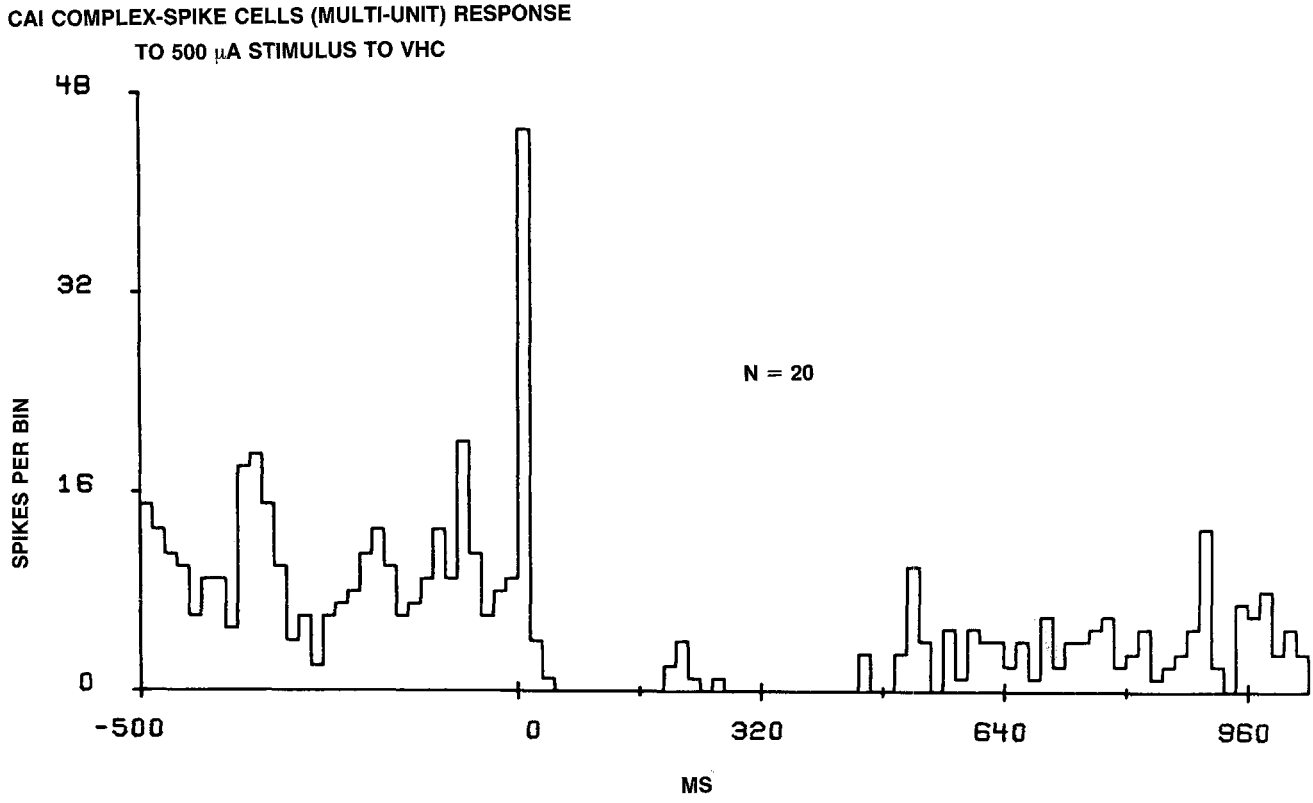

Fig. 4. Depression of spontaneous unit activity of multiple CA1 complex-spike cells following activation. Ventral hippocampal commissure stimulated (500 μA) at time zero. Histogram of twenty responses. Bin width is 16 ms. The number of action potentials in the bin immediately following the stimulus shown here is not a true representation of the data. There were actually more than are shown here. Population-spikes in response to these stimuli were too large to filter out data for individual units. The remainder of the data is an accurate representation of the original. All stimuli 200 μs in duration. See text for further details

similar duration (1.5–3 ms for the population-spike as compared to 0.5–1.0 ms for the units). It was common to find that threshold for population-spikes was lower than for driving of local units. Therefore, it was rare to find units which were driven at stimulus intensities at which they could be seen in a narrow band record. There was also difficulty with isolation since the cells are so closely packed in the cells layers. As a result, it was possible to demonstrate collision in only two complex-spike cells. In one of these sufficient data was collected to show that it was antidromically activated by the criteria of Fuller and Schlag (1976).

The six complex-spike cells recorded in CA1 were activated only orthodromically from stimulation of VHC. Their firing was highly correlated in time with the orthodromic population-spike in CA1. Figure 3 shows such a unit. A spontaneous complex-spike is shown in Fig. 3A. Figure 3B illustrates the response to VHC stimulation. The cell fires on the negative CA1 population-spike. Figure 3C demonstrates that the unit could also be driven synaptically (about 1 ms jitter at threshold) at short latency from EC-AB stimulation. The positive-going population-spike in Fig. 3C 2 is presumably volume conducted from dentate, and there is no CA1 population-spike, which explains why this CA1 cell does not fire on the population-spike. This is an atypical cell in that only three of 14 complex-spike cells were driven from both VHC and EC-AB stimulation at moderate intensities.

Spontaneous activity in all complex-spike cells was drastically reduced or eliminated following activation sufficient to produce a population-spike. This was most dramatic when multiple complex-spike cells were recorded simultaneously, since any single unit had a very low spontaneous rate. Figure 4 is a peristimulus time histogram of multi-unit complex-spike cell data recorded from CA1 during slow wave sleep. (Theta cells were not being recorded simultaneously from this electrode). Following a 500 μA stimulus to VHC, many complex-spike cells were activated at short latency, and then spontaneous activity was reduced for up to a second or more.

It was very difficult to drive complex-spike cells during this period of reduced spontaneous activity

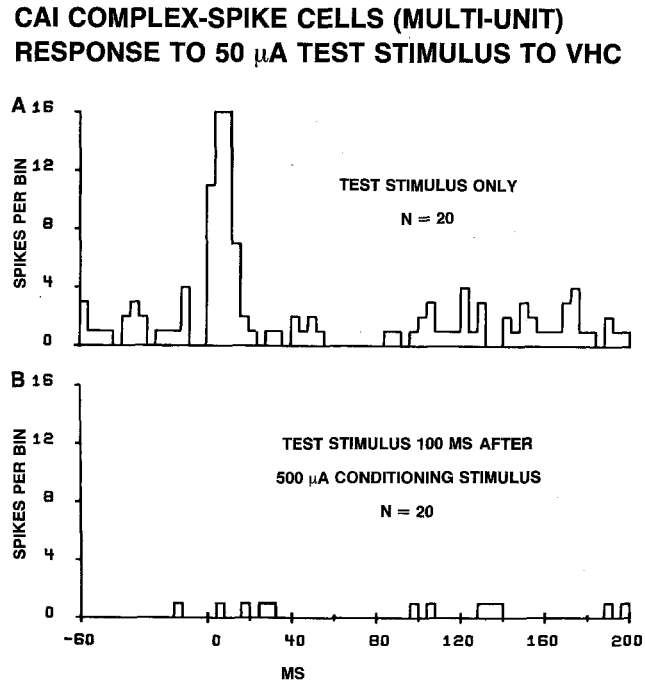

Fig. 5A, B. Depression of evoked unit activity of multiple CA1 complex-spike cells following activation. **A** Control response to 50 μA stimulus to ventral hippocampal commissure. Histogram of 20 responses. Bin width is 4 ms. This intensity, below the threshold for production of a measureable population-spike, was chosen in order to be certain that the number of evoked action potentials in the histogram was a true representation of the original data. When there is no population-spike, activation of complex-spike cells occurs over a longer time following the stimulus than when the population-sike occurs (at higher stimulus intensities). This explains the relatively long duration of activation seen here (~ 16 ms). **B** Response to 50 μA stimulus to ventral hippocampal commissure presented 100 ms after a 500 μA conditioning stimulus to the same site. Histogram of twenty responses. Bin width is 4 ms. Almost no response occurs. Also note that "spontaneous" activity in the 60 ms preceeding the test stimulus is greatly reduced. All stimuli 200 μs in duration

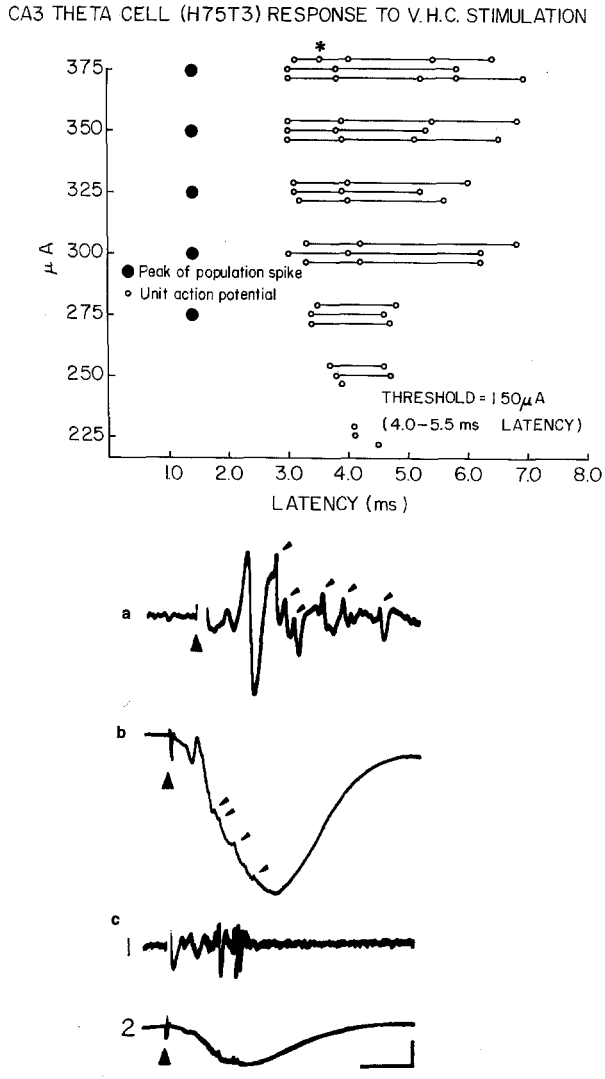

Fig. 6. Relation of evoked action potentials of CA3 theta cell to field potential. The graph shows latency of action potentials (open circles) in response to increasing stimulus intensities to ventral hippocampal commissure (three trials per intensity). The latencies of action potentials for each trial are joined by horizontal lines. Filled circles represent latency to peak of CA3 antidromic population-spike. Asterisk (*) marks latency of action potential of curiously short inter-spike interval (ISI) which was seen several times at higher stimulus intensities. *Trace a:* narrow band record of response to 425 μA stimulus (at triangle). Small arrows mark action potentials. Again note short ISI. Calibrations: 2 ms, 200 μV. *Trace b:* wide band record of response to 475 μA stimulus (at triangle). Small arrows again mark action potentials. Calibrations: 4 ms, 750 μV. *Traces c1* and *c2:* response to 250 μA stimulus (at triangle). *1:* narrow band; *2:* wide band. Two superimposed sweeps. Note that the unit is activated even though there is almost no population-spike. Calibrations: 4 ms, 1: 200 μV, 2: 500 μV. All stimuli 200 μs in duration

which followed activation. Figure 5 shows a histogram of the response to a small stimulus (50 μA) to VHC for the same CA1 multi-unit activity shown in Fig. 4. Figure 5 illustrates the effect of a 500 μA conditioning stimulus preceeding the 50 μA test stimulus by 100 ms. There was practically no response at all. Evoked activity of synaptically driven complex-spike cells, like the population-spike in field potential studies, was profoundly depressed or eliminated following a large conditioning stimulus in all five units (in four rats) in which this was tested. In all 14 cases the electrophysiological characteristics of complex-spike cells were similar to what would be predicted for projection cells from population-spike field potential data. Dual pulse *facilitation* of units was not able to be demonstrated due to contamination of the record by the population-spike.

Theta Cells. Twenty-six theta cells were studied, 17 in CA1, nine in CA3 and CA4. One unequivocally identified theta cell in CA1 was antidromically driven from stimulation of EC/AB about 4 mm away. The

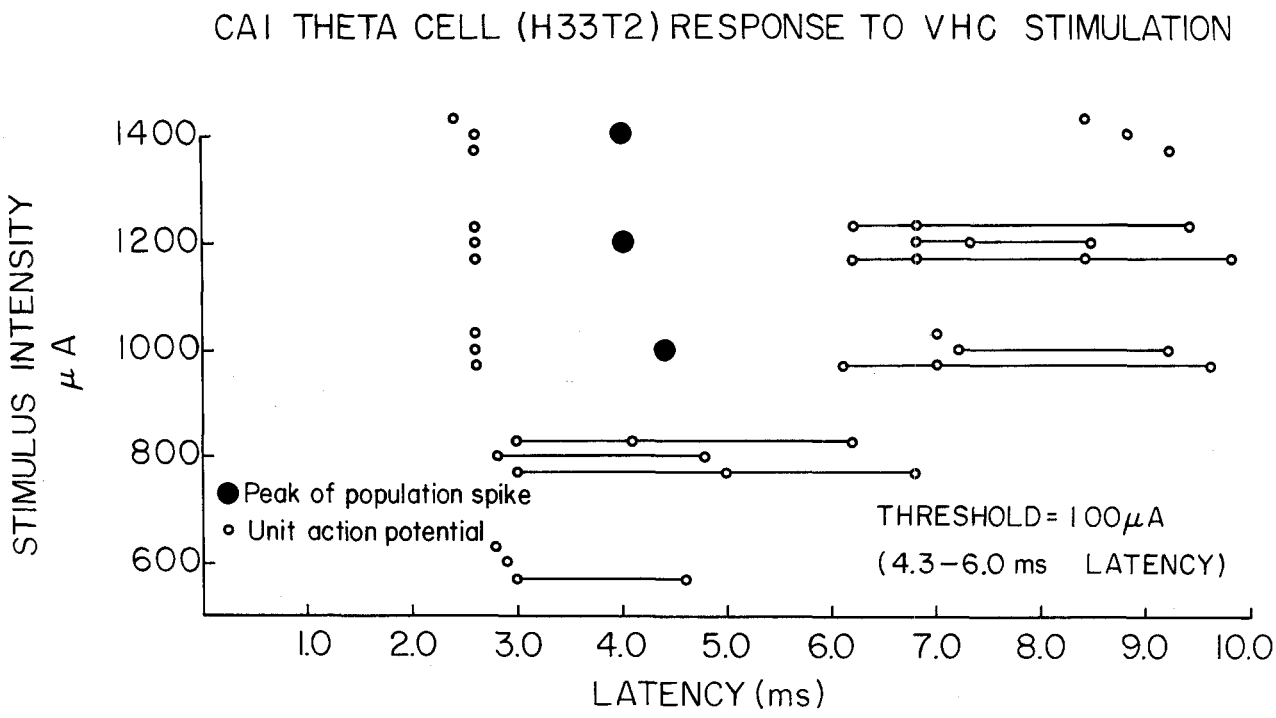

Fig. 7. Relation of evoked action potentials of CA1 theta cell to field potential. Filled circles represent peak of orthodromic CA1 population-spike. Otherwise, representations as in Fig. 6. Note evoked action potentials occurring prior to the peak of the population-spike. These also have the lowest threshold. Latencies of action potentials preceding the population-spike for a given trial are not joined with horizontal lines to those which follow the population-spike. All stimuli 100 μs in duration

antidromic driving met all criteria including the collision cirteria of Fuller and Schlag (1976). In all other theta cells activated there was never a hint of antidromic driving from any stimulation site. In particular, none of the eight theta cells in CA3 and CA4 were antidromically activated by any criterion (as compared to seven of eight complex-spike cells in CA3 and CA4). There was always ≥ 0.4 ms latency jitter at threshold, and latency always decreased with increasing stimulus intensity.

Theta cells had response patterns which were very different from those of complex-spike cells. Figures 6 and 7 show the responses of a CA3 theta cell and a CA1 theta cell, respectively, to increasing intensities of stimulation of VHC. At the intensities which were used, there was only an antidromic population-spike in CA3 and the theta cell fired multiple action potentials on the positive extracellular field potential that followed it. The threshold of the unit was lower than the threshold for producing an obvious population-spike (Fig. 6). In CA1, theta cell action potentials in response to stimulation of VHC occurred before, during and after orthodromic population-spikes (Fig. 7). Following large population-spikes, amplitude of unit action potentials was often decreased.[1] Latency decreased as stimulus intensity increased in both CA1 and CA3. The single CA4 theta cell which responded to VHC stimulation had characteristics very much like those in CA3. There were five theta cells in CA3 or CA4 which were driven by stimulation of EC/AB at minimum latencies of 2.5, 2.8, 5.4, 7.0, and 8.0 ms.

Spontaneous activity of theta cells was reduced following activation by stimulation of VHC, like complex-spike cells. Figure 8 is a peristimulus time histogram of the response of a CA1 theta cell to a 500 μA stimulus to VHC.

Dual pulse studies of theta cells revealed aspects of both facilitation and depression of response to a second (test) stimulus. Figure 9A illustrates the response of the same CA1 theta cell shown in Fig. 8 to increasing test stimulus intensities. Latency and

1 The amplitude of extracellularly recorded action potentials of theta cells does not decrease during "spontaneous" firing, only during electrically evoked firing. This evoked decreasing amplitude of theta cell spikes does not introduce an exception or ambiguity to the definitions of theta cells and complex-spike cells, which are based on "spontaneous" activity. Theta cells *never* have spontaneous complex-spikes (decreasing amplitude bursts)

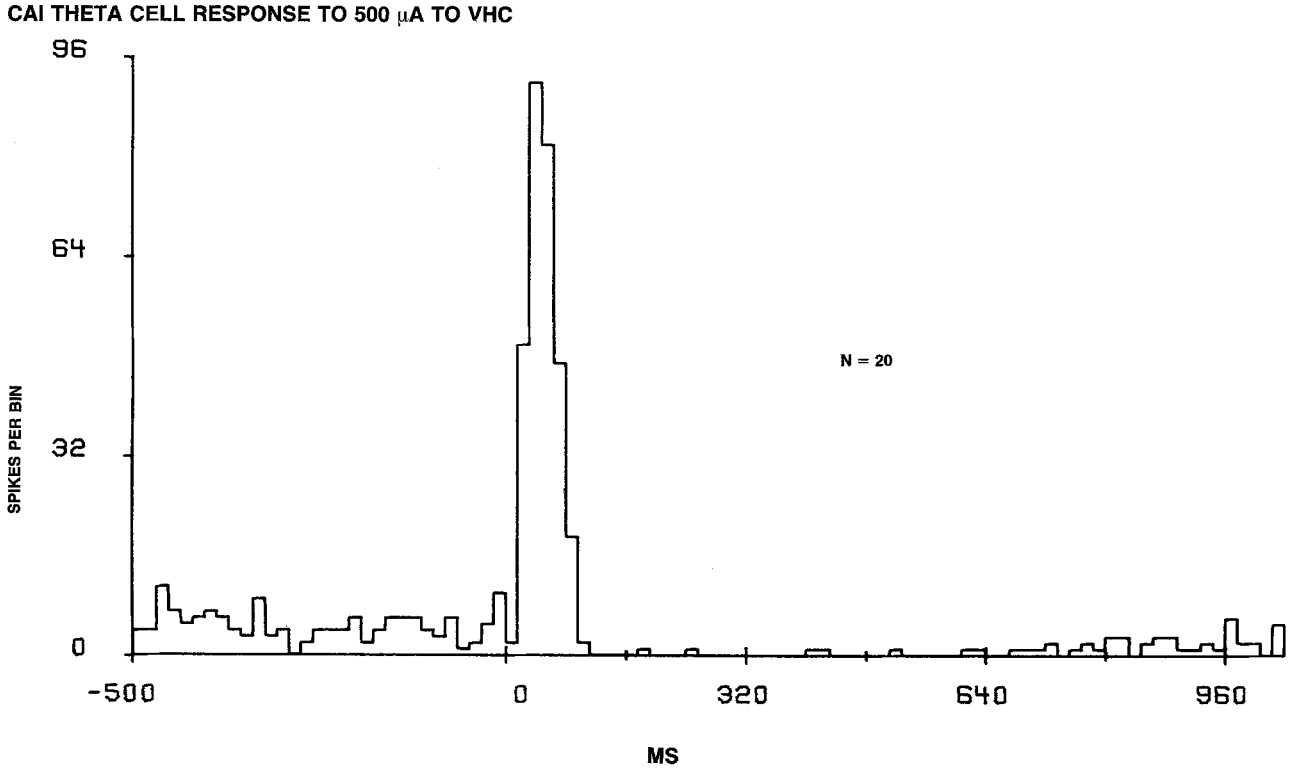

Fig. 8. Depression of spontaneous activity of a CA1 theta cell following activation. Ventral hippocampal commissure stimulated (500 μA) at time zero. Histogram of 20 responses. Bin width is 16 ms. The number of action potentials in the first bin following the stimulus is not a true representation of the data due to artifact during population-spike. The true value is unknown. The remainder of the data is accurate. All stimuli 200 μs in duration. See text for further details

number of post-population-spike action potentials were a function of stimulus intensity. When the test stimulus followed a 500 μA conditioning stimulus by 100 ms (Fig. 9B), the latency of the first action potential in response to the test stimulus was *decreased* at all intensities (especially at low intensities). Average latency in response to a 50 μA test stimulus decreased from 6.2 ms (with no conditioning stimulus) to 4.8 ms (following a 500 μA conditioning stimulus). On the other hand, the amplitude of the population-spike was decreased (presumably indicating decreased activation of projection cells), and the number of post-population-spike action potentials of the theta cell was decreased. Comparison of Fig. 9 with Fig. 8 shows that when spontaneous activity was profoundly reduced following activation, evoked activity was only slightly depressed (as was the case in eight of nine units tested); and by the measure of latency to first spike, evoked activity was facilitated (in six of the nine units from seven rats).

Other Characteristics

Synaptic driving of theta cells was highly effective. One was shown to follow trains of up to 50 pulses delivered to VHC at frequencies as high as 100/s. Action potentials were produced by dual stimuli with as short as 2.8 ms inter-stimulus interval. Synaptic driving at high frequency is not without precedent. Eccles et al. (1960) described a population of interneurons in spinal cord which followed 50 ms trains of pulses at up to 700/s.

Theta cells were more likely to be driven synaptically from more than one stimulating site than were complex-spike cells. Forty percent of the theta cells driven by VHC stimulation (ten of 26) also responded to stimulation of EC-AB, while only 20% of complex-spike cells driven by VHC stimulation (three of 14) responded to stimulation of EC-AB. Thresholds for activation of theta cells tended to be somewhat lower than for complex-spike cells, although the difference was only significant at the 0.08 level.

The numbers of units studied do not represent their true relative proportions in the various hippocampal areas (Fox and Ranck 1975). The fact that CA1 theta cells are over-represented reflects their ease of isolation, and the fact that two or three microelectrodes usually penetrated CA1 at some point, while only one of the three were usually

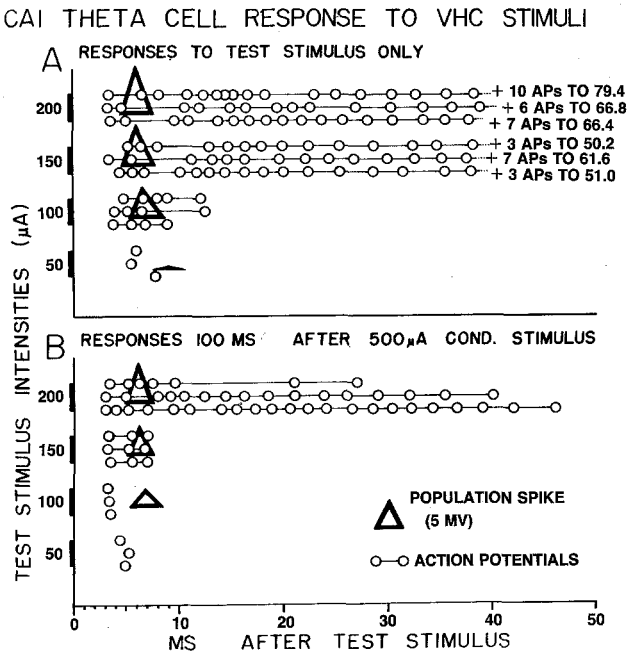

Fig. 9A, B. Facilitation and depression of evoked activity of a CA1 theta cell following activation. **A** Control response to increasing intensities of stimuli to ventral hippocampal commissure. Triangles represent orthodromic CA1 population-spike. The base represents onset and offset of population-spike. Vertex represents peak latency and amplitude. Otherwise, representations as in Fig. 6. Note that the duration of burst following the population-spike at high stimulus intensities was uncommonly long. **B** Responses to increasing intensities of stimuli to ventral hippocampal commissure presented 100 ms after a 500 μA conditioning stimulus to the same site. Representations as in **A**. Note that (1) latency to the first (pre-population-spike) action potential is decreased, (2) population-spike amplitude is decreased (as in Fig. 4), (3) number of post-population-spike action potentials is decreased. All stimuli 200 μs in duration. See text for further details

advanced to CA3 or CA4. Units driven from VHC are over-represented here because VHC stimulating electrode was almost always "on beam", while the EC/AB electrode was not.

Units Responding only to Stimulation of Entorhinal Cortex

Three complex-spike cells were recorded from the cell layer of CA1 which responded to stimulation of EC-AB at moderate intensities, but *not* to stimulation of VHC. Their responses to stimulation of EC-AB occurred at the same time as the negative wave in the field potential, which followed the inverted population-spike (apparently volume conducted from fascia dentata). In this respect their responses were similar to Fig. 3C (except that they could not be driven from VHC). Five of the six complex-spike cells driven by EC/AB stimulation fired at latencies between 5 and 10 ms suggesting monosynaptic activation.

One theta cell was recorded inside the hilus of fascia dentata which responded only to EC-AB stimulation. It fired multiple action potentials on the positive wave following the population-spike recorded from the granule cell layer.

Discussion

Complex-Spike Cells and Theta Cells

Table 1 is a list of characteristics of complex-spike cells and theta cells from this and previous studies. That they are two very different populations of cells is evident from the large number of differing characteristics. Taken together these differences between these two classes of cells are so clear that one expects to find equally clear anatomical distinctions. However, even though almost all action potentials recorded in Ammon's horn are clearly from complex-spike cells or theta cells, it does not follow that all (or almost all) neurons in Ammon's horn must belong to one of these two classes, for perhaps there are some neurons without action potentials.

There are two obvious anatomical criteria which can be applied to the neurons of Ammon's horn to divide them into two mutually exclusive sets. The first divides them into projection cells and interneurons by the limits of their axonal ramifications. The second divides them into pyramidal and non-pyramidal cells on the basis of somato-dendritic morphology. These two criteria have a great deal of overlap, for almost all pyramidal cells are projection cells and vice versa. However, we cannot assume that these criteria are equivalent. Chronister and De France (1979) have recently pointed out that both Ramón y Cajal (1968) and Lorente de Nó (1934) noted that some neurons which were not pyramidal cells seemed to send axons into the alveus, and hence might be projection cells.

The only electrophysiological characteristic which conclusively identifies a projection cell is antidromic driving with collision. There are no electrophysiological characteristics which conclusively identify a neuron as an interneuron. There are, however, certain characteristics in an electrophysiological study which one would expect for projection cells in hippocaompus and others for interneurons. Projection cells of Ammon's horn should: (1) be a vast majority, (2) have cell bodies predominantly in pyramidal cell layer, (3) be able to be driven antidromically from output tracts, (4) fire during population-spikes, and (5) have a recovery cycle which corresponds to the recovery cycle for population-spikes. Hippocampal interneurons should (1) be a

Table 1. Characteristics of Hippocampal Complex-spike and Theta Cells[a]

Characteristic	Complex-spike cells	Theta cells
1. Spontaneously occurring action potentials		
A: Type	Single *and* complex	*All* single
B: Extracellular negative spike:		
Duration (wide band)	0.4 to 1.0 ms	0.2 – 0.4 ms
Mean amplitude ± s.d.	267 ± 193 μV (N = 613)	164 ± 74 μV (N = 74)
2. Rates		
A: Behavior for maximum	Various specific behaviors or spatial locations	Theta mode behaviors (walking, jumping, etc.)
B: Maximum	Most < 20/s Sustained for < 2 s	Most 30 – 100/s Sustainable for min (Most 2X non-theta rate)
C: In slow wave sleep	Most 0.5 – 5/s	Most 10 – 40/s (non-theta)
D: In paradoxical sleep	Most < 0.1 – 1/s	Most 30 – 100/s (theta-mode)
3. Phase relation to theta	Usually[b]	Usually
4. Localization	Most in pyramidal and granule cell layers	Relatively more diffuse
5. Relative number	Most cells	< 10% of cells
6. Maximum number of evoked action Potentials (single stimulus)	1 – 2	Usually many
7. Relation of evoked action potentials to field potential	On population-spikes (when present)[c]	Most on post-population-spike positive waves
8. Antidromic activation	Common	Rare
9. Post-activation response		
A: Spontaneous activity	Profoundly reduced	Profoundly reduced
B: Synaptically evoked activity	Profoundly reduced	Facilitated or slightly reduced
10. Activation from multiple stimulation sites	Few	Many

[a] Data for characteristics I through V are summarized from previous papers (Ranck 1973b; Fox and Ranck 1975). The remainder of the characteristics are summarized from the data of this paper

[b] *Note:* (a common error) Both complex-spike cells and theta cells *do* have a phase relation to theta rhythm, but since theta cells usually fire much faster, their relation is visually more obvious than that of complex-spike cells

[c] Below the threshold for population-spike complex-spike cell action potentials in response to synaptic activation often occurred over a fairly broad time period following the stimulus (5–20 ms). Only when population-spikes were present in the field potential, was firing of complex-spike cells synchronous

minority of cells, (2) have cell bodies distributed all over hippocampus, but be concentrated in stratum oriens of CA1 and CA3a, apical dendritic layers of CA3b and c, and the hilus of fascia dentata (Fox and Ranck 1975), (3) never be antidromically driven from extra-hippocampal sites.

All of the characteristics of complex-spike cells are consistent with the notion that they are projection cells. Although conclusive evidence that complex-spike cells are projection cells (antidromic activation with collision) was only obtained for two cells, other evidence suggests that this is the general case. Most CA3 and CA4 complex-spike cells were antidromically activated from VHC by the criteria of latency and latency jitter. All complex-spike cells (including those in CA1 which could not be tested for antidromic driving from these stimulating sites) have electrophysiological characteristics which suggest that they are the cells responsible for population-spikes. Furthermore, purely on the basis of numbers and localization most complex-spike cells must be projection cells (and also pyramidal cells).

The case for theta cells is more difficult. In one case a theta cell was unequivocally a projection cell by collison criteria, so the hypotheses that all theta cells are interneurons and all projection cells are complex-spike cells must be rejected. Much of what we expect projection cells to do is based on field potential studies, which means it tells us what we expect *most* projection cells do. Theta cells are a small minority of hippocampal neurons and hence, some or even all could be projection cells whose behavior is not predicted by field potential studies.

Recently Chronister and De France (1979) examined the dentritic pattern of neurons in Ammon's horn which were labeled by retrograde uptake of horseradish peroxidase from a small injection into septal region – all such neurons are projection neurons. They found that some of these projection neurons are non-pyramidal neurons with their somas outside the stratum pyramidale. These were not just displaced pyramidal cells, but neurons with the polymorphic and fusiform cell bodies and dentritic tree previously thought to be characteristic of

interneurons. Perhaps complex-spike cells are cells with the dendritic pattern of pyramidal cells, and theta cells are non-pyramidal cells, at least a few of which are projection cells.

"Basket" Cells

Much of the electrophysiological interest in interneurons in hippocampus has been in presumed inhibitory interneurons involved in recurrent inhibition. Spencer and Kandel (1961) showed conclusively that there is recurrent inhibition in Ammon's horn. Andersen et al. (1964a, b) showed that the hyperpolarizing phase of this IPSP of recurrent inhibition corresponded in time to the extracellularly recorded positive wave in the cellular layers following population-spikes. This implies that this IPSP is at least partially generated by endings on the cell bodies of the pyramidal cells. Andersen et al. (1964b) have described cells in hippocampus which they suggest are inhibitory interneurons with endings on the cell bodies of projection cells, i.e., basket cells. Finch and Babb (1977) have described a neuron with similar characteristics in a cat.

The theta cells in this study have all the characteristics of the "basket" cells described by Andersen et al. (1964b) in anesthetized preparations: (1) multiple action potentials occurring during the positivity recorded in the cellular layers following population-spikes; (2) decrease in amplitude of extracellular spike with very rapid evoked firing; (3) no antidromic driving (with one noteable exception); (4) little depression of evoked activity following activation; (5) lower thresholds, and greater convergence from different stimulation sites than other units; (6) some apparently direct activation (i.e., not recurrent). We have intentionally made Figs. 6, 7, and 9 in a format similar to Fig. 194 of Andersen et al. (1969) to facilitate this comparison. It is clear that Andersen's "basket" cells and our theta cells have a very large overlap and are probably identical.

Schwartzkroin and Mathers (1978) have recorded intracellularly from units in hippocampal slices which have many of the electrophysiological characteristics of Andersen's "basket" cells and our theta cells. Horseradish peroxidase was injected into four of these cells in order to identify them morphologically. Their conclusion was that these units were interneurons, but that they were *not* basket cells. These data are important not only for supporting the notion that theta cells are "non-pyramidal", but also for showing that at least some neurons with locally ramifying axons have action potentials. On the other hand, horseradish peroxidase injection of a neuron in a slice can show dendritic pattern and local axonal ramification, but may not be able to show whether or not the cell is also a projection cell. We cannot think of any way to conclusively demonstrate by purely electrophysiological methods that a neuron is an interneuron, much less a special type such as a basket cell. The data of Schwartzkroin and Mathers indicate that at least some of our theta cells and Andersen's "basket" cells are non-pyramidal cells with locally ramifying axons and are not basket cells.

Even though it is not established, the notion that Andersen's "basket" cells and our theta cells are involved in recurrent inhibition is very attractive. If we keep in mind (a) that a neuron with a locally ramifying axon may also be a projection cell (Chronister and De France 1979) and (b) that there is substantial inhibition on pyramidal cells mediated by endings *not* on the cell body (Purpura et al. 1968; Fugita 1979; Dingledine and Langmoen 1980) then *all* of the data on Andersen's "basket" cells and our theta cells are consistent with these cells being involved in recurrent inhibition. We will not develop this argument fully here, as others have done it elsewhere (Andersen et al. 1964a, b, 1969; Andersen 1975). Briefly, after a stimulus to VHC or EC-AB these cells fire multiple action potentials, before, during and after the population-spike, especially during the positive field potential in the cell body layer occurring at the time of the hyperpolarizing part of the IPSP of recurrent inhibition.

Both complex-spike cells and theta cells have decreased spontaneous activity following strong activation. The complex-spike cells are presumably receiving recurrent inhibition. If the theta cells were mediating recurrent inhibition then they would receive less input from local projection cells at this time and would have a decreased firing rate due to disfacilitation. In addition, the smaller response to a test stimulus following a conditioning stimulus could also be disfacilitation, combined with decreased recurrent activation due to decreased evoked activity in projection cells. On the other hand, Schwartzkroin and Mathers (1978) have presented evidence that interneurons themselves have IPSP's after some kinds of activation. The decreased latency of the first action potential of theta cells after a conditioned test stimulus to VHC or EC-AB is unexplained. The fact that some theta cells fired before the synaptic population spike in CA1 suggested that they are driven monosynaptically by CA3 fibers, in addition to recurrent excitation from local pyramidal cells. Frequency following to 100 per s in a CA1 cell theta cell with VHC stimulation is further support for this.

Everything we have said above also holds for theta cells in the dentate hilus. The population-spike response of dentate to dual pulses to monosynaptic

(perforant path) input is very similar to population-spike response to dual pulses to monosynaptic inputs to CA1. Even though we do not have data on granule cells these dual pulse population-spike data suggest strongly that the facilitatory and inhibitory processes in dentate and CA1 are similar.

In conclusion: (1) the sharp distinction between two classes of neurons in Ammon's horn of rats identified by firing repertoire and relation to behavior holds up when their electrophysiological characteristics are studied. These characteristics are listed in Table 1. (2) Most complex-spike cells in Ammon's horn must be projection and/or pyramidal cells and vice versa. (3) One theta cell was a projection cell. (4) Theta cells and "basket" cells studied by Andersen have a very large overlap and probably are identical. (5) At least some of our theta cells and Andersen's "basket" cells are non-pyramidal cells with locally ramifying axons. However, we do not think that our theta cells and Andersen's "basket" cells can be conclusively identified as inhibitory interneurons, much less basket cells at present, even though the suggestion that they may be involved in recurrent inhibition is very attractive.

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Knowles and Schwartzkroin (Neurosci Abstr 6: 570 (1980)) have now demonstrated in hippocampal slices that intracellular stimulation of non-pyramidal cells produces IPSPs in CA1 pyramidal cells, while stimulation of pyramidal cells produces EPSPs or spikes in non-pyramidal cells. This lends more credence to the notion that our theta cells and Andersen's "basket cells" are involved in recurrent inhibition.