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IMPULSES AND RESTING MEMBRANE PROPERTIES OF SMALL CULTURED RAT HIPPOCAMPAL NEURONS

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SUMMARY

1. The impulses and resting membrane parameters of small (soma diameter $< 10\ \mu$M) cultured hippocampal neurons from rat embryos were studied with the tight-seal whole-cell recording technique.

2. Mean resting potential was -47 mV, mean input resistance 3·3 GΩ, mean capacitance 11 pF, and mean time constant 33 ms.

3. Rectangular suprathreshold current steps elicited regenerative potential responses. The amplitude and time course of the responses were clearly stimulus dependent: stronger current steps caused impulses of larger amplitude.

4. The current threshold was very low: rheobase current was < 15 pA.

5. The potential response depended on the preceding holding potential, responses from more negative potentials showing sharper peaks than those from more positive potentials.

6. Spontaneous impulses with pre-potentials similar to synaptically induced events were recorded from several cells. The amplitude of the spontaneous impulses varied similarly to that of the stimulus-induced responses.

INTRODUCTION

Most detailed studies of the electrical properties of neuronal cell bodies have, due to technical limitations, focused on relatively large neurons. Thus, in the hippocampus, pyramidal cells have been extensively investigated (e.g. Kandel, Spencer & Brinley, 1961; Schwartzkroin, 1975; Segal, 1983; Andersen, Storm & Wheal, 1987; Storm, 1987), while smaller cells have been less thoroughly investigated, in spite of their abundance. Even the studies of neurons in the granule cell layer of the dentate gyrus (Barnes & McNaughton, 1980; Brown, Fricke & Perkel, 1981; Fournier & Crepel, 1984; Mody, Stanton & Heinemann, 1988; Edwards, Konnerth, Sakmann & Takahashi, 1989) seem mainly to have been concerned with relatively large cells, as judged by their capacitance and resistance values.

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The tight-seal recording technique (Marty & Neher, 1983) is well suited for electrophysiological studies of small cells, permitting a control of the intracellular solution as well as minimizing membrane damage and leakage at the pipette–membrane seal. We have used this technique to study small cultured hippocampal neurons from rat embryos. Unexpectedly, we found action potentials with an amplitude and time course that strongly depended on the stimulating current. This finding clearly deviates from the 'all-or-nothing' principle.

The present paper describes the graded action potentials and the resting membrane properties of the cells studied. Two subsequent papers (Johansson and Århem, 1992*a*, *b*) will describe a voltage-clamp analysis of the currents underlying the graded action potentials and numerical computations revealing the factors determining the variations in amplitude and time course. Some preliminary results have been published (Johansson & Århem, 1990).

METHODS

Cell cultures

The neuronal cultures were prepared from hippocampi dissected from Sprague–Dawley rats (ALAB, Sweden) at embryonic day 18–21. Pregnant rats were killed by CO_2-induced asphyxia (in a container with dry ice), and the embryos removed under sterile conditions and killed by decapitation. After dissociation by trituration, the cells were plated on poly-D-lysine- (0·1 mg ml^{-1}) coated dishes, at a density of about one million cells per 35 mm circular dish. The cultures were kept at 37 °C, in a water-saturated atmosphere containing 5% CO_2, for 4–7 days before the experiments. The growth medium was Eagle's minimal essential medium (MEM, GIBCO, UK), glucose (6 mg ml^{-1}), 7·5% heat-inactivated fetal calf serum (Flow Laboratories, UK), penicillin (2·5 U ml^{-1}, GIBCO, UK) and streptomycin (2·5 μg ml^{-1}, GIBCO, UK).

The cultures contained several types of neuron (pyramidal, multipolar, fusiform and small round cells) and glial cells. Only cells with a soma diameter less than 10 μm were used for the experiments (except for a few control measurements, see Results). These cells varied morphologically, and may therefore correspond to several types of neuron in the intact hippocampus. However, the majority were relatively round. A large proportion of the small round cells in intact hippocampus are dentate granule cells (Brown & Zador, 1990). Most cells in our study had dendrite- or axon-like processes which in many cases formed close contacts with other cells. The mean surface area of the soma including the bases of larger dendrites, estimated under a light microscope (magnification ×400), was about 200 μm^2 (range 50–300 μm^2). All cells studied were electrically excitable.

Electrophysiological recordings

The tight-seal whole-cell technique (Marty & Neher, 1983) was used for electrophysiological recording. Borosilicate glass pipettes (GC 120F or 150TF, Clark Electromedical instruments, UK), with a resistance of 2–8 MΩ when filled with and immersed in the bathing solution (see below), were used. The pipette–cell membrane seal was obtained by close apposition and, in most cases, gentle suction. It had a resistance higher than 10 GΩ as measured in the cell-attached recording configuration (Hamill, Marty, Neher, Sakmann & Sigworth, 1981). This resistance often increased further, just before rupture of the membrane patch, due to the suction pulses used to establish the whole-cell configuration. The series resistance between the pipette interior and the cell interior (in the whole-cell configuration) is not critical for the potential recordings (due to the small currents), and is dealt with in the accompanying paper (Johansson & Århem, 1992*a*).

The signals were recorded using an EPC-7 electrometer (List-electronic, Germany) in the current-clamp mode. Pulses were generated and sampled with a TL-1 DMA interface (Labmaster, USA) and the pCLAMP software (Axon Instruments, USA). The signals were low-pass filtered at 3·3 kHz (−3 dB, 3-pole Bessel filter) before sampling. Spontaneous activity was recorded on an FM tape-recorder and later fed to the computer using an event detector. All experiments were performed at room temperature (21–23 °C).

Solutions

Throughout the experiments, the cells were bathed in a solution containing (in mM): 137 NaCl, 5·0 KCl, 1·0 $CaCl_2$, 1·2 $MgCl_2$ and 10 HEPES (*N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulphonic acid), pH 7·4. In a few experiments 5 mM-D-glucose was added to the bathing solution. The recording pipette was filled with either a solution containing (in mM) 140 KCl, 3·0 NaCl, 1·2 $MgCl_2$, 1·0 EGTA (ethyleneglycol-bis-(β-aminoethylether)*N,N,N',N'*-tetraacetic acid) and 10 HEPES, pH 7·2 or a solution containing 140 KOH/KH_2PO_4, 3·0 NaCl, 1·2 $MgCl_2$, 1·0 EGTA and 3·0 Na_2-ATP, pH 7·2. No systematic difference between the recordings made with the two pipette solutions was detected, nor between experiments with or without glucose in the bathing solution.

RESULTS

Resting membrane parameters

The resting membrane potential was estimated from the potential obtained within a few seconds after establishing contact between the pipette solution and the cytoplasm. The mean value for twenty-three cells was -47 ± 11 mV ($\pm$standard deviation). The recorded potential often showed relatively large fluctuations. In several cells the peak-to-peak fluctuations, occurring within 100 ms, exceeded 10 mV.

The input resistance, calculated from the steady-state potential response to a negative current step, was $3{\cdot}3 \pm 1{\cdot}4$ GΩ (mean $\pm$ standard deviation) for ten consecutive cells. The time constant of the approximately exponential potential change to the same current step was 33 ± 12 ms. From the resistance and the time constant, the membrane capacitance was calculated to be 11 ± 4 pF. Using the mean membrane area of 200 μm^2 (see Methods), we obtained a mean conductance per unit area of 0·15 mS cm^{-2} and a mean capacitance per unit area of 5·5 $\mu F\ cm^{-2}$.

Stimulus-induced action potentials

Amplitude and time course: stimulus dependence

All seventeen cells tested showed regenerative potential responses when stimulated with positive rectangular current pulses of sufficient strength (Fig. 1*A*). Unexpectedly, the amplitude and time course of the induced action potentials strongly depended on the stimulus current intensity. The peak amplitude was graded with stimulus intensity. A stronger current elicited an action potential of higher amplitude than did a weaker current. At the strongest currents used, the peak potential exceeded the zero level by 30–40 mV. The relation between peak potential and stimulus current is shown in Fig. 1*B*. The curve was typically slightly S-shaped in the suprathreshold range. The slope of the rising phase of the action potential increased, and thus the time-to-peak decreased, with increased stimulus current (cf. Fig. 1*A*).

The duration of the action potential also depended on current strength (Fig. 1*C*). Measured at half-peak amplitude (relative to the holding potential), the duration showed a complex current dependence, while if measured at 0 mV, a uniformly increasing duration–current curve was obtained. Irrespective of the measuring method, the duration varied markedly between cells. Measured at half-peak amplitude for a holding potential between -60 and -80 mV, the variation was

between 2 and 20 ms in different cells, partly, however, due to differences in holding potential (see below).

The falling phase of the action potential did not follow a simple exponential time course, but showed an early fast and a late slower phase (Fig. 1*A*; more clearly seen

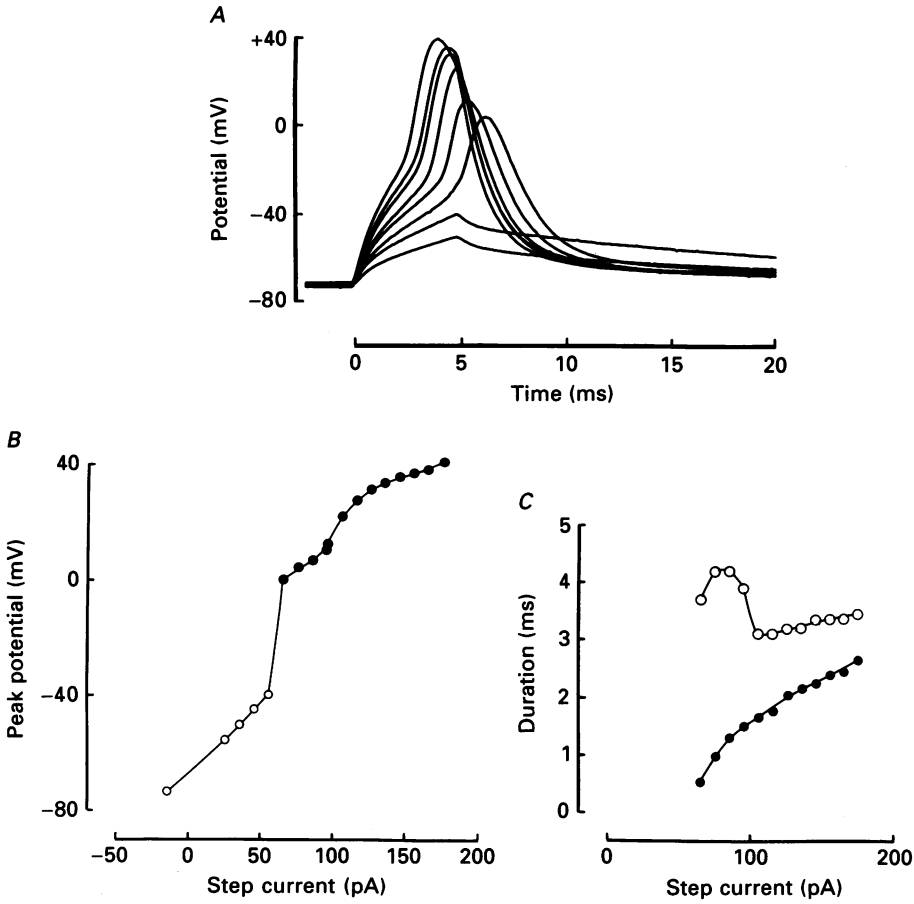

Fig. 1. *A*, action potentials. The cell was stimulated with positive rectangular current pulses of 5 ms duration. Current steps (associated with curves from bottom to top): 50, 70, 90, 110, 130, 150, 170 and 190 pA above the holding current of −15 pA. *B*, peak potential *versus* stimulus strength. Data from the same cell as shown in Fig. 1*A*. Open circles (○) indicate the responses to subthreshold stimuli, filled circles (●) the action potentials. Note the S-shape of the curve joining the filled circles. *C*, impulse duration as a function of stimulus current strength. ○, duration measured at half-peak amplitude. ●, duration measured at 0 mV. Same cell as for *A* and *B*.

in the lowest curve in Fig. 4). The late phase was approximately exponential, with a time constant close to the membrane time constant. The early phase was much faster, as shown in Fig. 2 where the falling phase of the action potential reaches values more negative than the subthreshold response. No after-hyperpolarization was seen within 500 ms after the impulse peak.

Threshold

Regenerative potential responses, although graded in amplitude, were elicited exclusively when the stimulus current exceeded a threshold value (Fig. 1*A* and *B*). The threshold was remarkably low, about 10–50 pA for 10 ms pulses. With longer

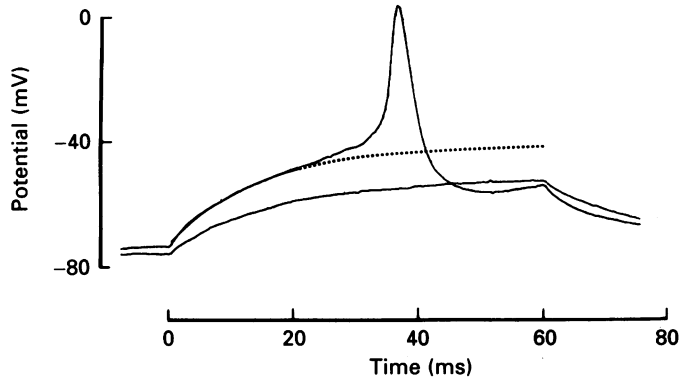

Fig. 2. Fast falling phase of action potential. Sub- and suprathreshold responses to rectangular current stimuli of 60 ms duration. The dotted line is an exponential curve fitted to the first 18 ms of the suprathreshold response in order to indicate the presumed electrotonic response to the same stimulus. Current steps: 10 and 15 pA above the holding current of −8 pA.

pulses the values were still lower. Figure 3*A* shows the potential response in a cell where a 60 ms pulse of only 3 pA was sufficient to elicit an impulse. Some threshold values at different stimulus durations are shown in Fig. 3*B*. The rheobase current was below 15 pA.

E–ects of holding potential

The effect of the holding potential on the impulse was studied, since many recordings were made from neurons held at potentials more negative than the resting potential. Current pulses to a constant step value were given from different levels of steady-state current injection. The time course of the action potential clearly depended on the holding potential (Fig. 4). The rise and the decay phases were faster when the impulse was elicited from more negative potentials than from the resting potential. The difference between the early fast and the late slow falling phase of the action potential was also more marked. (The transition between the fast phase and the slow phase is indicated with an arrow-head in the lowest trace in Fig. 4). However, stimulus-dependent amplitude differences, of the type shown in Fig. 1*A*, were seen when the impulses were elicited from more negative potentials as well as from the resting potential.

Comparison with larger neurons

To find out how far the present results were due to the culture or the experimental conditions, we made a few recordings from larger cells. Five neurons with a largest diameter of 20–25 μm showed a mean resting potential of -58 ± 7 mV. Two of these

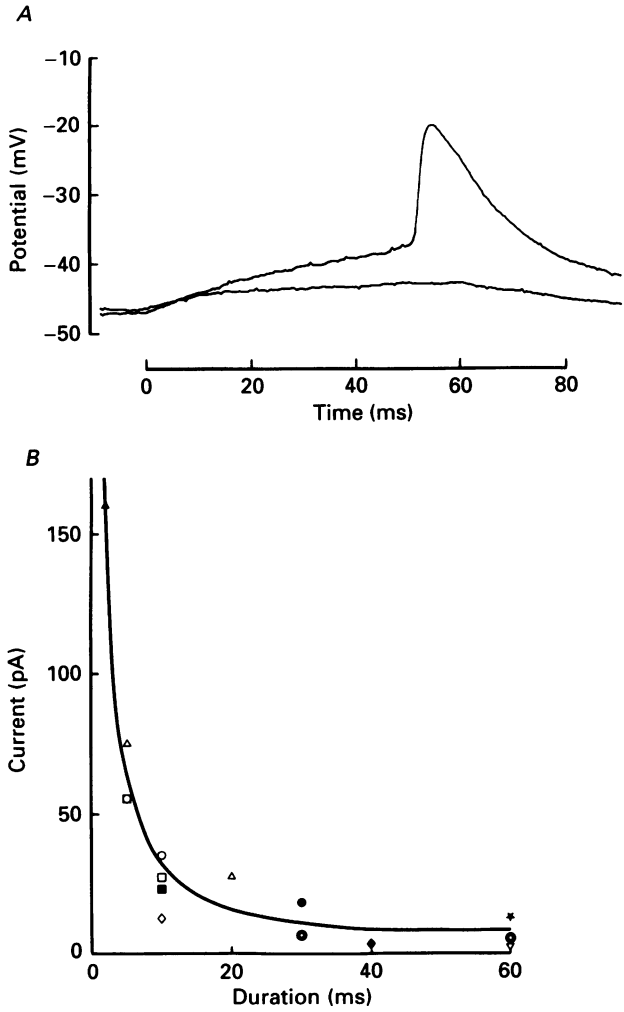

Fig. 3. *A*, example of an action potential elicited by an extremely small rectangular current step (3 pA) 60 ms in durations. The subthreshold response to a 2 pA pulse is shown for comparison. No holding current. *B*, relation between stimulus duration and current threshold. The threshold strength for a rectangular current pulse is plotted *versus* the duration of the pulse. Data from ten different cells as indicated by symbols. The different cells were in a few cases held at different membrane potentials (ranging from −45 to −80 mV) between the test steps. However, no systematic dependence on the holding potential was detected when the thresholds in the different cells were compared. Line drawn by eye.

neurons showed particularly stable resting potentials and were chosen for an analysis of impulse amplitude. They both generated essentially all-or-nothing impulses.

Spontaneous action potentials

In many cells spontaneous impulses were observed. They always occurred irregularly, and showed no clear burst pattern. Impulses of the most common type (analysed in four cells chosen for their relatively high firing frequency) were

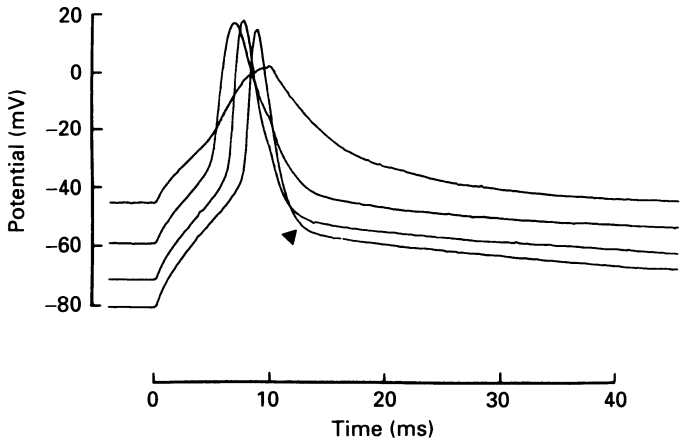

Fig. 4. Effects of holding potential. The holding potential was varied by injection of a constant current. Potential responses to rectangular current steps to +45 pA from holding currents of −15, −10, −5 and 0 pA (corresponding to curves from bottom to top, at start and end of curve) are shown. The transition between the two phases is indicated with an arrow-head on the lower curve.

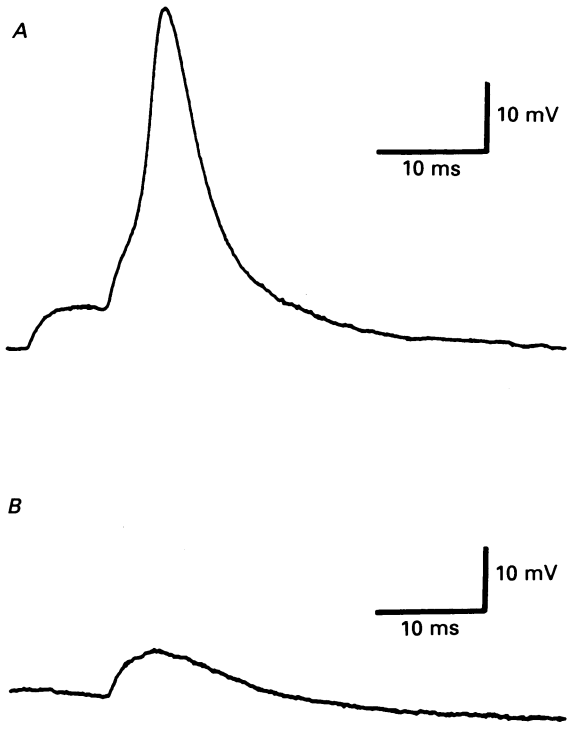

Fig. 5. Spontaneous impulse (*A*) and isolated potential fluctuation (*B*) from the same cell.

correlated with small positive fluctuations, which preceded and presumably induced the regenerative responses (Fig. 5). These fluctuations showed a fast rise phase (time-to-peak 2·5–5 ms) and a slower decay phase (decay time to half-amplitude 6–11 ms)

(Fig. 5*A* and *B*). They resembled the excitatory synaptic potentials described for other hippocampal neurons (e.g. Schwartzkroin, 1975; Dudek, Deadwyler, Cotman & Lynch, 1976; MacVicar & Dudek, 1982; Fournier & Crepel, 1984; Andersen *et al.* 1987). They showed temporal summation, and the number of potential events

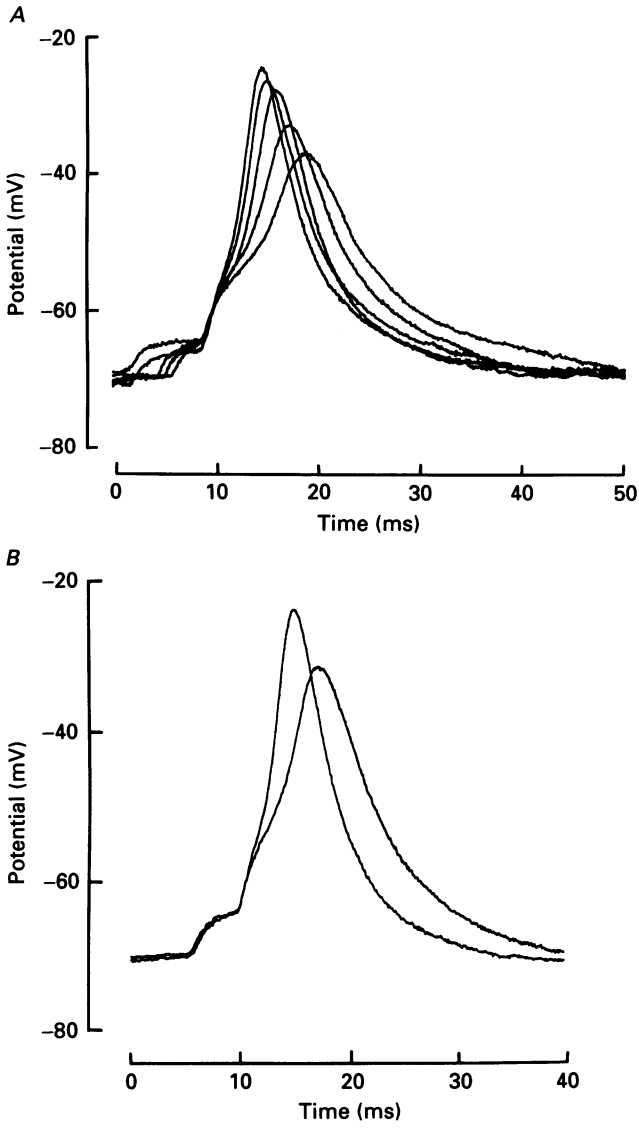

Fig. 6. Amplitude variation of spontaneous action potentials. *A*, superimposed traces. *B*, two different action potentials elicited from the same membrane potential.

needed to elicit an impulse varied from one to three. Similar potential events that did not elicit impulses were also observed (Fig. 5*B*). The amplitudes of the fluctuations varied from the level of the resting potential noise to suprathreshold potentials.

Spontaneous impulses associated with plateau potentials, and impulses that arose smoothly from the resting potential without any clearly separable preceding potential event, were also recorded from several cells. They will be analysed in more detail elsewhere.

Amplitude variation of spontaneous impulses

The spontaneous impulses varied considerably in amplitude. However, most cells that showed spontaneous firing also showed large resting potential fluctuations. Since the shape of the action potential depended on the preceding membrane potential, it was difficult to draw any conclusions about the relation between this shape and the inducing event *per se*.

However, cells with an atypically low membrane resistance showed more stable resting potentials. We therefore analysed the amplitudes of spontaneous action potentials in such cells. Spontaneous action potentials elicited from approximately the same membrane potential showed considerable variation in amplitude (Fig. 6*A*), even when the time course of the preceding potential was essentially the same on different occasions (Fig. 6*B*). The different impulse amplitudes were not correlated to the time interval (peak-to-peak interval > 80 ms for impulses analysed) after the preceding impulse.

DISCUSSION

Our most unexpected finding was that the small cells systematically generated action potentials that depended on stimulus strength. Thus, they did not follow the 'all-or-nothing' principle generally assumed to describe the action potential (e.g. Adrian & Forbes, 1922; see also Hille, 1984; Kandel & Schwartz, 1985).

The present investigation does not necessarily imply that the corresponding neurons in the hippocampus show graded action potentials *in vivo*. At present, solid physiological evidence for graded impulses in vertebrate neurons is lacking (Bullock, 1981). However, it seems likely that such neurons occur in the mammalian cortex and other stratified nervous structures (Bullock, 1981).

Comparison with other preparations

Little information is available on the electrical properties of small cells. Thus, it is difficult to determine how far the neurons studied were affected by the experimental conditions, and impossible to determine how far the properties described are limited to the developmental stage studied (4–7 days *in vitro* after plating 1–4 days before parturition).

However, larger hippocampal neurons in culture show electrophysiological properties similar to those observed *in situ* or in slice preparations (Segal, 1983). The larger neurons in the present cultures showed essentially all-or-nothing action potentials and a more negative resting potential than the smaller cells. Thus, the properties of the cells studied, including the ability to generate graded impulses, were not general consequences of the present culture conditions.

Resting potential

The mean resting membrane potential for the small cells studied (−47 mV) was more positive than reported for most other neurons. However, a direct comparison with other studies may be misleading for at least two reasons. First, in most studies only potentials exceeding a defined value are accepted in the data collection (e.g. Kandel *et al.* 1961; Schwartzkroin, 1975; Barnes & McNaughton, 1980; Segal, 1983; Fournier & Crepel, 1984; Mody *et al.* 1988). Secondly, most measurements have been obtained with microelectrodes containing 2–4 M-K^+. This means that a substantial K^+ leak into the cell is expected (Pusch & Neher, 1988), and thus, in small cells, affected resting potential.

Input resistance

The mean input resistance (3·3 GΩ) is high compared with that reported in most other studies (see Table 4 in Coombs, Curtis & Eccles, 1959; for hippocampal neurons see Spencer & Kandel, 1961; Barnes & McNaughton, 1980; Brown *et al.* 1981; Segal, 1983; Fournier & Crepel, 1984; Mody *et al.* 1988). This also appears to be the case when the small membrane area is taken into account (specific resistance about 6·6 kΩ cm^2). However, most earlier studies of neuronal membrane properties have employed 'classical' microelectrodes (Ling & Gerard, 1949) which may cause membrane damage and increased leakage (Marty & Neher, 1983). Several studies with the tight-seal technique applied to other small excitable cells also show a high input resistance (Fenwick, Marty & Neher, 1982; Hockberger, Tseng & Connor, 1987; Cull-Candy, Marshall & Ogden, 1989; Johansson, Rydqvist, Swerup, Heilbronn & Århem, 1989).

Membrane capacitance

The membrane capacitance (mean value 11 pF) was low compared to that of other neurons, probably due to the small membrane area. The estimated specific value (5·5 μF cm^{-2}) agrees well with that estimated for many other neuronal cell bodies (e.g. Table 4 in Coombs *et al.* 1959; Durand, Carlen, Gurevich, Ho & Kunov, 1983).

Threshold

The current-threshold values obtained were extremely low, in many cases just a few picoamps (1 pA corresponds to about 0·5 μA cm^{-2}). This should be compared with the 100–500 pA given as rheobase current for hippocampal neurons by Spencer & Kandel (1961). On the other hand, very low threshold values have been found in other small excitable cells (Fenwick *et al.* 1982; Johansson *et al.* 1989).

Spontaneous impulses

Spontaneous impulses of different amplitudes were generated by endogenous stimuli under the present experimental conditions. Most impulses seemed to be induced by synaptic events. The preceding potential fluctuations were similar to the spontaneous synaptic events reported from neurons in the hippocampal granule cell layer (Dudek *et al.* 1976; MacVicar & Dudek, 1982; Fournier & Crepel, 1984). This is consistent with the observation (see Methods) that the cells studied often formed close contacts.

Relevance for neuronal information processing

The finding of graded action potentials seems to be of general interest for the study of information processing in mammalian neurons. The importance for information transmission depends on how the graded impulses influence synaptic regions. It is obvious that the information content of the graded action potentials will be transmitted directly to regions that are electrically close, but several studies suggest that impulses of different amplitudes (Huxley, 1959; Zettler & Järvilehto, 1971; Bush, 1981) or decremental impulses (Lorente de Nò & Condouris, 1959; Cooley & Dodge, 1966) may propagate along axons. Thus, graded action potentials may in some systems reach and affect even remote synaptic regions.

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