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The effect of neuronal population size on the development of epileptiform discharges in the low calcium model of epilepsy

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Abstract

The CA1 region of the rat hippocampal slice generates spontaneous electrographic seizures (field bursts) when exposed to ACSF containing ≤0.2 mM calcium. It has been proposed that, particularly during the early part of a field burst, synchronised activity in small independent aggregates of neurons results in low amplitude irregular population spikes and subsequent fusion of aggregates generates high amplitude, regular discharging spikes. In the present experiments, we have tested the hypothesis that progression from aggregate formation to aggregate fusion requires a critical mass of participating neurons. We found that isolated CA1 segments >2 mm are still able to generate high amplitude, regular discharging population spikes, but when segment length is reduced to 1–2 mm, only 29% generate spikes with these characteristics; in the remainder, the field burst shows a DC shift ± low amplitude irregular population spikes. No field bursts were seen in segments <0.7 mm or in 50% of those 0.7–1 mm in length (in the remaining 50%, only the DC component of the field burst was present). Exposing 1–2 mm segments to hypo-osmolar perfusate induced a return of high amplitude rhythmic discharging population spikes in the field burst. We interpret these observations by indicating that progression from aggregate formation to aggregate fusion requires a critical neuronal mass and can be enhanced by reducing osmolarity of the perfusate.

Keywords: Epilepsy; Epileptiform; Hippocampus; Osmolarity; Field-effect; Aggregate

The CA1 region of the isolated hippocampal slice, when exposed to artificial cerebrospinal fluid (ACSF) containing a low (≤0.2 mM) concentration of calcium, generates spontaneous field bursts [12,14,22,26]. These consist of a shift in DC potential lasting ∼15 s, superimposed on which there may be repetitive population spikes consisting of synchronised neuronal discharges [1,24]. Particularly at the onset of a field burst, the population spikes are irregular, with a high instantaneous frequency and low amplitude; these are usually replaced by high amplitude, regular population spikes, with a lower frequency (25–70 Hz) [5,6]. In a previous paper [5], we proposed that the irregular low amplitude population spikes are generated by synchronised action potentials in (semi-)independent neuronal aggregates and that progression to the rhythmic, low frequency discharges results from the fusion of activity in these aggregates. This implies that generation of the slow, rhythmic discharges which bear a resemblance to the activity seen in human EEGs during an epileptic attack, is a two stage process consisting of aggregate formation followed by aggregate fusion. In the present experiments, we have further tested this hypothesis by investigating whether progression from individual aggregate activity to fused population discharges can be blocked by reducing the size of the participating neuronal population.

Transverse hippocampal slices (400 μm) were prepared from male Sprague–Dawley rats (180–225 g; anaesthetized with ketamine (7.4 mg kg^{-1} i.p.) and medetomidine (0.7 mg kg^{-1} i.p.); killed by cervical dislocation), using a Vibroslice (Campden Instruments Ltd., Loughborough, UK). All experiments were performed under the Animals (Scientific Procedures) Act 1986 of the UK. The slices were stored at room temperature, submerged in a holding chamber filled with "normal" ACSF consisting of (in mM): 125 NaCl, 26 $NaHCO_3$, 3 KCl, 2 $CaCl_2$, 1.0 $MgCl_2$, 1.25 NaH_2PO_4 and 10 glucose, aerated with 95% O_2, 5% CO_2 mixture. After >60 min, slices were transferred to an interface recording chamber (35 °C) perfused with "low-calcium" ACSF consisting of (in mM): 125 NaCl, 26 $NaHCO_3$, 5 KCl, 0.2 $CaCl_2$, 1.0 $MgCl_2$, 1.25 NaH_2PO_4 and 10 glucose, bubbled

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with 95% O_2, 5% CO_2 mixture. After control recordings had been made, the CA1 region was sectioned radially (using iridectomy scissors or a scalpel blade) to give segments, 0.5–3.5 mm long (the longest segment was essentially an isolated, complete CA1 region of the slice); segments were minimally separated from the remainder of the slice to ensure isolation.

Extracellular field potentials were recorded with glass micropipettes (2–8 MΩ) filled with low-calcium ACSF and positioned in the CA1 pyramidal cell layer. Signals were amplified and low-pass filtered (1 kHz) with an Axoclamp-2B or -2A (Axon Instruments, Union City, USA) and Neurolog NL-106 and NL-125 amplifiers (Digitimer, Welwyn, UK), digitised using a Power 1401 and Signal and Spike2 software (Cambridge Electronic Design Ltd., Cambridge, UK), and analysed with Signal and Spike2.

In some experiments, slices were exposed to hypo-osmolar solutions; in these, NaCl concentration was reduced to 105 or 90 mM (−40 and −70 mOsm). Results are expressed as mean ± S.E. except where otherwise stated.

In earlier work [10], it was observed that regular discharging population spikes began after their amplitude reached approximately 1.5 mV. For the purposes of the present paper, a field burst was said to have activity in independent aggregates when 20 consecutive population spikes had a maximum amplitude <1.5 mV, a mean interspike interval (ISI) <10 ms and the mean of the consecutive interspike interval differences (MCD) was >50% mean ISI; fused aggregates were identified by the presence of 20 consecutive population spikes with a minimum amplitude >1.5 mV, a mean ISI >10 ms and an MCD <15% mean ISI [5].

All of the intact slices used in the present experiments generated spontaneous field bursts (duration = 18.9 ± 1.2 s, DC amplitude = 4.6 ± 0.36 mV, interburst interval = 77 ± 7.3 s; $n = 15$) containing periods of both fused aggregate (maximum spike amplitude = 7.1 ± 0.8 mV; minimum frequency = 29 ± 2.5 Hz; Fig. 1A) and independent aggregate (maximum amplitude = 1.0 ± 0.1 mV; ISI = 8.1 ± 0.6 ms; MCD = 5.4 ± 0.7 ms) activity.

When the slice was sectioned, isolated segments 2.0–3.5 mm in length generated field bursts which were very similar to those in the complete slice (duration = 17.9 ± 1.2 s; DC amplitude = 4.7 ± 0.39 mV; interburst interval = 75 ± 9 s; $n = 10$); in particular, they continued to generate regular discharging population spikes (maximum population spike amplitude = 8.0 ± 1.2 mV; minimum frequency = 25 ± 1.5 Hz).

When slice length was reduced below this, however, aggregate fusion frequently failed in the isolated segment (Table 1).

Table 1
Field burst characteristics in segments of different length

	DC shift: regular population spikes	DC shift: irregular population spikes only	DC shift only	No bursts
0.5–0.7 mm	0/9	0/9	0/9	9/9
0.7–1 mm	0/6	0/6	3/6	3/6
1–2 mm	4/14	5/14	5/14	0/14
2–3.5 mm	10/10	0/10	0/10	0/10

Segments between 1 and 2 mm length still generated field bursts (duration = 11.1 ± 0.9 s; DC amplitude = 2.7 ± 0.4 mV; interburst interval = 45 ± 5.3 s; differences from controls were statistically significant, paired t-test, $p < .001$ for each measure, $n = 14$), but only 4/14 continued to generate regular discharging, high amplitude population spikes; field bursts in the other 10 slices either showed only the DC shift or population spikes which remained irregular and of low amplitude (Fig. 1B). Segments <1 mm in length ($n = 15$) produced no high amplitude regular discharging population spikes and segments <0.7 mm ($n = 9$) failed to generate even the DC component of the field bursts.

Reducing osmolarity of the ACSF partially reversed the effects of reduced segment size on burst characteristics. Five of the 1–2 mm segments, in which field bursts only contained irregular discharging low amplitude spikes, were perfused with ACSF in which osmolarity had been reduced by 40 mOsm. Four of these slices then showed regular discharging population spikes (Fig. 1C), suggesting that reduction in osmolarity had facilitated aggregate fusion.

In small (<1 mm) segments showing no bursts in normal osmolar ACSF, perfusing with −40 mOsm or −70 mOsm ACSF induced field bursts in 2/4 and 4/4 segments, respectively, but they consisted only of a DC shift or DC shift + small, irregular discharging population spikes; regular discharging population spikes failed to become established.

Alveus stimulation provided an assessment of slice viability. In intact slices, the response to alveus stimulation depended on the timing of the stimulus in relation to the last spontaneous field burst. A stimulus presented midway between spontaneous bursts evoked only a brief discharge of high frequency population spikes (frequency = 193 ± 8 Hz; duration = 37 ± 2.5 ms) and a brief shift in DC potential (amplitude = 0.4 ± 0.17 mV; duration = 1.2 ± 0.3 s); a stimulus presented during the last quarter of the predicted interburst period evoked a high frequency discharge followed by a field burst. In segments which were too small to generate spontaneous bursts, alveus stimulation still evoked a high frequency discharge of population spikes (amplitude of the first spike was 31 ± 6% of that seen in the intact slice; frequency = 207 ± 6.4 Hz; duration of high frequency discharge = 28 ± 4.3 ms) and a small shift in DC potential (amplitude = 0.3 ± 0.1 mV; duration = 1.1 ± 0.7 s). The stimulus never, however, triggered a full field burst.

Our observation that isolated segments of CA1 longer than 0.7 mm were able to generate spontaneous field bursts which were independent of activity in the remainder of the hippocampal slice compares with a size of 0.5–0.7 mm, which Miles et al. [18] regarded as the minimum necessary for the generation of picrotoxin induced epileptiform discharges in CA3. In the neocortex, there is evidence that the minimum cortical area necessary to support penicillin induced epileptic activity is 0.5–0.6 mm^2 [3,16]. This length corresponds to 2000–3000 neurons [7,17,25], but this may represent an overestimate, since some damage is inevitable at the section site, resulting in further loss or inactivation of neurons.

Reducing the size of the participating neuronal pool (at least in segments <2 mm) resulted in a reduction of the amplitude of

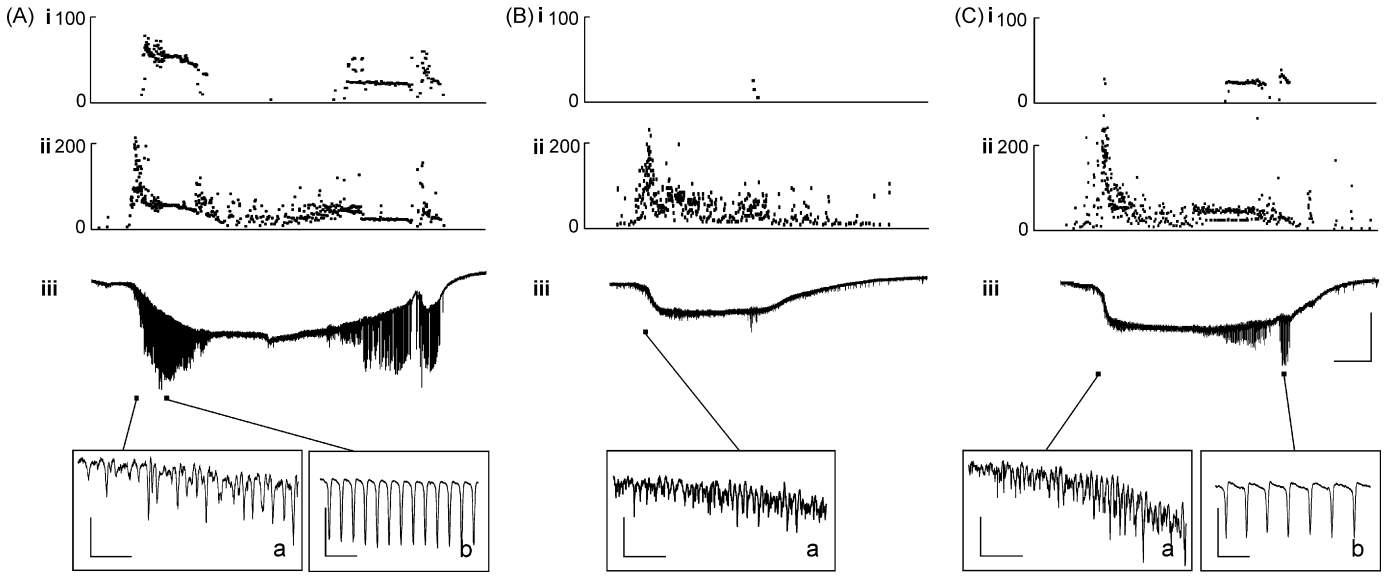

Fig. 1. Effect of sectioning CA1 and subsequent reduction of osmolarity on the structure of low-calcium field bursts. Instantaneous frequency analyses for population spikes >1.5 mV (i) and >0.45 mV (ii) are shown above the voltage trace (iii) and are aligned with its time axis. Vertical axes of instantaneous frequency plots are labelled in Hz; calibration bars (5 mV and 2 s) apply to A, B and C; insets (a) show the irregular activity at burst onset (1 mV and 50 ms calibration bars) and (b) regular higher amplitude spikes which occur later in the burst (5 mV and 50 ms calibration bars). (A) Intact hippocampal slice. (B) After making an isolated 1.6 mm segment, the slice still generated field bursts with small irregular population spikes (ii and iii), but higher amplitude regular discharging spikes failed to become established (i). (C) Exposure to ACSF with osmolarity reduced by 40 mOsm resulted in a return of regular discharging, higher amplitude population spikes (i and iii).

the DC shift, implying that in these segments, field bursts produced smaller potassium transients. There was a concomitant reduction in burst duration and interburst interval. Previous work has shown that interburst interval can be modified by factors which influence excitability [12,27]; in the present experiments smaller potassium transients would cause a reduced excitation, explaining the observed reductions in burst duration and interburst interval.

A distinction should be made between the generation of field bursts and the synchronisation of neuronal discharges within the burst. We have previously presented evidence that during the early part of a field burst, action potentials are synchronised in small, semi-independent aggregates of neurons; the fusion of activity in these aggregates results in the appearance of rhythmic, low frequency, high amplitude population spike potentials [5]. The present experiments demonstrate that the transition to rhythmic high amplitude population spikes only occurs when segment size reaches a critical level. This transition only occurred reliably in segments greater than 2 mm in length. Between 1 and 2 mm lengths, neuronal discharges could become synchronised to the extent of generating small population potentials, but they failed to progress into high amplitude population spikes. We interpret the apparent irregularity of these small spikes as resulting from the simultaneous recording of several neuronal aggregates, each of which is discharging independently [5].

Generation of high amplitude population spikes frequently fails during the middle part of a field burst and we have previously shown that this is due, at least in part, to depolarisation block [6]. The presence of irregular low amplitude potentials at this time (Fig. 1) suggests that some neurons are still active and that aggregates are able to persist or reform. As the phase of depolarisation block finishes, high amplitude, regular discharging population spikes return, implying that the change in excitability has enabled activity in the aggregates to again become fused.

The effect of a reduction in the number of neurons in a segment can be partly reversed by reducing the osmolarity of the perfusate. Exposure to hypo-osmolar ACSF is known to increase the potassium transient during field bursts [10] and induces cell swelling with a concomitant change in extracellular resistance [2,8,10,20], which enhances field effect interactions between neurons [8,11]. These effects would facilitate both the initial development of neuronal aggregates and their fusion into larger populations of synchronously discharging neurons.

In epilepsy, an abnormally high level of neuronal synchronisation results in the generation of rhythmic spike discharges in the EEG and the low calcium model shows that non-synaptic mechanisms can contribute to this synchronisation [4,8,9,13,15,19,21,23]. In the present experiments, we have shown that non-synaptic synchronisation, at least in the low calcium model, is a two stage process (aggregate formation and aggregate fusion) which can be experimentally separated.

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