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Fast network oscillations induced by potassium transients in the rat hippocampus *in vitro*

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Brief pressure ejection of solutions containing potassium, caesium or rubidium ions into stratum radiatum of the CA1 or CA3 regions of the hippocampal slice evoked a fast network oscillation. The activity evoked lasted ~3–25 s with the predominant frequency component being in the gamma frequency range (30–80 Hz), although beta frequency (15–30 Hz) and ultrafast (> 80 Hz) components could also be seen. The gamma frequency component of the oscillation remained constant, even when large changes in power occurred, and was synchronous across the CA1 region. Measurements with potassium ion-sensitive electrodes revealed that the network oscillation was accompanied by increases (0.5 to 2.0 mM) in the extracellular potassium concentration $[K^+]_o$. Bath application of the *N*-methyl-D-aspartate (NMDA) receptor antagonists D(−)-2-amino-5-phosphonopentanoic acid (D-AP5; 50 μM) had no significant effect but the α-amino-3-hydroxy-5-methyl-4-isooxazolepropionic acid (AMPA)/kainate receptor antagonist 2,3,-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulphonamide disodium (NBQX; 20 μM) caused a significant reduction (86.7 ± 4.5 %) in the power in the gamma frequency range. Residual rhythmic activity, presumably arising in the interneuronal network, was blocked by the $GABA_A$ receptor antagonist bicuculline. The putative gap junction blocker octanol caused a decrease in the power of the gamma frequency component of 75.5 ± 5.6 %, while carbenoxolone produced a reduction of only 14 ± 42 %. These experiments demonstrate that a modest increase in exogenous $[K^+]_o$ in the hippocampus *in vitro* is sufficient to evoke a fast network oscillation, which is an emergent property of the synaptically and electrically interconnected neuronal network.

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Rhythmic oscillations, including those at gamma (30–80 Hz), beta (15–30 Hz) and ultrafast (> 80 Hz) frequencies, are thought to be important in a variety of cognitive processes. Gamma frequency oscillations have been recorded from a number of cortical areas, most notably sensorimotor (Murthy & Fetz, 1996), auditory (Barth & MacDonald, 1996) and visual cortices (Roelfsema *et al.* 1997). In the neocortex gamma frequency activity has been proposed as a mechanism for associative binding between large ensembles of neurons, particularly during visual processing (Gray *et al.* 1989; Singer & Gray, 1995). Gamma frequency activity has also been recorded in the hippocampus *in vivo* and *in vitro* under a range of different conditions, although the function of this network activity in this region remains unclear. In the hippocampus gamma frequency oscillations were seen in conjunction with theta (4–12 Hz) oscillations in both anaesthetised (Soltesz & Deschenes, 1993; Sik *et al.* 1997; Ylinen *et al.* 1995; Penttonen *et al.* 1998) and awake behaving rats (Bragin *et al.* 1995). Transient gamma frequency activity is also seen *in vivo* following physiological sharp waves (Buzsaki *et al.* 1986; Ylinen *et al.* 1995) and in association with limbic seizures (Bragin *et al.* 1997). In the hippocampus *in vitro* gamma frequency activity occurs after tetanic stimulation of CA1 pyramidal cells (Whittington *et al.* 1995, 1997) or following bath application of the cholinergic muscarinic receptor agonist carbachol (Fisahn *et al.* 1998). Beta frequency (15–30 Hz) oscillations have been observed after evoked gamma oscillations in sensory evoked potential recordings (Pantev, 1995) and may play a role in long range synchronisation *in vivo* (Roelfsema *et al.* 1997) and *in vitro* (Traub *et al.* 1996). Ultrafast (>80 Hz) 'ripple' oscillations have also been seen in the hippocampus *in vivo* (Buzsaki *et al.* 1992) and *in vitro* (Draguhn *et al.* 1998). It has been suggested that the ultrafast ripple activity may play a role in the storage and retrieval of memories (Chrobak & Buzsaki, 1996).

Several mechanisms have been proposed to be involved in the generation of these different frequencies of oscillatory network activity (for review see Jefferys *et al.* 1996; Traub *et al.* 1999; Whittington *et al.* 2000). Gamma and beta frequency activities are critically dependent on synaptic interactions but at least two types of synaptic circuitry are now known to be involved. Inhibition based gamma rhythms can arise from a network of interneurons and

does not require the involvement of pyramidal cells (Whittington *et al.* 1995, 1997). Other models of gamma frequency activity, for example those produced by bath application of carbachol, require activation of both interneurons and pyramidal cells (Fisahn *et al.* 1998). In addition to these different synaptic interactions signalling via gap junctions (Traub *et al.* 2000) and ephaptic field effects (Bracci *et al.* 1999; Whittington *et al.* 2001) can also contribute to the generation of oscillatory network activity under certain conditions.

One factor that may aid our understanding of the role of these fast network oscillations in the hippocampus is to know what conditions can give rise to oscillatory activity. One mechanism that could contribute to network oscillations is a change in extracellular potassium (Kaila *et al.* 1997). Fast neuron-to-neuron signalling mediated by increased extracellular $[K^+]_o$ transients has previously been demonstrated (Kaila *et al.* 1997; Smirnov *et al.* 1999). These authors proposed that activity induced $[K^+]_o$ shifts could play a role in the generation of neuronal oscillations. The initial aim of this study was, therefore, to determine whether brief increases in $[K^+]_o$ would be sufficient to trigger a network oscillation. We now show that focal application of potassium, in either the CA1 or CA3 region, is indeed able to elicit a transient episode of fast hippocampal network activity. The potassium-evoked fast oscillation consists of gamma, beta and ultrafast frequency components, and involves both fast glutamatergic and GABAergic synaptic signalling, with a possible contribution from gap junctions. Preliminary data from this study have been published in abstract form (LeBeau *et al.* 2000).

METHODS

Preparation of slices

Adult male Wistar rats (~150–200 g) were anaesthetised with inhaled isoflurane followed by injection of ketamine (⩾ 100 mg kg^{-1}) and xylazine (⩾ 10 mg kg^{-1}) I.M. Following the abolition of all pain reflexes, the animals were perfused intracardially with ~50 ml of modified artificial cerebrospinal fluid (ACSF) which was composed of (mM): 252 sucrose, 3 KCl, 1.25 NaH_2PO_4, 24 $NaHCO_3$, 2–4 $MgSO_4$, 2 $CaCl_2$ and 10 glucose. Following brain removal, 450 μm thick horizontal slices were cut. Slices were then trimmed and transferred to a holding chamber where they were maintained at room temperature at the interface between normal ACSF (where sucrose was replaced with 126 mM NaCl) and humidified 95 % O_2–5 % CO_2. For recording, slices were transferred to an interface chamber maintained at 34–35 °C. The procedure was in accordance with the UK Animals (Scientific Procedures) Act 1986.

Drugs

Bicuculline methochloride (20 μM), 2, 3,-dioxo-6-nitro-1, 2, 3, 4,-tetrahydrobenzo[f]quinoxaline-7-sulphonamide disodium (NBQX; 20 μM), (*S*)-α-methyl-4-carboxyphenylglycine (MCPG; 0.5–1 mM), D(−)-2-amino-5-phosphonopentanoic acid (D-AP5; 50–100 μM) and tetrodotoxin (TTX; 1 μM) were from Tocris Cookson (UK). Atropine (10 μM), carbenoxolone (100–200 μM), octanol (0.5–2 mM), lithium chloride, caesium chloride, potassium acetate (0.25–1.5 M) and potassium ionophore cocktail B were from Fluka. *N*,*N*,-Dimethyltrimethylsilylamine was from Sigma (UK). Rubidium chloride and potassium methylsulphate (0.25–1.5 M) were from ICN (Aurora, OH, USA).

Recording, data acquisition and analysis

Intracellular recordings were made using 1.5 M KCH_3SO_4-filled glass microelectrodes pulled to resistances of ~70–90 MΩ. Extracellular recording electrodes were filled with ACSF (resistance 2–5 MΩ). Pressure application of potassium solution (0.25 to 1.5 M potassium methylsulphate) was made with 1 mm borosilicate glass pipettes using a pneumatic picopump (WPI) (~40–60 p.s.i.; duration 3–60 ms). Extracellular potassium concentration was measured using K^+-sensitive microelectrodes that were silanised by exposing the tips to *N*,*N*,-dimethyltrimethylsilylamine vapour for ~30 min. Electrodes were then tip-filled with potassium ionophore cocktail B and back filled with 10 mM potassium chloride. Potassium recording electrodes were placed in stratum pyramidale (s. pyramidale) and were calibrated at the end of each recording by bath application of 3, 5 and 10 mM KCl.

Data were recorded with either one or two Axoprobe-1A amplifiers (Axon Instruments) and recorded on computer via an ITC-16 interface (Instrutech, USA). Data were acquired and analysed on computer using Axograph software (Axon Instruments). Fourier analysis gave the peak frequencies of the oscillations. A measure for power in a given frequency band was determined as the area under the peak in the power spectra between 15 and 30 Hz for beta frequency oscillations, 30–80 Hz for gamma frequency oscillations and 80–150 Hz for the ultrafast oscillations. Power spectra were constructed using a 2–3 s epoch at the point where the oscillation amplitude was maximal. Cross- and autocorrelations were calculated over a 1 s epoch. Results are expressed as means ± standard error and statistical significance was determined with Student's *t* test or the Mann-Whitney rank sum test. A significance level of $P < 0.05$ was chosen.

RESULTS

Pressure ejection of potassium, rubidium and caesium solutions evokes oscillatory network activity in CA1 and CA3

Pressure ejection of potassium methylsulphate (0.25–1.5 M) onto the surface of stratum radiatum (s. radiatum) of either the CA1 or CA3 hippocampal subfields elicited a transient (~3–25 s period of oscillatory network activity that could be recorded with an extracellular field electrode. Figure 1 shows extracellular and intracellular recordings from the CA1 region following ejection of potassium solution. The onset of oscillatory activity was preceded by an ~1–8 mV negative extracellular potential shift that recovered quickly to baseline, usually within 1–2 s. Oscillatory activity occurred after the DC shift had recovered (Fig. 1*A*i) and simultaneous intracellular recordings from a CA1 pyramidal cell showed a 10 mV depolarisation but, in this example, no action potential firing (Fig. 1*A*ii). The oscillatory activity consisted of rhythmic field potentials in the extracellular recording (Fig. 1*B*i) that occurred simultaneously with intracellularly recorded post-synaptic potentials (PSPs) (Fig. 1*B*ii). The corresponding power spectra (Fig. 1*C*i and ii) exhibited a prominent peak in the gamma frequency range (30–80 Hz)

and the intracellular PSPs were temporally correlated with the extracellular activity (Fig. 1*D*). In all extracellular recordings from both the CA1 and CA3 regions (57 slices/35 animals) similar gamma frequency peaks were seen in the spectra. The resting membrane potential of pyramidal cells was depolarised by a mean of 13.6 ± 8.6 mV ($n = 11$) following ejection of potassium. Action potential firing during the oscillation was variable, depending on the duration of the potassium ejection, but spiking on every cycle of the oscillation was rarely observed with most cells usually firing action potentials sporadically or not at all. The oscillatory activity evoked with potassium ejection was also highly reproducible on repeated ejections of potassium with similar epochs of gamma frequency activity being evoked for up to 6 h of recording.

In addition to potassium methylsulphate, pressure ejection of several other salt solutions (concentrations 0.25–1.5 M), including potassium chloride ($n = 7$), potassium acetate ($n = 4$) caesium chloride ($n = 3$) and rubidium chloride ($n = 9$), also evoked a gamma frequency oscillation. No oscillatory activity was observed following pressure ejection of either sodium chloride (1.5 M; $n = 6$) or lithium chloride (1.5 M; $n = 2$) (data not shown), but the ability of those slices to show oscillatory activity was confirmed by subsequent pressure ejection of potassium methylsulphate into the same region. Pressure ejection of sucrose solution (1 M), which has been shown to increase neurotransmitter release (Bekkers & Stevens, 1989), did not elicit any oscillatory activity in three slices tested.

Properties of potassium-evoked fast oscillations

An important feature of the gamma frequency activity elicited with potassium ejection was that the frequency of the oscillation was largely constant throughout the period of the oscillation (Fig. 2*A*i). In six slices (CA1 region), in

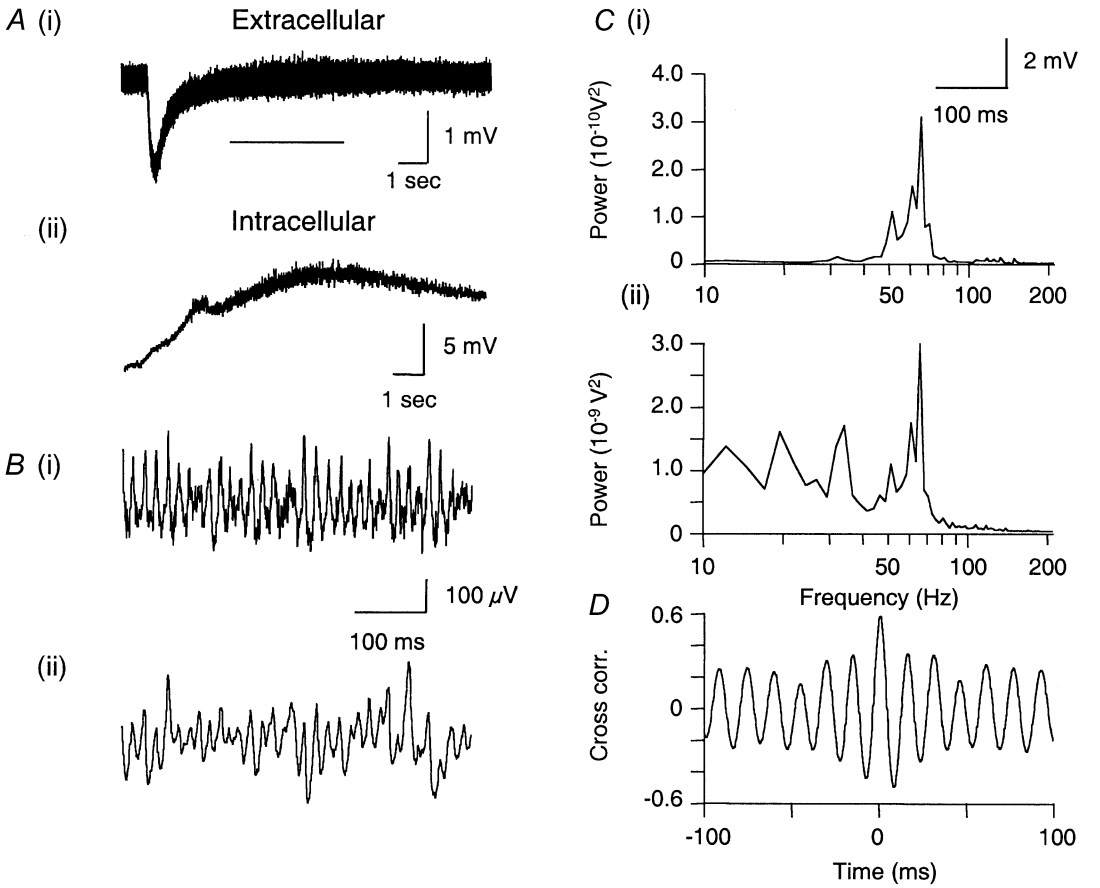

Figure 1. Pressure ejection of potassium evokes a transient episode of gamma frequency oscillatory activity

A, extracellular and intracellular recordings from stratum radiatum in the CA1 region following pressure application of potassium methylsulphate (1.5 M). In the extracellular trace (*A*i) oscillatory activity occurs (region indicated by the dark bar) upon recovery from the transient negative DC shift. Simultaneous intracellular recording (*A*ii) from a CA1 pyramidal cell (resting membrane potential −57.5 mV) shows a modest depolarisation to −47 mV. Details of the oscillatory activity are shown in the expanded traces (0.5 s) below for extracellular (*B*i) and intracellular (*B*ii) recordings. The corresponding power spectra (*C*i and ii) show prominent peaks in the gamma frequency range (30–80 Hz). The cross-correllogram (*D*) shows that intracellularly recorded post-synaptic potentials were correlated with the extracellular response (~1 ms delay).

which the oscillation lasted 8 s, frequency and power were determined for 1 s epochs from the onset of the oscillation. The peak frequency of the gamma (30–80 Hz) component of the oscillation 4–5 s after oscillation onset was 62.6 ± 4.8 Hz, which was not significantly different ($P > 0.05$) from the frequency at the onset of the oscillation, which was 62.1 ± 5.5 Hz. In contrast, the power (see Methods) of the gamma frequency activity (Fig. 2*A*ii) increased steadily over the first few seconds of the response, reaching a maximum at 4–5 s after oscillation onset when the mean power was 1.7 ± 0.9 mV^2 compared to 0.58 ± 0.3 mV^2 at 0–1 s. Thereafter, the power of the activity gradually decreased as the oscillation slowly subsided. In addition to the gamma frequency component, which was always

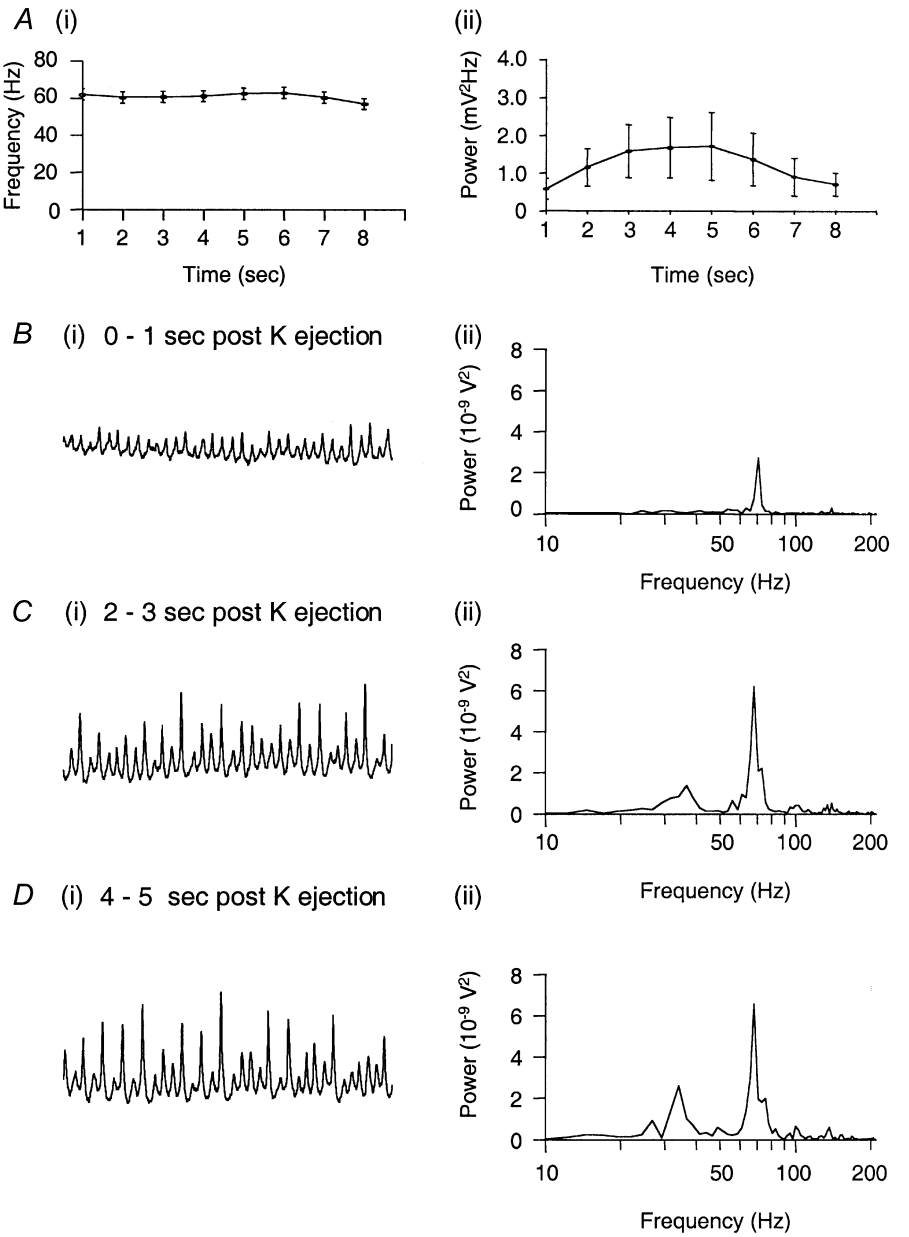

Figure 2. Changes in frequency and power during the oscillation

The frequency (*A*i) and power (*A*ii) of the oscillatory activity were determined over 1 s epochs from the onset of the oscillation. Frequency was unchanged during the course of the oscillation despite a large increase and subsequent decrease in the power of the activity. *B–D*, beta frequency oscillatory activity emerges with increased oscillatory power. Extracellular field recordings from the same slice show oscillatory activity following pressure ejection of potassium into the CA1 subfield. *B*i, at the onset of the oscillation (0–1 s post-potassium ejection) only a small gamma frequency component is evident in the corresponding power spectrum (*B*ii). *C*i, as the power of the activity increased (2–3 s post-potassium ejection) a beta frequency component can be seen in the power spectrum (*C*ii) and this becomes more prominent later in the oscillation (4–5 s post-potassium ejection; *D*i-ii).

present in the activity evoked following ejection of potassium, two additional components could also be seen in the spectra. One component was at half the frequency of the gamma frequency activity and thus appeared to correspond approximately to the beta frequency range (~15–30 Hz) although the so called 'beta' frequency activity in this study ranged from ~20 to 37 Hz. The second component was in the ultrafast (> 80 Hz) frequency range. Activity within the 'beta' frequency range was present in 33 of 57 recordings. In some cases the emergence of a 'beta' component appeared to depend on the degree of depolarising drive. Figure 2*B–D* shows an example of one experiment included in the analysis for frequency and power changes (Fig. 2*A*) during the course of the oscillation. When the power of the oscillation is low immediately after potassium ejection, i.e. 0–1 s post-potassium ejection, only a single gamma frequency peak is seen in the power spectrum (Fig. 2*B*ii). However, as the power of the activity increases 2–3 s post-potassium ejection (Fig. 2*C*ii) a second 'beta' frequency peak becomes evident, which is even larger 4–5 s post-oscillation onset (Fig. 2*D*ii). In this case the power in the 'beta' range increased by 288 % at 4–5 s post-potassium ejection compared with 0–1 s post-potassium ejection. An ultrafast component was present in 35 of 57 slices (e.g. Figs 4, 5 and 7) and could occur either in conjunction with a mixed gamma/beta frequency response or with just a gamma frequency response. Unlike the beta frequency component, however, its appearance showed no obvious correlation with the power of the gamma frequency oscillation. Although 'beta' and ultrafast frequency peaks could clearly be detected in some spectra, for the experiments described below we have restricted our analysis to investigate changes in the gamma frequency component.

Changes in extracellular potassium concentration during gamma frequency activity

In order to quantify the changes in extracellular potassium $[K^+]_o$ following pressure ejection of potassium methyl-sulphate we made a series of measurements using potassium ion-sensitive electrodes (see Methods). With the ion-sensitive electrode positioned as close as possible (< 50 μM) to the field recording electrode at a depth of ~100 μM in s. pyramidale in CA1 and CA3 ($n = 3$), the changes in $[K^+]_o$ were measured (Fig. 3). The changes in $[K^+]_o$ detected during an episode of gamma frequency activity ranged from 0.5 to 2.0 mM (Fig. 3*B*).

Synchronization of gamma frequency oscillations across the CA1 area

Using multiple extracellular field electrode recordings we mapped the spatial distribution of the gamma frequency oscillation evoked following potassium ejection. With multiple field electrodes placed in s. radiatum of CA1, oscillations, evoked by potassium application midway along the horizontal axis of CA1, could be recorded in four electrodes spanning over ~600 μm (Fig. 4*A*, E1–E4). The activity was highly synchronised (Fig. 4*C*) across CA1 with a mean phase lag between the most separated electrodes of only 0.6 ± 0.4 ms ($n = 2$).

Gamma frequency activity depends on fast excitatory and inhibitory neurotransmission

In order to elucidate the mechanisms that generate this transient gamma frequency oscillatory response we investigated the contribution of chemical synaptic transmission and gap junction-mediated signalling. To determine which neurotransmitter systems were involved in the generation of the potassium-evoked gamma frequency activity, various receptor antagonists were bath

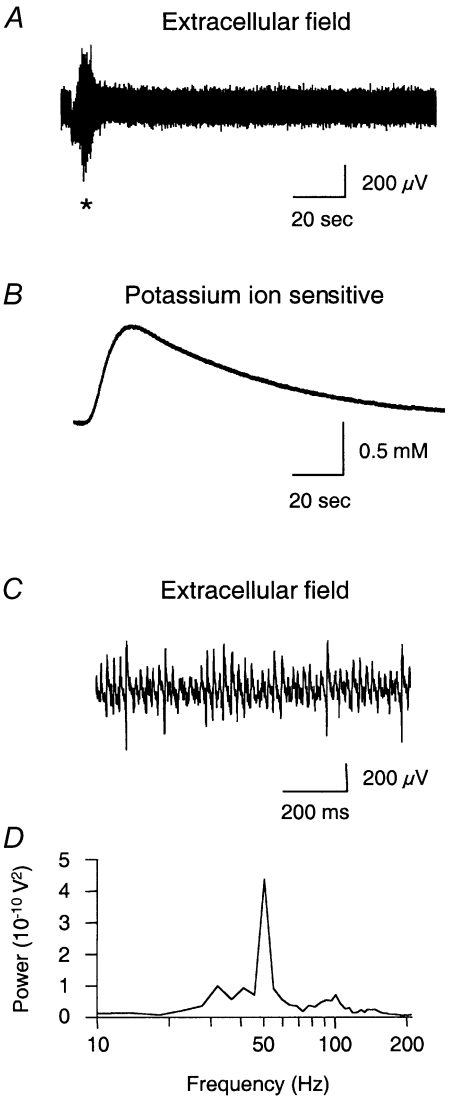

Figure 3. Potassium ion sensitive recordings show increases in extracellular potassium

A, extracellular field recordings from stratum pyramidale in CA3 show oscillatory activity (*) occurring on recovery from the negative DC shift. *B* shows the simultaneous extracellular potassium ion sensitive recording during this response. *C* shows an expanded section of the field recording trace in *A* during the peak of the oscillation (*) and *D* shows the corresponding power spectra.

applied. In all cases oscillatory activity was evoked in both the CA1 and CA3 regions and the results were initially assessed separately. However, no significant differences in the effects of the receptor antagonists on either the frequency or power of the activity were found between the two hippocampal subfields. The data presented below, therefore, represent the pooled results for CA1 and CA3. In Fig. 5 the effects of sequentially blocking fast glutamatergic, and then GABA-ergic inhibition, are illustrated for one experiment recorded in the CA1 region. Bath application of the NMDA receptor antagonist D-AP5 (50 μM) caused a small decrease (28 %) in the power of the activity in this example compared to the control but only a moderate decrease (4 %) in frequency (Fig. 5*B*i–ii). In contrast the AMPA/kainate receptor antagonist NBQX (20 μM) caused a substantial reduction in the power of the oscillation (Fig. 5*C*i–ii). Interestingly, a residual network oscillation was still evident in the presence of D-AP5 and NBQX that is likely to represent interneuronal network gamma activity (Whittington *et al.* 1995). This interneuronal network gamma activity was abolished after application of the $GABA_A$ receptor antagonist bicuculline (20 μM) to the superfusion medium (Fig. 5*D*i–ii). The potassium-evoked oscillation recovered, at least partially, on wash (Fig. 5*E*i–ii). Overall blockade of NMDA receptors caused no significant ($P > 0.05$) change in either the frequency or power of the oscillations (Fig. 5*F* and *G*), which were reduced by 1.6 ± 1.5 % and 10.4 ± 22.8 % respectively ($n = 9$). In contrast blockade of AMPA/kainate receptors with NBQX caused a significant ($P < 0.05$) reduction in the power of the oscillation, which decreased by 86.7 ± 4.5 % ($n = 15$). Bath application of bicuculline (BIC) totally abolished all oscillatory activity

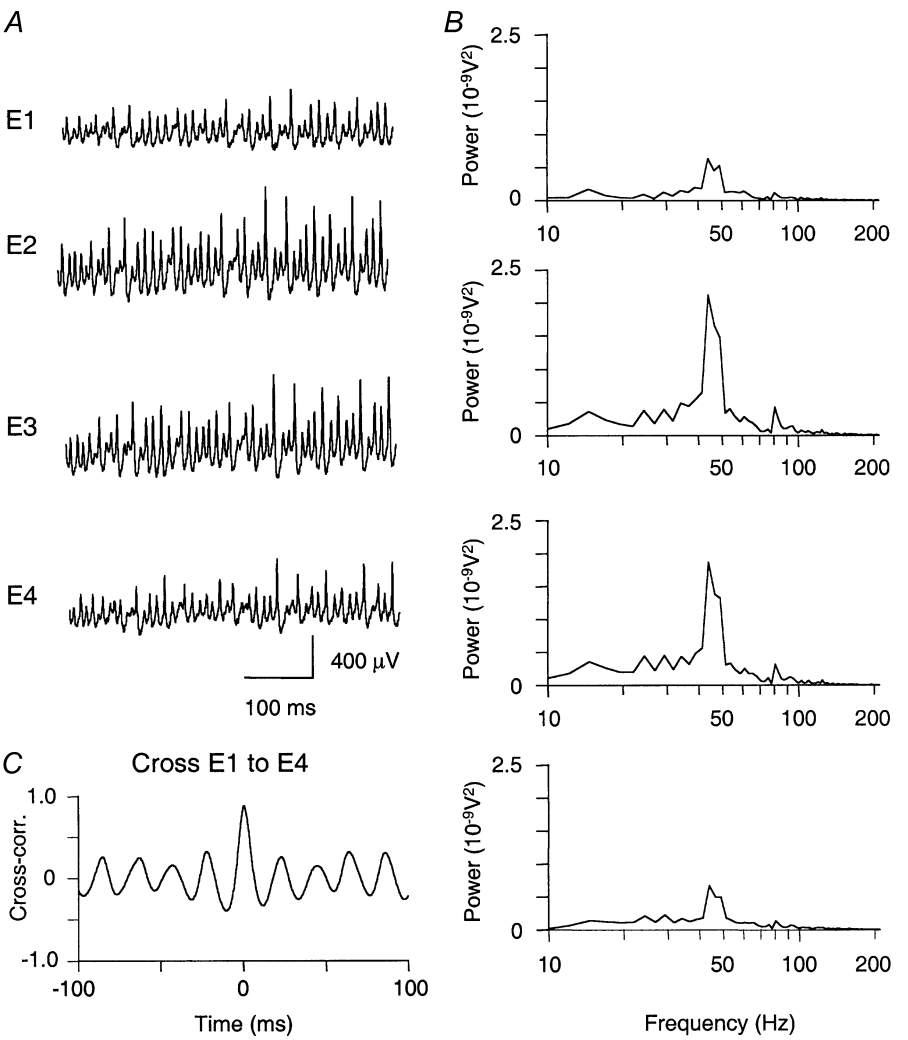

Figure 4. Oscillations are highly synchronised over distance

A, multiple extracellular field recordings (E1 to E4) from stratum radiatum along the horizontal extent of CA1 with each electrode ~200 μm apart. Pressure ejection of potassium midway, between E2 and E3, produced an oscillation with a peak frequency (*B*) of 43 Hz that was recorded at each electrode. *C*, the cross-correllogram between the most spatially distant electrodes (E1 and E4) shows this activity was synchronised over ~600 μm with a phase lag of 0.6 ± 0.4 ms ($n = 2$).

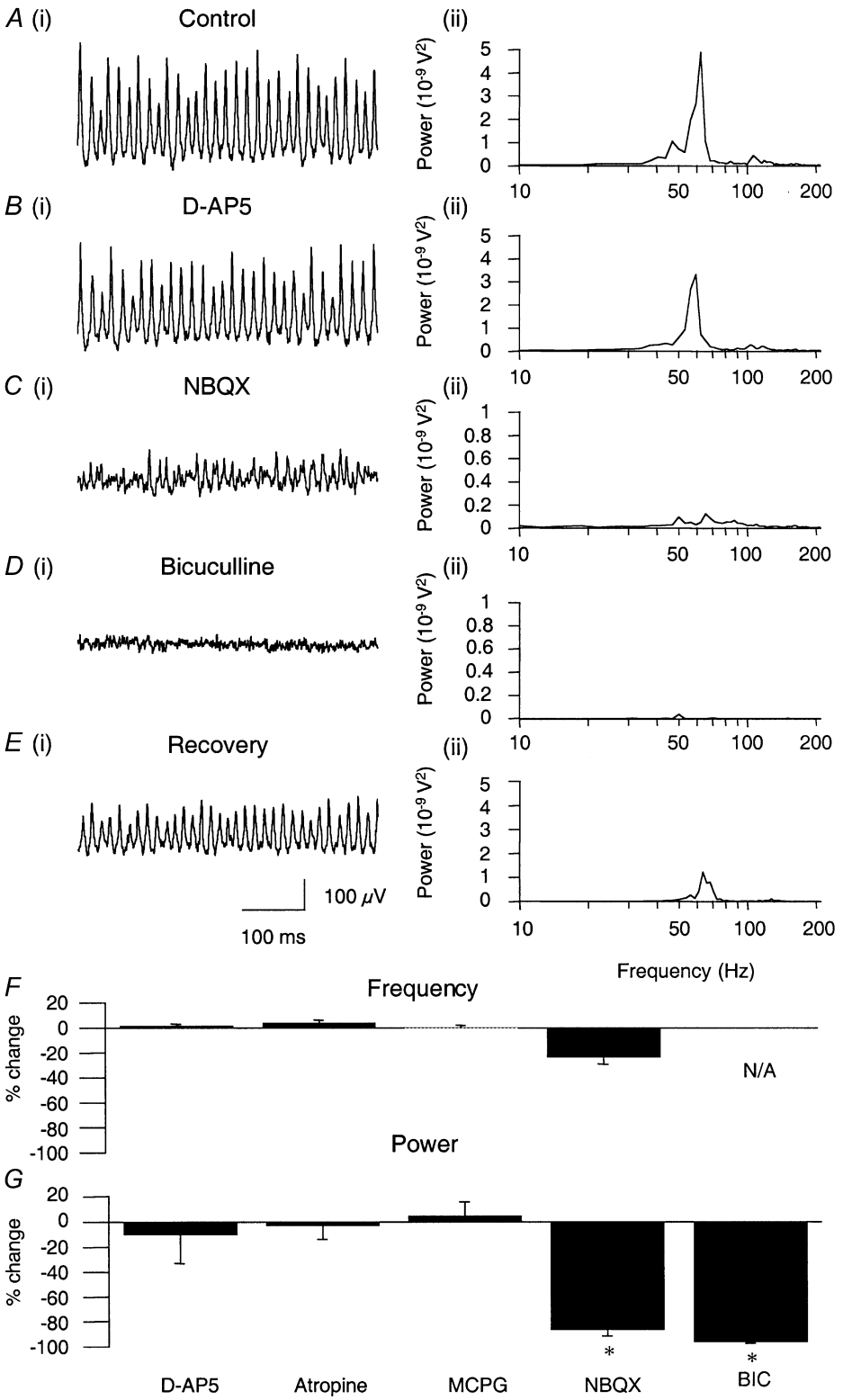

Figure 5. Potassium-evoked oscillations are dependent on both fast glutamatergic and fast $GABA_A$ receptor-dependent neurotransmission

*A–E*i, extracellular field recordings from one slice in the CA1 region with the corresponding power spectra (*A–E*ii). *A*, in normal ACSF pressure ejection of potassium produces a network oscillation at 60 Hz. *B*, bath application of D-AP5 (50 μM) caused a small reduction in the power of the activity. *C*, however, the subsequent addition of NBQX (20 μM) dramatically reduced the power of the response. *D*, the residual activity is completely abolished by addition of bicuculline (20 μM) that was partially reversible on wash (*E*). Group data show the percentage change in frequency (*F*) and power (*G*) of activity for all the receptor antagonists tested. Asterisks indicate statistical significance ($P < 0.05$).

with a reduction in power of 96.3 ± 1.2 % ($n = 7$). In addition TTX (1 μM) blocked all oscillatory activity ($n = 5$; data not shown). Pressure ejection of potassium could result in the release of either glutamate or acetylcholine as a result of the depolarisation of either pyramidal cells, or possibly nerve terminals. Furthermore, both of these neurotransmitters have been shown to induce a gamma frequency oscillation via activation of metabotropic receptors (Whittington *et al.* 1995) or cholinergic muscarinic receptors (Fisahn *et al.* 1998), respectively.

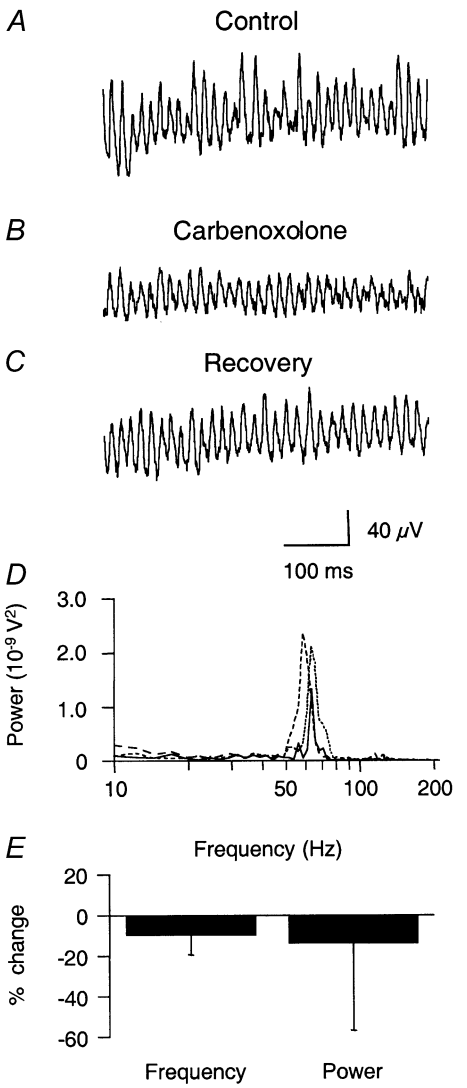

Figure 6. Effects of carbenoxolone on the generation of potassium-evoked gamma frequency oscillations

Extracellular field recordings from the CA1 region show oscillatory activity following pressure ejection of potassium in control (*A*) and following bath application of carbenoxolone (200 μM) for 60 min (*B*). Carbenoxolone caused a reduction in power in this example but did not abolish the oscillation. The effect was reversible on wash (*C*). *D*, the corresponding power spectra are shown (control, dotted line; carbenoxolone, continuous line; recovery, dashed line). *E*, group data ($n = 7$) show considerable variability in carbenoxolone's effect with no significant changes in either frequency or power ($P > 0.05$).

However, neither bath application of the broad spectrum metabotropic glutamate receptor antagonist MCPG (0.5–1 mM; $n = 5$) nor of the muscarinic cholinergic receptor antagonist atropine (10 μM; $n = 9$) had any significant effect on the frequency or power of the oscillatory activity (Fig. 5*F* and *G*). These results demonstrate that there is no significant involvement of either metabotropic glutamate or muscarinic receptors in the generation of the potassium-evoked gamma oscillations.

Effects of gap junction blockers on gamma frequency oscillations

Gap junctions have been implicated in a variety of network activities including several models of epilepsy (Perez-Velazquez *et al.* 1994), gamma frequency oscillations (Traub *et al.* 2000) and ultrafast oscillations (Draguhn *et al.* 1998). We therefore assessed the effects of two different putative gap junction blockers, carbenoxolone and octanol, on potassium-evoked gamma frequency oscillations. Again, the effects of these gap junction blockers on the responses recorded from the CA1 and CA3 regions were analysed separately but have subsequently been grouped together as no differences were observed. The effect of carbenoxolone (100–200 μM) on the potassium-evoked gamma frequency oscillation was very variable. In 3/7 slices tested bath application of carbenoxolone for 60 min caused a reduction in the power of the oscillation that was either fully or at least partially reversible on wash (60–90 min). Figure 6 shows one example from the CA1 region where after 60 min application of 200 μM carbenoxolone (Fig. 6*B*) a gamma frequency oscillation is still present, albeit reduced in power from control (Fig. 6*D*). In 2/7 cases the gamma frequency activity was completely abolished by carbenoxolone but no recovery was obtained after 60–90 min of wash, and in a further 2/7 cases the power of the activity actually increased in carbenoxolone. The group data (Fig. 6*E*) show a 9.9 ± 9.4 % reduction in the frequency and a 14.0 ± 42 % reduction in the power of the oscillation with carbenoxolone. In view of this variability the change in the power of the activity was not significant ($P > 0.05$). The efficacy of the carbenoxolone solution used in this study was tested in a parallel series of experiments (data not shown) in which we were consistently able to block kainate-evoked gamma frequency activity with carbenoxolone. In view of the lack of any consistent effect with carbenoxolone we also used octanol, which has been shown to block carbachol-evoked oscillations (Traub *et al.* 2000). In contrast to the results obtained with carbenoxolone, the effect of octanol (1–2 mM) was highly consistent with at least some reduction in power in all cases ($n = 8$) that was either fully or at least partially reversible on wash (Fig. 7). Overall after 40 min bath application of octanol the power of the oscillation was reduced by 75.6 ± 5.6 % while frequency was decreased by a mean of 15.5 ± 7.9 % compared with the control. Interestingly, in a few cases ($n = 3$) the decrease in frequency in the presence

of octanol was more noticeable and could, as in the example from the CA1 region shown, occur before any substantial reduction in the power of the response was evident (Fig. 7*B*i-ii).

DISCUSSION

We have found that a brief, focal application of potassium, caesium, or rubidium solutions into s. radiatum of either the CA1 or the CA3 regions of the hippocampus *in vitro* evoked a transient episode of oscillatory network activity (Fig. 1). This activity, studied in detail using pressure ejection of potassium methylsulphate, consisted of at least three different frequency components. The main frequency component, present in all cases, was in the gamma range (30–80 Hz) but in many cases a beta (15–30 Hz) (Fig. 2) and/or ultrafast (> 80 Hz) frequency component (Figs 4, 5 and 7) was also present.

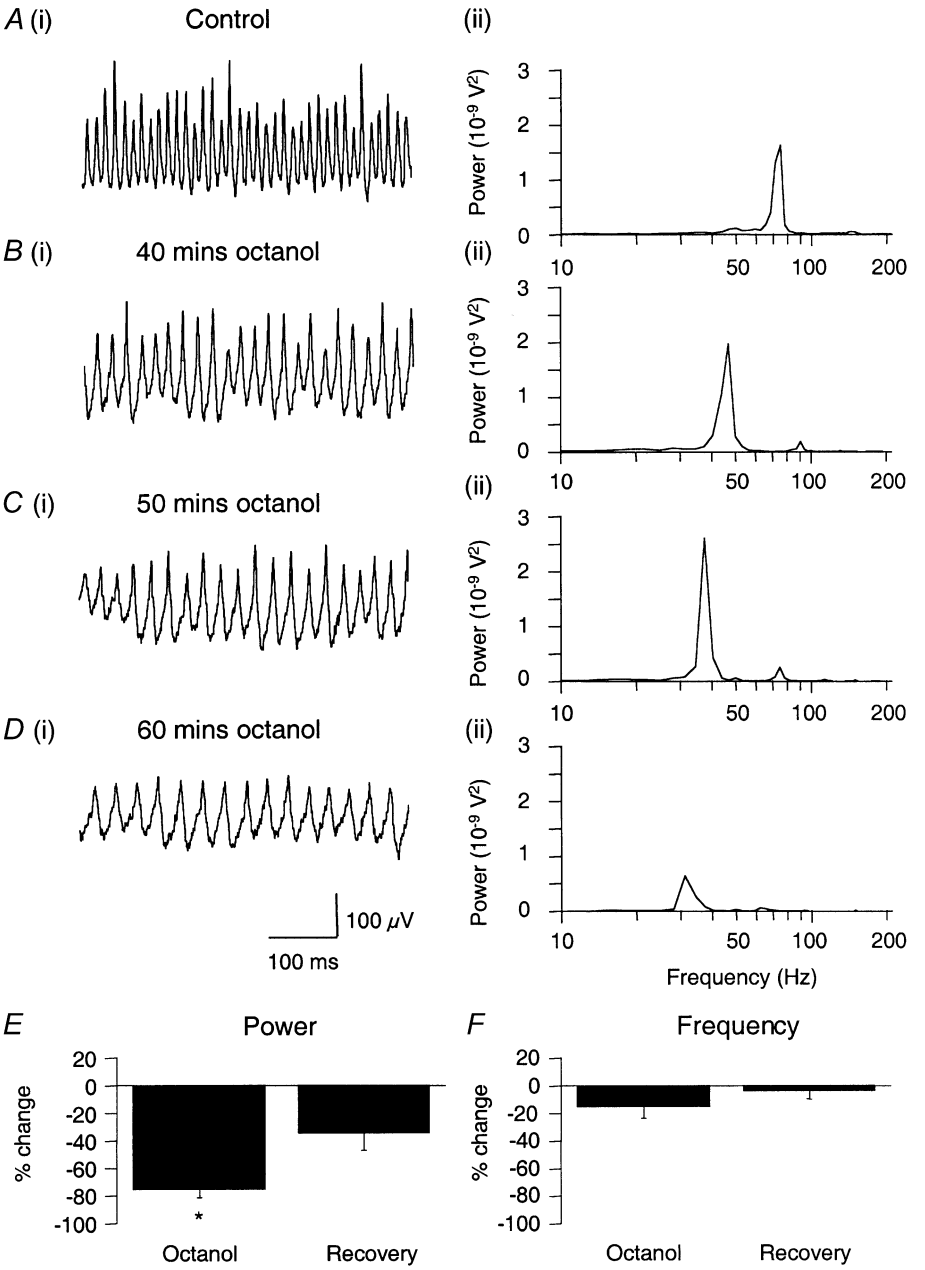

Figure 7. Effects of octanol on the generation of potassium-evoked gamma frequency oscillations

*A*i, extracellular recording from CA1 shows a gamma frequency oscillation following ejection of potassium. With increasing time into bath application of octanol (*B–D*) the frequency of the oscillation slows substantially and the power of the oscillation is also reduced. *E*, group data ($n = 7$) show that octanol caused a significant ($P < 0.05$) reduction in power in all cases that was partially reversible. *F*, octanol also slowed the frequency of activity in a reversible manner in some cases but overall this change was not significant ($P > 0.05$).

It is well documented that increasing $[K^+]_o$ in the bathing medium may cause epileptiform bursting in hippocampal slices (e.g. Rutecki *et al.* 1985; Korn *et al.* 1987; Traynelis & Dingledine, 1988). Focal application of potassium has also been used previously to evoke spreading depression (Herreras *et al.* 1994). However, the significance of modest, focal increases in potassium on network oscillatory activity has not previously been assessed. Kaila and colleagues (Kaila *et al.* 1997; Smirnov *et al.* 1999) demonstrated that high frequency stimulation induced a biphasic response in CA1 pyramidal cells consisting of a $GABA_A$ receptor-mediated phase followed by an activity-induced $[K^+]_o$ transient which depolarised the pyramidal cells. These authors suggested that this endogenous activity-dependent $[K^+]_o$-evoked depolarisation could play a role in the generation of gamma frequency activity. We have now shown that a transient elevation of $[K^+]_o$ is indeed sufficient to evoke a brief episode of fast oscillatory activity in both the CA1 and CA3 regions. However, a tonic increase of $[K^+]_o$ to similar levels is not effective in eliciting oscillatory activity (authors' unpublished observations). One possible explanation for this difference might be due to the fact that increases in $[K^+]_o$ have been shown to cause a positive shift in the GABA reversal potential which subsequently decreases somatic IPSPs recorded from pyramidal cells (McCarren & Alger, 1985; Korn *et al.* 1987; Thompson & Gahwiler 1989). Importantly, in these experiments the IPSPs occurring during the oscillation remain hyperpolarising, at least in the pyramidal cells (Fig. 1). The depolarising drive which initiates the oscillation, therefore, appears to derive directly from the modest increase in $[K^+]_o$ rather than from a tonic depolarising GABAergic response, which has been suggested to play a major role in other experimental models of gamma frequency network oscillations (Bracci *et al.* 1999, 2001; Whittington *et al.* 2001). Interestingly, Herreras *et al.* (1994) found that focal injection of potassium into the CA1 region *in vivo* elicited what they termed 'sawtooth wavelets' and population spikes prior to the onset of spreading depression. The oscillatory activity they observed occurred transiently prior to the onset of spreading depression, with a mean frequency of 68 Hz, and therefore presumably represents a gamma frequency network oscillation similar to that reported here *in vitro*. Importantly, the fast network oscillations evoked with potassium in this study were not accompanied by any epileptiform burst discharges or spreading depression. Using ion-sensitive electrodes we have demonstrated that the changes in $[K^+]_o$ occurring in stratum pyramidale following ejection of potassium are modest with increases of only 0.5–2 mM above control levels being seen (Fig. 3). We have recently shown that > 50 % of this increase in $[K^+]_o$ is activity dependent, with the remainder of the increase resulting directly from the ejection of potassium (Towers *et al.* 2002). Larger transient increases in potassium almost certainly occur immediately adjacent to the potassium ejection electrode and we cannot, therefore, exclude the possibility that a greater more localised increase in $[K^+]_o$ might contribute to the initiation of the gamma frequency activity. Small (1–3 mM) increases in $[K^+]_o$ have been reported in the hippocampus following single stimulation (Krnjevic *et al.* 1982). In contrast, increases in $[K^+]_o$ up to 6–9 mM have been reported during intense synaptic activity (Benninger *et al.* 1980; Krnjevic *et al.* 1982) and global increases up to 6.5–8.5 mM are used to evoke epileptiform discharges (Rutecki *et al.* 1985; Korn *et al.* 1987; Traynelis & Dingledine, 1988). During spreading depression extracellular potassium can exceed 30 mM (Somjen & Giacchino, 1985).

The ability to trigger an episode of gamma frequency activity was not, however, specific for potassium as both caesium and rubidium were equally effective. This suggests that the mechanism(s) responsible for eliciting this activity are not unique to potassium and, therefore, are most likely to be a consequence of providing a simultaneous depolarising drive to a large population of cells. Rubidium might be expected to have similar effects to potassium as it is permeable through potassium channels (Hille, 1992). The effects of caesium are probably complex, as low doses (1–3 mM) of caesium block the mixed cation current I_h (Maccaferri *et al.* 1993), which would hyperpolarise cells, but also produces epileptiform activity suggesting an increase in excitability (Janigro *et al.* 1997). Caesium also produces a depolarising effect through blockade of potassium channels (Hille, 1992). Our results therefore suggest that the oscillation emerged as a consequence of the properties and connectivity of the activated network. The frequency of the gamma component appears to be relatively constant within any given slice and independent of the amount of potassium ejected over the ranges tested here (Fig. 2*A*). However, beta frequency activity was occasionally evoked when there was an increase in depolarising drive (Fig. 2*B*). Interestingly, Herreras *et al.* (1994) also reported that the frequency of the sawtooth wavelet activity they observed *in vivo* in CA1 following potassium injection was of a constant frequency 'within any one experiment'.

Contribution of chemical synaptic interactions to potassium-evoked network oscillation

The network activity evoked by potassium ejection, while sharing some characteristics with previously described models of gamma frequency activity *in vitro*, also differed in several important respects. Gamma frequency activity evoked *in vitro* either by tetanic stimulation (Whittington *et al.* 1997) or bath application of carbachol (Fisahn *et al.* 1998) has been shown to depend on metabotropic glutamate receptors and cholinergic muscarinic receptors, respectively. However, neither receptor appeared to play a role in the generation of the gamma frequency activity evoked by potassium. Neither the broad spectrum metabotropic receptor antagonist MCPG (0.5–1.0 mM)

nor the muscarinic receptor antagonist atropine (10 μM) had any effect on the power or frequency of the network oscillation (Fig. 5*F* and *G*). In addition, NMDA receptors are not essential because there was no significant change in either power or frequency of the oscillation. Interestingly, in contrast to gamma frequency activity evoked by carbachol (Fisahn *et al.* 1998), the potassium-evoked oscillation was not fully blocked by addition of D-AP5 and NBQX (Fig. 5*C*i). The same duration of potassium ejection in the presence of these blockers of fast glutamatergic transmission was still able to elicit an oscillatory response, albeit of reduced power to that occurring in normal ACSF (Fig. 5*C*ii). This activity represents interneuronal network gamma and is thought to arise from networks of tonically excited and mutually coupled interneurons (Whittington *et al.* 1995; Traub *et al.* 2001). The dependence of the remaining oscillation on the interneuronal network was confirmed by the fact that this residual oscillatory activity was completely abolished by the $GABA_A$ receptor antagonist bicuculline (Fig. 5*D*i). These results demonstrate that, as with other *in vitro* models of gamma frequency activity, the potassium-evoked oscillation is critically dependent on both pyramidal cell and interneuronal activity (Whittington *et al.* 1995; Fisahn *et al.* 1998). However, unlike the carbachol model of gamma frequency oscillation (Fisahn *et al.* 1998), in the absence of fast glutamatergic input the network is still able to generate a rhythmic oscillation. Potassium ejection, therefore, provides a novel way in which to study coherent activity generated within the interneuronal network.

Interestingly, the complete blockade of the gamma frequency activity seen in this study with bicuculline is at variance with a recent study in the dentate gyrus (Towers *et al.* 2002) where application of bicuculline did not always block the oscillatory activity evoked by potassium ejection. One explanation for this difference is that in the dentate gyrus there is a substantial contribution from non-synaptic, i.e. electrical and/or ephaptic, interactions to the oscillatory activity. In the CA1 region we have previously reported (Traub *et al.* 2001) that pressure ejection of potassium in stratum pyramidale in the presence of blockers of both fast glutamatergic and GABAergic transmission can evoke an ultrafast oscillation (> 100 Hz) similar to that reported previously by Draguhn *et al.* (1998). However, gamma frequency oscillations in the CA1 or CA3 regions are always blocked by bicuculline.

Contribution of electrical signalling to potassium-evoked network oscillation

There is increasing evidence that gap junctions play an important role in synchronised network activity. Gap junction blockers have been shown to abolish or reduce several forms of epileptiform activity in the hippocampal slice (Perez-Velazquez *et al.* 1994; Ross *et al.* 2000; Traub *et al.* 2001) and spreading depression (Largo *et al.* 1997). In addition, ultrafast hippocampal oscillations are blocked by halothane both *in vivo* (Ylinen *et al.* 1995) and *in vitro* (Draguhn *et al.* 1998). Gamma frequency oscillations evoked by carbachol are also reversibly blocked by octanol (Traub *et al.* 2000) or carbenoxolone (E. H. Buhl & A. Fisahn, unpublished observations). However, the effect of gap junction blockers on potassium-evoked fast network oscillations was variable. Carbenoxolone caused a reversible decrease in the oscillations in 3/7 cases (Fig. 6) but overall the effects were inconsistent, although octanol did consistently abolish or reduce the activity (Fig. 7). Other studies have also reported slow onset, poor recovery and variability in the effects of carbenoxolone (Ross *et al.* 2000). In contrast, octanol was always able to block the potassium-evoked activity, and in some experiments also caused a marked slowing of the frequency of the activity (Fig. 6). However, octanol also has a number of non-specific membrane effects and alters the kinetics of the $GABA_A$ response (Tatebayashi *et al.* 1998) and a slowing of the $GABA_A$ decay constant would be expected to result in a slowing of the network frequency (Whittington *et al.* 1996). Gap junctions comprise different proteins, the so-called connexins (Dermietzel & Spray, 1993), and little is known about the possible specificity of different gap junction blockers for connexin subtype, and which subtypes are important for generating which types of activity. These factors may well prove to be critical for our understanding of the role of gap junctions in generating the potassium-evoked rhythmic activity but our results would suggest that, at least in some cases, electrical signalling via gap junctions contributed to the generation of this network oscillation.

The fact that a transient elevation of potassium alone is able to evoke a network oscillation, whose frequency components may vary as a function of the depolarising drive, has important implications for hippocampal function. Using this approach to evoke gamma frequency activity it will be possible to assess the effects of local changes in $[K^+]_o$ on hippocampal network activity. In addition, the persistence of an interneuronal network gamma oscillation following blockade of fast glutamatergic transmission provides a novel way to assess the interneuronal network activity.

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