Spike after-depolarization and burst generation in adult rat hippocampal CA1 pyramidal cells

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1. Intracellular recordings in adult rat hippocampal slices were used to investigate the properties and origins of intrinsically generated bursts in the somata of CA1 pyramidal cells (PCs). The CA1 PCs were classified as either non-bursters or bursters according to the firing patterns evoked by intrasomatically applied long ($\geqslant$ 100 ms) depolarizing current pulses. Non-bursters generated stimulus-graded trains of independent action potentials, whereas bursters generated clusters of three or more closely spaced spikes riding on a distinct depolarizing envelope.

2. In all PCs fast spike repolarization was incomplete and ended at a potential ~10 mV more positive than resting potential. Solitary spikes were followed by a distinct after-depolarizing potential (ADP) lasting 20–40 ms. The ADP in most non-bursters declined monotonically to baseline ('passive' ADP), whereas in most bursters it remained steady or even re-depolarized before declining to baseline ('active' ADP).

3. Active, but not passive, ADPs were associated with an apparent increase in input conductance. They were maximal in amplitude when the spike was evoked from resting potential and were reduced by mild depolarization or hyperpolarization ($\pm$ 2 mV).

4. Evoked and spontaneous burst firing was sensitive to small changes in membrane potential. In most cases maximal bursts were generated at resting potential and were curtailed by small depolarizations or hyperpolarizations ($\pm$ 5 mV).

5. Bursts comprising clusters of spikelets ('d-spikes') were observed in 12% of the bursters. Some of the d-spikes attained threshold for triggering full somatic spikes. Gradually hyperpolarizing these neurones blocked somatic spikes before blocking d-spikes, suggesting that the latter are generated at more remote sites.

6. The data suggest that active ADPs and intrinsic bursts in the somata of adult CA1 PCs are generated by a slow, voltage-gated inward current. Bursts arise in neurones in which this current is sufficiently large to generate suprathreshold ADPs, and thereby initiate a regenerative process of spike recruitment and slow depolarization.

Early intracellular recordings from hippocampal pyramidal cells (PCs) in lightly anaesthetized cats have indicated that spontaneously active PCs generate an admixture of solitary spikes and bursts (Kandel & Spencer, 1961*a*). Each solitary spike was followed by a distinct after-depolarizing potential (ADP) of about 10 mV amplitude and 30 ms duration. A burst was a tightly packed cluster of several fast spikes riding on a slow depolarizing wave. Bursts were considered to be intrinsic events, since they were evoked also by passing positive current pulses through the recording microelectrode. It was hypothesized that bursts are produced by a process of ADP 'summation', in which consecutive spike ADPs sum temporally to produce a slow depolarizing potential that maintains spike recruitment despite the increase in firing threshold, until the accumulated spike inactivation terminates the discharge (Kandel & Spencer, 1961*a*).

Subsequent work in adult rodent hippocampal slices perfused with salines containing slightly supranormal concentrations of K^+ (usually 5 mM) indicated that only a fraction of PCs manifest some burst firing characteristics. In the CA3 field, approximately half of the PCs displayed a capability to burst in response to certain depolarizing current pulses injected into the soma (Bilkey & Schwartzkroin, 1990). Burst generation in these neurones seemed to involve an underlying slow Ca^{2+} spike, whereas burst termination was attributed primarily to activation of Ca^{2+}-gated K^+ conductances (Wong & Prince, 1978). A

smaller fraction of bursters was found in the CA1 field (Schwartzkroin, 1975; Masukawa, Benardo & Prince, 1982). To account for this regional difference, it was suggested that burst firing in CA3 PCs occurs in both soma and dendrites, whereas in CA1 PCs it is an exclusive property of the apical dendrites (Wong, Prince & Basbaum, 1979; Benardo, Masukawa & Prince, 1982; Wong & Stewart, 1992).

In the strict sense, a burster is a neurone that responds to a threshold-straddling stimulus by generating a self-sustained burst. However, previous studies have employed various stimulation protocols in order to evoke burst responses in PCs, thereby obscuring the differences between unconditional bursters and neurones that burst only under special circumstances. Recently we have surveyed the burst characteristics of CA1 PCs in the CA1 *b* subfield of adult rat hippocampal slices (Jensen, Azouz & Yaari, 1994). In saline containing a normal concentration of K^+ (3·5 mM), 17% of the PCs were capable of burst firing in response to long ($\geqslant$ 100 ms), threshold-straddling depolarizing pulses injected into the soma. Raising extracellular K^+ to 7·5 mM increased the overall fraction of bursters to 42%. The propensity for burst firing also increased, so that many of the PCs fired bursts also in response to brief (3–5 ms) depolarizing current pulses or even spontaneously.

Burst firing cells are amplifying elements in neuronal networks and have been strongly implicated in the generation of epileptiform discharges and in other forms of electroencephalographic sharp waves (reviewed by Traub & Miles, 1991). Therefore, the mechanisms that underlie and modulate burst activity in mammalian cortical structures have attracted great interest (e.g. Metherate, Cox & Ashe, 1992; Wang & McCormick, 1993; Azouz, Jensen & Yaari, 1994). In this and in the subsequent paper (Azouz, Jensen & Yaari, 1996) we have investigated the membrane events that lead to the formation of spike ADPs and bursts in somata of adult CA1 PCs.

METHODS

Slice preparation and solutions

Transverse hippocampal slices were prepared from adult Sabra rats (150–200 g body weight). Animals were anaesthetized with ether before decapitation. Both hippocampi were dissected out and kept in cold (0–4 °C) saline. Slices (450 μm thick) were cut with a vibratome, placed on a nylon mesh support in an interface chamber at 33·5 °C and perfused from below with oxygenated (95% O_2–5% CO_2) saline. The upper surface of the slices was exposed to the humidified gas mixture. The slices were allowed to recover for at least 1 h before a recording session was started.

Standard saline contained (mM): NaCl, 124; KCl, 3·5, NaH_2PO_4, 1·25; $MgSO_4$, 2; $CaCl_2$, 2; $NaHCO_3$, 26; and D-glucose, 10. After testing the viability of the slices with extracellular recordings of evoked orthodromic field potentials, the slices were perfused with high-K^+ saline, containing 7·5 KCl. In intact slices, raising extracellular K^+ induces brief network-driven bursts in the CA3 field, which secondarily propagate into CA1 (e.g. Rutecki, Lebeda & Johnston, 1985). To avoid this complication, the glutamate receptor antagonists 6-cyano-7-nitro-quinoxaline-2,3-dione (CNQX; 15 μM; Tocris Cookson) and 2-amino-5-phosphonovaleric acid (APV; 50 μM; Sigma) were added to the saline. As previously shown, these drugs entirely blocked glutamatergic excitatory postsynaptic potentials (Andreasen, Lambert & Jensen, 1989) and prevented the development of brief network-driven bursts (Jensen & Yaari, 1991).

Intracellular recordings

Intracellular recordings were made from within the CA1 pyramidal layer (including all CA1 subfields) using potassium acetate (4 M)-filled glass microelectrodes (50–80 MΩ). An active bridge circuit in the amplifier (Axoprobe; Axon Instruments)

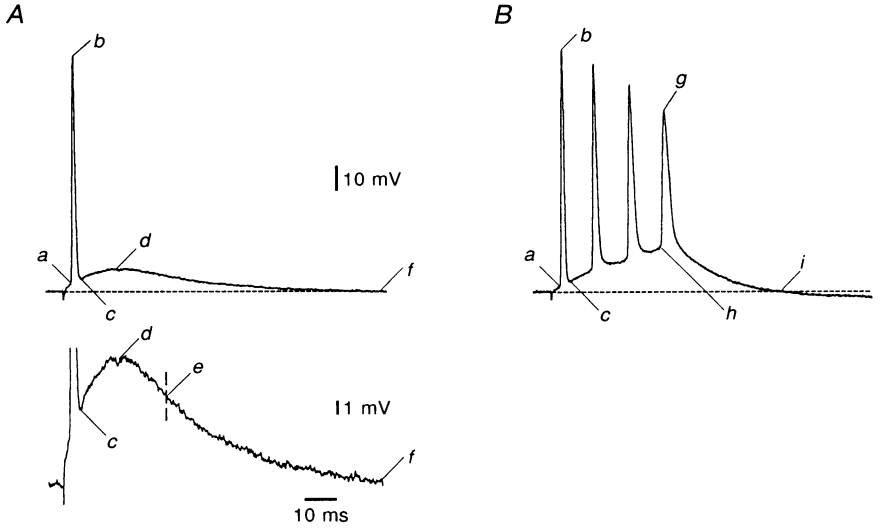

Figure 1. Reference points used to measure various functional parameters of solitary spikes (*A*) and bursts (*B*) from digitized traces

See text for further description.

allowed simultaneous injection of current and measurement of membrane potential. The bridge balance was carefully monitored and adjusted before each measurement. The intracellular signals were recorded on a digital tape recorder (VT-100; Instrutech), digitized and stored by a personal computer using a data acquisition system (TL-1 DMA; Axon Instruments). Off-line data analyses were performed using pCLAMP software (Axon Instruments).

Neurones were identified as PCs if they responded with short-latency spikes to stimulation of the alveus and manifested strong spike frequency accommodation during sustained depolarization. The PCs accepted for this study had a stable resting potential of at least −55 mV and overshooting action potentials. The mean resting membrane potential, input resistance (R_N) and spike amplitude of 127 CA1 PCs in saline containing 7·5 mM K^+ were −62·7 ± 2·9 mV, 25·5 ± 6·9 MΩ and 84·9 ± 8·9 mV, respectively.

Data measurement and analysis

The reference points used to measure various functional spike and burst parameters from digitized traces are depicted in Fig. 1. The spike parameters included the following (Fig. 1*A*): (1) spike threshold (*a*); (2) spike amplitude (the voltage difference between *a* and *b*); (3) membrane potential at the end-point of fast spike repolarization, i.e. the peak of the fast after-hyperpolarization (AHP; *c*); (4) membrane potential at the peak of the re-depolarizing ADP component, if present (*d*); (5) duration of the ADP, from the peak of the fast AHP (*c*) to the time point when membrane potential returns to, or crosses, resting potential (*f*); and (6) time constant of ADP decay, i.e. the time constant of the monoexponential function best fitted to the monotonic component of the ADP decay (from *e* to *f*, *e* being the time at which ADP amplitude declines to two-thirds of its peak).

We considered as a burst any spontaneously generated or stimulus-evoked cluster of three or more closely spaced action potentials, riding on a distinct depolarizing envelope. The measured burst parameters included, in addition to those of the first spike (parameters 1–3 above), the following (Fig. 1*B*): (7) number of spikes in a burst; (8) mean firing frequency in the burst (derived from parameter 7 and the interval between first and last intra-burst spikes, i.e. time segment between *b* and *g*); (9) peak amplitude of the slow depolarizing envelope (burst envelope) underlying the cluster of spikes (the voltage difference between the threshold of the last intraburst spike, *h*, and resting potential); and (10) burst envelope duration (time segment between *a* and *i*, the time point when membrane potential returns to, or crosses, resting potential.

To measure passive membrane properties, the PCs were injected with small (0·1–0·5 nA) 200 ms square negative current pulses. The input resistance (R_N) was provided by the slope of the linear regression line fitted through the linear portion of the steady-state voltage *versus* current amplitude plot. The apparent membrane time constant (τ_m) was taken as the slowest component (τ_0) of multi-exponential functions fitted to the charging curve produced by application of a small negative current step (from onset to steady state), as suggested by Rall (1977). The R_N and τ_m values thus obtained are most probably underestimations due to somatic leak produced by the impaling microelectrode (Spruston & Johnston, 1992).

In order to disclose changes in input conductance during the spike after-depolarization (ADP), square negative current pulses (5 ms; 0·5–1·0 nA) were injected during the ADP. The resultant voltage deflections were compared with those produced by the same pulses at resting and at depolarized potentials corresponding to the peak of the ADP.

Statistical analysis

Averaged data are expressed as means ± S.D. The significance of the differences between the measured parameters was evaluated using one-way analysis of variance (ANOVA). When significant differences were indicated in the F ratio test ($P < 0·05$), the Tukey method for multiple comparisons was employed to determine those pairs of measured parameters that differed significantly within the pair ($\alpha < 0·05$).

RESULTS

Of the 127 PCs included in this study, twenty-nine neurones were regular firing cells (non-bursters), generating accommodating trains of independent action potentials in response to all suprathreshold stimuli. The remaining ninety-eight neurones displayed the gradient of burst characteristics previously described (Jensen *et al.* 1994). Of these, fifteen PCs fired in a burst mode only in response to strong suprathreshold stimuli ('borderline' bursters); thirty PCs generated a burst in response to threshold straddling, long (100–400 ms) depolarizing current pulses, but fired a solitary spike in response to a brief (3–5 ms) depolarizing current pulse (grade I bursters); thirty PCs also fired bursts in response to a brief pulse (grade II bursters); and the remaining twenty-three PCs fired bursts in response to any suprathreshold stimulus and also spontaneously (grade III bursters). The marked predominance of bursters in the present series reflects a sample bias, since many non-bursters impaled were discarded.

Electrophysiological features of PC subgroups

The passive membrane properties (resting potential, R_N and τ_m) of non-bursters *versus* bursters were compared (Table 1). Non-bursters differed significantly from bursters only in having a shorter τ_m (8·1 ± 2·1 *versus* 14·3 ± 4·8 ms, respectively). No significant differences in action potential threshold, amplitude and end-point of fast repolarization (peak of the fast AHP) were found between non-bursters and bursters (Table 1). The large and similar spike amplitudes in non-bursters (88·7 mV) and bursters (84·9 mV) exclude the possibility that in the latter group cell impalements were unintentionally made in dendrites. Confirmed recordings from apical dendrites of CA1 PCs in saline containing 6·25 mM K^+ indicated that dendritic spikes are small (50–65 mV; Wong *et al.* 1979; Bernado *et al.* 1982).

Variation in spike ADPs

Properties of the ADPs were analysed in seventy-four PCs which generated solitary spikes in response to brief depolarizing current pulses. Of these, twenty-nine neurones were non-bursters, whereas the remaining forty-five cells generated bursts only when injected with long depolarizing current pulses ('borderline' and grade I

bursters). As in saline containing 3·5 mM K^+ (Jensen *et al.* 1994), two characteristic types of ADPs, designated 'passive' and 'active' ADPs, were noted in high-K^+ saline.

Passive ADPs, observed in twenty-six PCs (35·1%), lasted 24·1 ± 7·2 ms. They declined monotonically to baseline from the peak of the fast AHP with a decay time constant of 12·2 ± 3·2 ms (Fig. 2*A*). In the remaining forty-eight PCs (64·9%) the spike ADPs were of the active type, beginning with a period of protracted depolarization or even re-depolarization (Fig. 2*B*), before slowly repolarizing to baseline. The amount of re-depolarization varied from 0·5 to 5 mV and in twelve of the forty-eight PCs (25·0%) it triggered a second spike (not shown). Both the duration (41·3 ± 8·8 ms) and the decay time constant of the declining phase of the ADP (20·3 ± 8·2 ms) were almost twofold longer than those of passive ADPs.

Spike ADPs in different PC subgroups

Clear differences in spike ADPs between non-bursters and bursters were found. In most non-bursters (21 of 29 cells; 72·4%; Fig. 2*A*), but only in a few bursters (5 of 45 cells; 11·1%), the ADPs were passive. In contrast, the ADPs in most bursters (40 of 45 cells; 88·9%; Fig. 2*B*) were active. Thus, when injected with long depolarizing current pulses, PCs with active spike ADPs were much more likely to burst than PCs manifesting passive ADPs.

Pyramidal cells with strong burst characteristics (i.e. grade II and III bursters) generated bursts also in response to brief depolarizing current pulses. Therefore, the duration of the first spike ADP could not be measured in these neurones. However, abating the burst discharge immediately after the first spike with an intervening negative current pulse disclosed a prolonged (duration, 56·4 ± 2·1 ms; $n = 5$) active ADP also in these neurons (Fig. 3).

Conductance changes during the ADP

The observation that some of the ADPs re-depolarize before slowly declining to baseline suggested that slow conductance changes may be involved in generating these potentials. Re-depolarization may result from either a decrease in outward current or an increase in inward current. We tested these possibilities by monitoring the voltage deflections produced by constant 5 ms negative current pulses applied during the ADP and at rest. Changes in the size of these deflections were assumed to reflect proportional changes in input resistance.

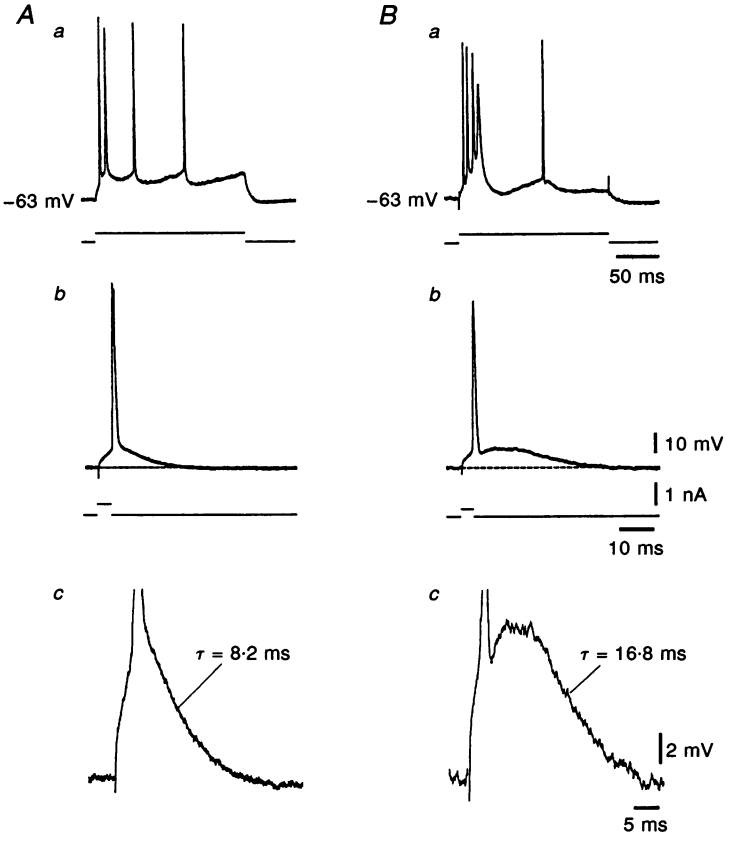

Figure 2. Variation in shape and size of spike ADPs in CA1 PCs

The records in *A* and *B* were obtained from two different PCs. For each cell, the upper (*a*) and middle (*b*) panels depict the PC responses to long and brief stimuli, respectively. ADPs are shown on an expanded voltage scale in the lower panels (*c*). Note that the ADP in the non-burster (*A*) is passive, decaying monoexponentially with a time constant of 8·2 ms. By contrast, the ADP in the burster (*B*) re-depolarizes before decaying slowly to baseline.

Table 1. Electrophysiological properties of non-bursters *versus* bursters in the CA1 field

	V_m (mV)	R_N (MΩ)	τ_m (ms)	R_m (kΩ cm^2)	Spike threshold (mV)	Spike amplitude (mV)	Fast AHP peak (mV)
Non-bursters	−63·1 ± 2·8 (29)	25·3 ± 7·6 (29)	8·1 ± 2·1* (29)	8·1* (29)	−55·6 ± 3·1 (29)	88·7 ± 4·9 (29)	−52·8 ± 5·3 (29)
Bursters†	−62·5 ± 3·0 (98)	25·6 ± 6·8 (94)	14·3 ± 4·8* (88)	14·3* (88)	−56·2 ± 4·1 (98)	84·9 ± 8·9 (98)	−53·6 ± 4·8 (98)
All neurones	−62·7 ± 2·9 (127)	25·5 ± 6·9 (123)	12·8 ± 4·8 (117)	12·8 (117)	−55·8 ± 3·9 (127)	85·3 ± 7·9 (127)	−53·4 ± 4·9 (127)

All entries are expressed as means ± S.D. V_m and R_N are resting membrane potential and input resistance, respectively. τ_m is the apparent passive time constant of the membrane measured at resting V_m. R_m is the apparent specific membrane resistance, calculated from $\tau_m = R_m C_m$ and assuming that the specific membrane capacitance, C_m, equals 1 μF cm^{-2}. † Bursters include all PCs displaying minimal to maximal burst characteristics. 'Resting' membrane properties of spontaneous (grade III) bursters were obtained after suppressing spontaneous bursts with the minimal steady hyperpolarizing current. Spike parameters in bursters pertain to the first spike. * Significantly different ($P < 0·05$) from the corresponding subgroup. Numbers of neurones are given in parentheses.

In PCs having passive spike ADPs, the current-induced voltage deflections slightly increased during the early portion of the ADP (Fig. 4*A* and *C*; $n = 4$), indicating a small increase in resistance. By contrast, in PCs having active ADPs the voltage deflections markedly decreased during the ADP (Fig. 4*B* and *D*; $n = 7$), suggesting a considerable decrease in resistance. This change was maximal during the re-depolarizing phase and closely paralleled the time course of the ADP. Depolarizing the PCs by 10 mV with constant current injection caused a mild increase (~8%) in the voltage deflections in all cases (lower traces in Fig. 4*A* and *B*; $n = 10$), indicating a small *increase* in resistance (Fig. 4*C* and *D*).

These data suggest that the waveform of passive ADPs reflects primarily passive charging of the membrane. The small resistance increase during these ADPs can be accounted for by the non-linear behaviour of the membrane near resting potential (cf. Spruston & Johnston, 1992). In contrast, the large resistance decrease seen during active ADPs strongly implicates a slow inward current in their generation.

Modulation of ADPs by membrane potential

To clarify the mechanisms of the ADP further, the effects of altering resting membrane potential on ADP waveform were examined. In all cases tested ($n = 10$), depolarization or hyperpolarization of up to 6 mV with steady current injection changed the peak hyperpolarization of the fast after-hyperpolarization (AHP) very little (± 2 mV), if at all (Fig. 5*A* and *B*; cf. Storm, 1987). At all these potentials, passive ADPs decayed monotonically to baseline. Their amplitude measured at any fixed time after the peak of the fast AHP changed linearly with membrane potential, decreasing with depolarization and increasing with hyperpolarization (Fig. 5*A*). The monoexponential decay time

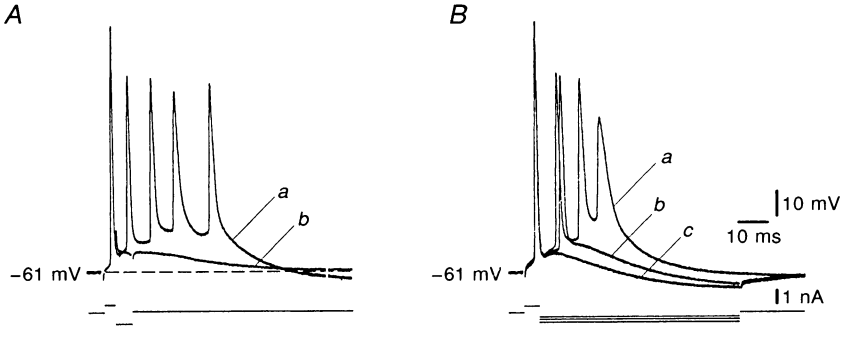

Figure 3. Blocking burst discharge by hyperpolarizing pulses unmasks prolonged active ADPs
The neurone in *A* fired a burst of five spikes upon brief stimulation (trace *a*). Injecting a small 5 ms negative current pulse immediately after the first spike blocked subsequent action potentials and unmasked a prolonged active ADP (trace *b*). Note the similarity in duration of the ADP and the native burst. The neurone in *B* fired a burst of four spikes upon brief stimulation (trace *a*). Injecting long negative current pulses of increasing intensity immediately after the first spike progressively curtailed the burst discharge (traces *b* and *c*). Blocking the late spikes disclosed a prolonged active ADP.

constant of the ADPs appeared to be voltage dependent, increasing from 8·4 ± 1·5 to 12·1 ± 2·5 ms (a 1·4-fold increase; $n = 4$) upon depolarization from −70 to −60 mV.

Active ADPs were strongly and non-linearly dependent on membrane potential. They were maximal when evoked from about resting potential and decreased with polarization of ± 2 mV or more (Fig. 5*B*; $n = 6$).

Modulation of burst firing by membrane potential

Bursts evoked from resting potential by long or brief depolarizing current pulses consisted of three to seven closely spaced spikes (Fig. 3). The number of spikes per burst and the mean spiking frequency during a burst were quite similar in the different burster types and averaged 3·7 ± 0·8 and 123·7 ± 38·2 Hz ($n = 98$),

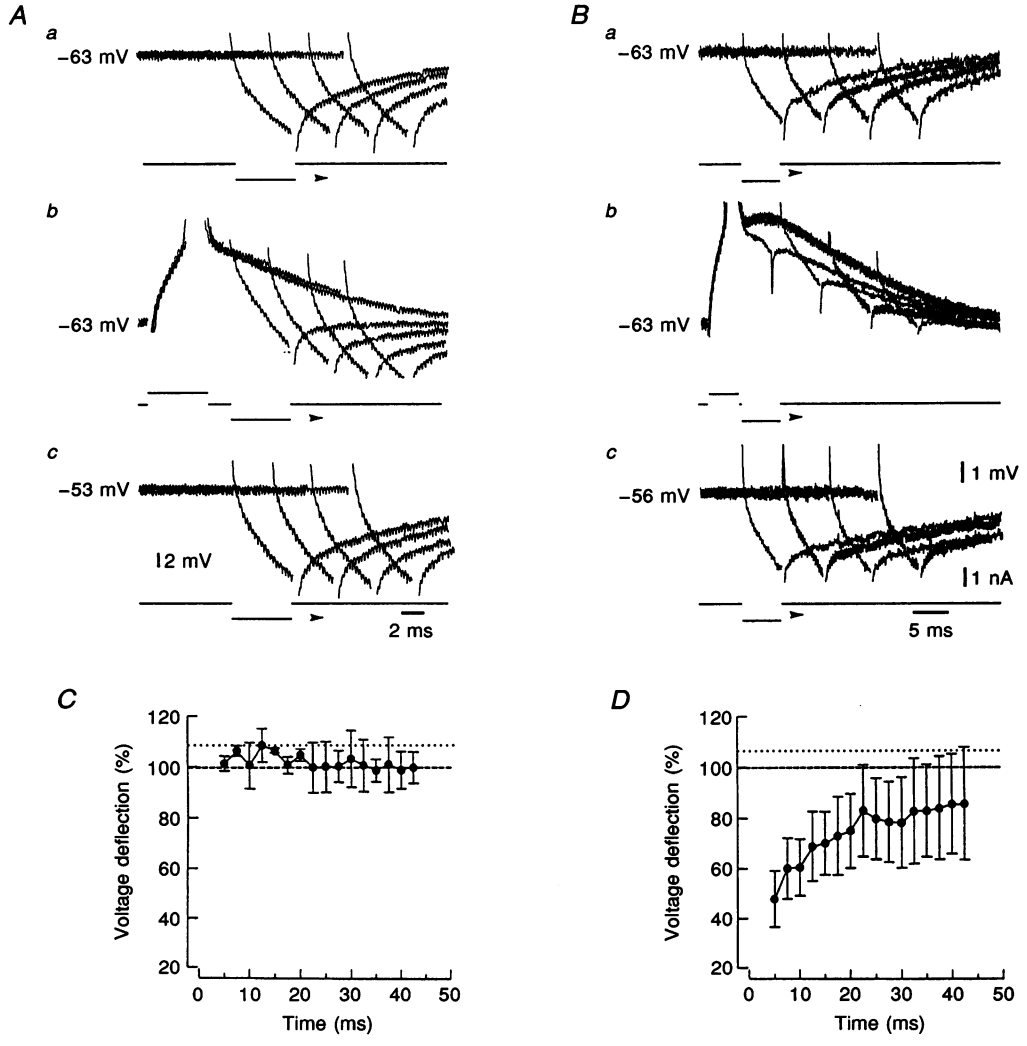

Figure 4. Changes in input resistance during spike ADPs

Single spikes were evoked by injecting brief positive current pulses. Constant 5 ms negative current pulses were delivered at rest and at various times during the ADPs. Changes in the amplitudes of the resultant hyperpolarizing deflections were assumed to reflect proportional changes in input resistance. *A* and *B* illustrate the changes in resistance associated with a passive and an active ADP, respectively, in two different neurones. In both cases, the superimposed traces show pulsing at the resting potential (−63 mV; *Aa* and *Ba*), during the ADP (*Ab* and *Bb*), and at a depolarized membrane potential (*Ac* and *Bc*). The voltage deflections are slightly increased during the early part of the passive ADP (*Bb*), but markedly reduced throughout the active ADP (*Ab*). In both cases they increase when the membrane is moderately depolarized by constant current injection (*Ac* and *Bc*). *C* and *D*, pooled data from four and six cells, respectively, depicting the time course of changes in voltage deflections during passive and active ADPs. Each point represents the mean ± S.D. of the normalized amplitudes of the voltage deflections evoked at the indicated times after the peak of the fast AHP. The dashed lines indicate the control response at resting potential. The dotted lines represent the averaged responses to pulses applied after depolarizing the membrane potential by 10 mV with steady current injection.

respectively. Spike threshold increased during a burst and consecutive spikes became progressively smaller and slower (Fig. 3). Since each spike was triggered from a more depolarized potential than its predecessor, the cluster of spikes appeared to ride on a growing slow depolarizing potential. The peak amplitude and duration of this burst envelope were $18{\cdot}1 \pm 6{\cdot}3$ mV and $63{\cdot}0 \pm 16{\cdot}5$ ms, respectively.

The effects of changing membrane potential on bursts evoked by brief pulses were investigated in thirty PCs. As illustrated in Fig. 6*A*, modestly depolarizing (panel *a*) or hyperpolarizing the neurone (panel *c*) induced intermittent failures of the latter spikes in the burst, unmasking prolonged ADPs. Further polarization blocked the burst entirely (panel *d*). Thus burst responses resembled active ADPs in being of maximal size when evoked from about resting potential.

In spontaneously burst firing PCs (grade III bursters) burst frequencies ranged from 0·12 to 2·0 Hz, averaging $1{\cdot}15 \pm 0{\cdot}62$ Hz ($n = 18$). The spontaneous bursts were similar to the bursts evoked in the same neurone by injecting brief depolarizing current pulses. Likewise, they were markedly modulated by membrane potential (Fig. 6*B*). Depolarizing the neurones decreased the size of the bursts (number of spikes and amplitude of the slow depolarizing potential), while increasing their frequency (panel *b*). Further depolarization converted burst firing into solitary spiking (panel *a*). Slightly hyperpolarizing the neurones (ca 4 mV) was usually sufficient to suppress spontaneous burst activity entirely (panel *d*).

Bursts of d-spikes

In a small percentage of CA1 bursters (12 of 98; 12·2%), bursts appeared as clusters of small spikelets. The spikelet amplitudes ranged from 5 to 15 mV and were rather insensitive to changes in resting membrane potential (Fig. 7). These previously described spikelets have been referred to as d-spikes because of their supposed dendritic origin (Kandel & Spencer, 1961*b*). Within a burst, some of the d-spikes were subthreshold, whereas others triggered full somatic spikes (Fig. 7). Bursts evoked by brief depolarizing current pulses always began with a rapidly rising somatic spike, followed by a series of d-spikes that intermittently triggered full somatic spikes (Fig. 7*A*). In contrast, spontaneous bursts usually began with a subthreshold d-spike, and full spikes were recruited only later in the burst (Fig. 7*Bb*). Depolarizing these PCs by a few millivolts facilitated the recruitment of full spikes by the d-spikes and enhanced the rate of spontaneous bursts (panel *Ba*). Conversely, hyperpolarization abolished all full spikes and reduced the frequency and size of the d-spike clusters (panel *Bc*). A bigger hyperpolarization was

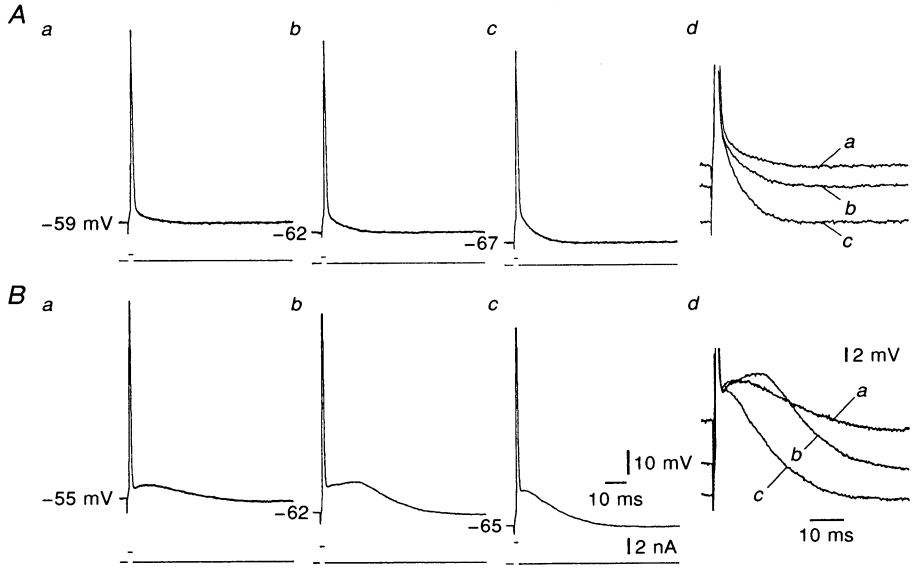

Figure 5. Modulation of spike ADPs by membrane potential

The records in *A* and *B* were obtained from two different PCs displaying passive and active spike ADPs, respectively. Resting membrane potential in both PCs was −62 mV. Membrane potential was polarized with constant positive or negative current injection to the indicated levels. *A*, modest polarization (up to ± 8 mV) did not change the peak of the fast AHP, so that the size of these passive ADPs (i.e. the difference between membrane potential and the peak of the fast AHP) increased linearly with hyperpolarization. The records evoked at −59 (*A a*), −62 (*A b*), and −67 mV (*A c*) are superimposed in *A d* for comparison. *B*, the active ADPs were strongly modulated by membrane potential even though the peak of the fast AHP did not change. They were maximal in amplitude at resting potential (*Bb*), and reduced by small depolarization (*Ba*) or hyperpolarization (*Bc*). For comparison, see the superimposed traces in *Bd*.

required to suppress spontaneous d-spike bursts entirely (ca 7 mV; panel *Bd*) than to suppress ordinary spontaneous bursts (ca 5 mV; Fig. 6*B*), supporting the view that the former bursts are initiated more remotely than the latter bursts.

DISCUSSION

In this study we have investigated intrinsic burst firing in somata of adult rat PCs recorded from all CA1 subfields. High-K^+ saline (7·5 mM K^+) was used in order to increase the size of the burster population, which in normal-K^+ saline (3·5 mM K^+) is rather small, precluding thorough quantitative analyses of burst characteristics (Jensen *et al.* 1994). The main conclusions of the present study are that bursts arise in the somata of CA1 PCs through a local regenerative depolarization mediated by a slow, voltage-gated inward current. The companion paper (Azouz *et al.* 1996) identifies this current as a persistent Na^+ current.

Electrical membrane properties in non-bursters *versus* bursters

The resting membrane potential and input resistance were almost identical in non-bursters and bursters (Table 1). However, the value of τ_m was 1·8-fold slower in the latter PCs (Table 1). Assuming a similar specific membrane capacitance (C_m) of the order of 1 mF cm^{-2} (Rall, 1977), this difference suggests that bursters may have a proportionately higher specific membrane resistance (R_m) than non-bursters (since $\tau_m = R_m C_m$). In the CA3 field, the estimated R_m values in burst firing PCs are also significantly (1·5-fold) higher than in non-bursters (Bilkey & Schwartzkroin, 1990). If that is the case, then what accounts for the similarity in the input resistance of bursters and non-bursters? One likely explanation is that bursters are larger in size than non-bursters.

Passive and active ADP components

We have confirmed previous findings *in vivo* (Kandel & Spencer, 1961*a*; Fujita, 1975) and *in vitro* (Schwartzkroin, 1975; Wong & Prince, 1981) that somatic spikes in adult PCs are followed by distinct ADPs that last tens of milliseconds. The ADPs were classified as either 'passive' or 'active' types according to their waveform. Passive ADPs declined monoexponentially from the peak of the fast AHP, whereas active ADPs first re-depolarized before slowly repolarizing.

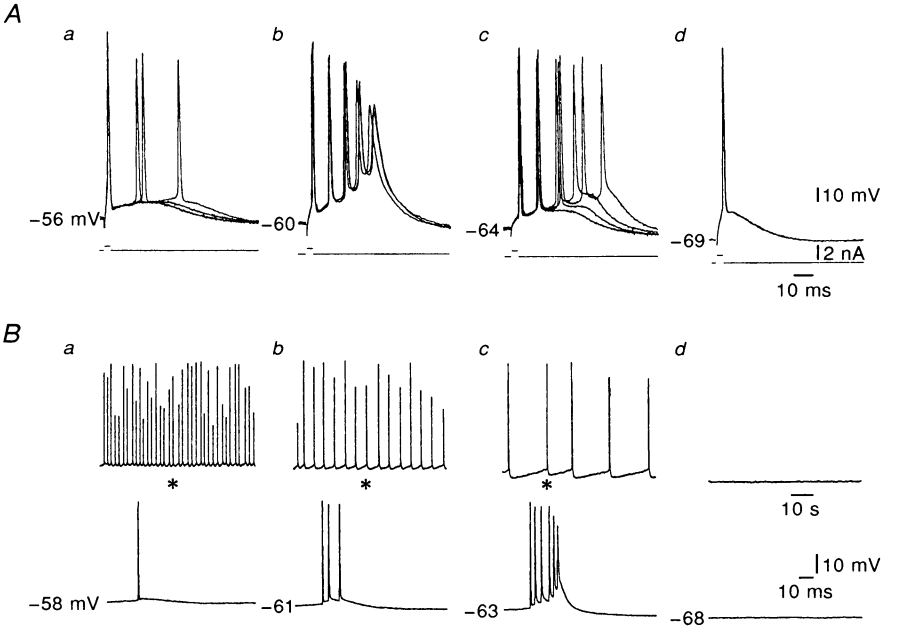

Figure 6. Modulation of evoked and spontaneous bursts by membrane potential

A, bursts were evoked by brief stimuli, while membrane potential was polarized (± 10 mV) by constant current injection. At resting membrane potential (−60 mV), the neurone fired a stereotyped burst of five action potentials (*A b*). Slightly (4 mV) depolarizing (*A a*) or hyperpolarizing (*A c*) the neurone reduced the spike frequency in the burst and produced intermittent failures of late spikes. Note the unmasking of prolonged active ADPs and the constancy of burst envelope duration at a given membrane potential. Further depolarization evoked repetitive firing of solitary spikes (not shown). Further hyperpolarization blocked burst generation (*A d*). *B*, spontaneous bursts in another PC were recorded without current injection (*Bc*) and during the injection of constant positive (*Ba* and *Bb*) or negative current (*Bd*). The events marked with an asterisk in the upper traces are expanded in the corresponding lower traces. Slightly depolarizing the neurone transformed burst firing into solitary spike discharge (*Ba*), whereas a 5 mV hyperpolarization suppressed spontaneous discharge entirely (*Bd*).

It was previously argued that passive ADPs in hippocampal PCs are caused by passive charging of the somatic membrane capacitance following incomplete spike repolarization, since their decay time constant was found to be similar to τ_m (Spencer & Kandel, 1961; Wong & Prince, 1981). This argument assumed that the somatic membrane is passive near the resting potential. However, it has been shown that voltage-dependent conductances are active near the resting potential of CA1 PCs and influence the measured values of R_N and τ_m (Hotson, Prince & Schwartzkroin, 1979; Spruston & Johnston, 1992). Small depolarizations from resting potential increased both R_N and τ_m. This inward rectification may contribute to the apparent increase in R_N during the early portion of passive ADPs and to the slowing of ADP decay upon mild depolarization of the neurone.

Unlike passive ADPs, active ADPs were associated with an apparent decrease in R_N. Also they were strongly modulated by small changes in membrane potential ($\pm$ 2 mV) and were abated by brief hyperpolarizing pulses. These properties suggest that the generation of active ADPs involves an interplay of slow voltage-gated currents that are active near spike threshold. The strong voltage sensitivity of these ADPs suggests that the conductance changes occur primarily at or near the soma.

The low threshold-activated (T-type) Ca^{2+} current (Yaari, Hamon & Lux, 1987) and the persistent Na^+ current (French, Sah, Buckett & Gage, 1990) have properties that could actively re-depolarize the neurons from the peak of the fast after-hyperpolarization. The T-type Ca^{2+} current has been implicated in the generation of active ADPs in immature hippocampal granule cells (Zhang, Valiante & Carlen, 1993). However, it is unlikely that it plays a role in active ADPs in adult CA1 PCs, since active ADPs could be evoked from depolarized membrane potentials (e.g. −55 mV in Fig. 5*B*) at which this current fully inactivates (Karst, Joëls & Wadman, 1993). Moreover, as shown in the companion paper (Azouz *et al.* 1996), active ADPs were not

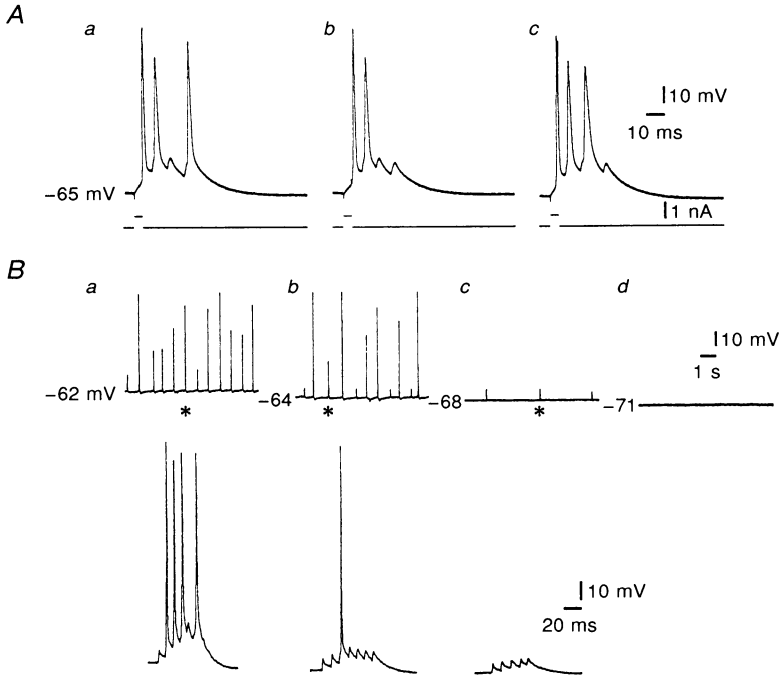

Figure 7. Evoked and spontaneous bursts of d-spikes

The records in *A* and *B* were obtained from two different PCs. *A*, the bursts evoked in this neurone by brief stimuli consisted of four spikes, as depicted in the three selected traces (*A a*, *A b* and *A c*). In all cases the first spike was a directly evoked somatic spike, i.e. it emerged abruptly from the stimulus-induced voltage deflection and was not preceded by a fast prepotential. All subsequent spikes were d-spikes that occasionally triggered full somatic spikes. *B*, spontaneous d-spike bursts recorded while changing membrane potentials by constant current injection ($\pm$ 0·5 nA). The upper traces depict the spontaneous activity on a slow time base. Selected records of bursts are shown on an expanded time scale in the lower traces. At resting potential (−64 mV; *B b*) the spontaneous bursts consisted of clusters of seven to ten d-spikes that occasionally triggered full somatic spikes. The threshold for full spikes was attained by temporal summation of several d-spikes (*B b*). Depolarizing the neurone to −62 mV increased both burst frequency and the number of d-spikes attaining the somatic spike threshold (*B a*). Conversely, hyperpolarizing the neurone to −68 mV blocked all somatic spikes and reduced burst frequency and size (*B c*). However, d-spike bursts were entirely suppressed only with further hyperpolarization to −71 mV (*B d*).

blocked by replacement of extracellular Ca^{2+} with Mn^{2+}, but were abolished by tetrodotoxin. These data suggest that the persistent Na^+ current is likely to be involved in generating active ADPs.

The persistent Na^+ conductance increases sigmoidally with depolarization from −70 to −30 mV (French *et al.* 1990). Since fast spike repolarization ends at −53·4 mV (Table 1), about half of this conductance would be active immediately after the spike. Whether from this point onward the neurone would depolarize (active ADP) or repolarize (passive ADP) would depend on the ratio between this current and slow repolarizing K^+ currents, which also are activated in this voltage range (e.g. the muscarine-sensitive K^+ current, I_M; Storm, 1990). This ratio most probably varies across CA1 PCs and in each neurone depends critically on resting potential. If the ratio favours depolarization, than a regenerative process may ensue, in which depolarization further activates the persistent Na^+ current, which further depolarizes the neurone, and so forth, until voltage- and time-dependent K^+ conductances increase sufficiently to terminate this process and repolarize the cell. Otherwise, the neurone repolarizes monotonically to its resting potential.

Relationship between ADPs and bursts

Previous studies of burst mechanisms in PCs have suggested a close relationship between active ADPs and bursts (Kandel & Spencer, 1961*a*; Fujita, 1975; Wong & Prince, 1981; Jensen *et al.* 1994). This notion is also supported by the data reported here. First, the ADPs were passive in most non-bursters and active in most bursters. Secondly, active ADPs and bursts were similarly modulated by changes in resting potential. Both were reduced by hyperpolarization or depolarization beyond a narrow voltage window. Finally, in the companion paper (Azouz *et al.* 1996), we show that active ADPs and bursts are similarly affected by ionic manipulations and drugs. The simplest explanation for this relationship is that bursts are produced by exceptionally large active ADPs.

Accordingly, we envisage the sequence of events that occur during a burst cycle as an exaggeration of the regenerative process that underlies an active ADP. If the first ADP is large enough, it triggers a second spike, which, being initiated at a more depolarized potential than the first spike, rises more slowly and attains a smaller amplitude (due to increased inactivation of fast Na^+ channels). It also repolarizes more slowly and less completely (due to less activation and/or inactivation of repolarizing K^+ currents, such as the fast transient K^+ current, I_A; Storm, 1990). Consequently, the second ADP begins at a more depolarized potential than the first, so that a larger persistent Na^+ current is recruited to sustain the depolarized state. If the second ADP persists long enough to intersect with the declining spike threshold, an even slower third spike is triggered and the process repeats itself again, and so forth. Activation of slow K^+ currents (Storm, 1990) may eventually terminate this process and repolarize the neurone.

According to this hypothesis of burst generation, the ratio of persistent Na^+ conductance to slow K^+ conductances is a critical determinant of the propensity to burst in CA1 PCs. Increasing this ratio would be expected to increase this propensity and vice versa. We have previously suggested that induction of intrinsic burst firing by high-K^+ saline (Jensen *et al.* 1994) may be due to direct enhancement of persistent Na^+ current (Cannon, Brown & Corey, 1991) and/or to a decrease in driving force of K^+ currents (Jensen, Cherubini & Yaari, 1993), both of which would enhance the ratio of persistent Na^+ conductance to slow K^+ conductances. Conversely, suppression of burst firing by muscarinic receptor stimulation (Azouz *et al.* 1994) may be due to suppression of the persistent Na^+ conductance.

Site of somatic burst initiation

Previous studies have reported that somata of immature CA1 PCs (Wong & Stewart, 1992) and isolated somata of adult CA1 PCs (Benardo *et al.* 1982) do not fire bursts spontaneously nor when injected with long depolarizing current pulses. Therefore, it was concluded that bursts recorded in the soma are, in fact, initiated in the distal dendrites and spread from there into the soma in a spike-for-spike manner (Wong & Stewart, 1992).

Our data suggest that somatic bursts in most bursters are initiated locally. First, threshold-straddling stimuli evoked full-blown bursts in all these neurones. It is unlikely that such stimuli evoke a short-latency burst in the dendrites before eliciting a somatic spike, because spike threshold is lower in the somata of CA1 PCs than in their dendrites (Spruston, Schiller, Stuart & Sakmann, 1995). It may be argued that the first spike in the burst cluster arises in the soma and secondarily recruits a dendritic burst, which in turn propagates back into the soma. Modelling this situation predicts that the first interspike interval in the somatic burst would be substantially longer than the others (see Fig. 6 in Traub, Wong, Miles & Michelson, 1991). However, such a gap was not observed. Indeed, in most bursters the first interspike interval in the burst was shorter than, or equal to, the subsequent interspike intervals in the burst.

The second argument against a dendritic origin of somatic bursts is their remarkable sensitivity to small changes (ca 2 mV) in somatic membrane potential. It is unlikely that bursts generated remotely would be blocked entirely by the minimal hyperpolarizing current pulses required to suppress somatic spikes. This argument is supported by the few cases in which somatic bursts were associated with remotely generated d-spikes. In these cases, bursts of d-spikes persisted even when all somatic spikes were blocked by hyperpolarization.

Although d-spikes have been viewed as non-invading dendritic spikes since their original description *in vivo* (Kandel & Spencer, 1961*b*) and *in vitro* (Schwartzkroin, 1977), other views of their origin may be entertained. Indeed, in confirmed paired recordings from soma and dendrites of immature CA1 PCs, bursts evoked by injecting current into dendrites did not evoke d-spikes in the soma (see Figs 3 and 4 in Wong & Stewart, 1992). An alternative origin of d-spikes may be electrotonically coupled neurons, as may be the case in dentate granule cells (MacVicar & Dudek, 1982). Interestingly, burst firing adult CA1 PCs were recently reported to be electrotonically coupled via gap junctions to other neurones (Church & Baimbridge, 1991). Such a coupling may be more common among immature CA1 PCs, since local coupling via gap junctions prevails in developing cortex (e.g. Peinado, Yuste & Katz, 1993).

Taken together, these data suggest a developmental switch in the site of burst generation in CA1 PCs. A postnatal increase in persistent Na^+ current in acutely dissociated neocortical PCs has been described (Huguenard, Hamill & Prince, 1988). If such an increase occurs preferentially in the soma of CA1 PCs, than it may account for an increased somatic burst firing capability during postnatal maturation.

Physiological and pathophysiological implications

It was suggested that the output of immature CA1 PCs reflects the location of excitatory input to the cell: excitation of distal dendrites elicits a dendritic burst which propagates into the soma, whereas excitation of the soma and proximal dendrites elicits a stream of repetitive firing in the soma (Wong & Stewart, 1992). Since in most adult CA1 bursters somatic bursts are initiated at or near the soma, both suprathreshold dendritic and somatic inputs would activate the burst mechanism. Therefore, the output pattern of adult CA1 PCs (burst firing *versus* regular repetitive firing) cannot faithfully code for the location of their excitatory input.

The question arises whether intrinsic burst firing plays a role in normal and pathological behaviour of adult CA1 PCs. In normal-K^+ saline the vast majority of bursters would burst only during a sustained depolarization (Jensen *et al.* 1994). Therefore, repetitive synaptic excitation may be required to evoke burst firing in these neurones. Even then, burst recruitment may be limited by the powerful GABAergic feedforward and recurrent inhibitory inputs to CA1 PCs (Masukawa & Prince, 1984). However, conditions which raise extracellular K^+ and/or reduce GABAergic inhibition may unleash the intrinsic burst mechanism and markedly augment the output of CA1 PCs, thereby contributing to the development of epileptiform discharges (Traub & Wong, 1983).

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