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Fast Network Oscillations in the Rat Dentate Gyrus In Vitro

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[1]*School of Biomedical Sciences, University of Leeds, Leeds LS2 9NQ;* [2]*Medical Research Council Anatomical Neuropharmacology Unit, University of Oxford, Oxford OX1 3TH, United Kingdom; and* [3]*Department of Physiology and Pharmacology, SUNY Health Science Center, Brooklyn, New York 11203*

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Towers, Stephen K., Fiona E. N. LeBeau, Tengis Gloveli, Roger D. Traub, Miles A. Whittington, and Eberhard H. Buhl. Fast network oscillations in the rat dentate gyrus in vitro. *J Neurophysiol* 87: 1165–1168, 2002; 10.1152/jn.00495.2001. The dentate gyrus is a prominent source of gamma frequency activity in the hippocampal formation in vivo. Here we show that transient epochs of gamma frequency network activity (67 ± 12 Hz) can be generated in the dentate gyrus of rat hippocampal slices, following brief pressure ejections of a high-molarity potassium solution onto the molecular layer. Oscillatory activity remains synchronized over distances >300 μm and is accompanied by a modest rise in $[K^+]_o$. Gamma frequency oscillations were abolished by a $GABA_A$ receptor antagonist demonstrating their dependence on rhythmic inhibition. However, in many cases, higher frequency oscillations (>80 Hz) remained in the absence of synaptic transmission, thus demonstrating that nonsynaptic factors may underlie fast oscillatory activity.

INTRODUCTION

Gamma oscillations (30–80 Hz) in the rodent hippocampus are often nested in the theta band of the electroencephalograph (EEG; 4–12 Hz), being frequently associated with exploratory behavior (Bragin et al. 1995). Moreover, in the awake rat it appears that the dentate gyrus is the dominant source of gamma frequency activity in the hippocampal formation, with oscillations exhibiting a higher power and frequency than in all other regions. However, following bilateral entorhinal lesions the magnitude and frequency of dentate gamma oscillations is dramatically reduced, suggesting a physiological role of entorhinal rhythms in entraining dentate gamma band activity (Bragin et al. 1995). It is therefore uncertain whether the isolated dentate network is capable of generating and sustaining gamma oscillations and, if so, what the underlying mechanisms are. Consequently, we have developed a novel experimental model to elicit short epochs of oscillatory activity and proceed to investigate some of the salient mechanisms that govern gamma oscillations in the dentate gyrus. Preliminary data have been published in abstract form (Towers et al. 1999).

METHODS

Adult (~150 g) Wistar rats were anesthetized with inhaled isoflurane prior to intramuscular injection of ketamine (≥100 mg/kg) and xylazine (≥10 mg/kg). Following the cessation of all pain reflexes, they were perfused intracardially with chilled sucrose-containing artificial cerebrospinal fluid (sACSF) composed of (in mM) 3 KCl, 1.25 NaH_2PO_4, 2 $MgSO_4$, 2 $CaCl_2$, 24 $NaHCO_3$, 10 glucose, and 252 sucrose. Following brain removal, 450-μm-thick hippocampal slices were cut and maintained at 34°C in a recording chamber at the interface between humidified carbogen gas (95% O_2-5% CO_2) and normal ACSF in which sucrose was replaced by equiosmolar (126 mM) NaCl. In calcium-free ACSF, $CaCl_2$ was omitted, and $MgSO_4$ was raised to 4 mM.

Picospritzer apparatus was used for pressure ejection of high-molarity (1.5 M) KCH_3SO_4 through glass microelectrodes (tip diameter <2 μm) onto the outer third of stratum moleculare (30–70 psi; duration 5–100 ms). Extracellular potassium concentration $[K^+]_o$ was measured using ion-sensitive microelectrodes containing potassium ionophore cocktail B (Sigma) as a liquid membrane and back-filled with 10 mM KCl. Recording procedures, data acquisition, and analysis closely followed previously described procedures (Fisahn et al. 1998). Results are expressed as means ± SD, and statistical significance was determined using the Mann-Whitney *U* test.

RESULTS

Gamma frequency oscillatory network activity (67 ± 12 Hz, means ± SD; $n = 91$) could be reliably and repeatedly induced following pressure ejection of high-molarity potassium solution (1.5 M KCH_3SO_4) onto the outer third of stratum moleculare. Extracellular field recordings in stratum granulosum revealed the occurrence of transient periods of oscillations (Fig. 1*A1*), with both amplitude (≤8 mV maximum) and duration (≤10 s) of rhythmic activity depending on ejection duration. Concomitant sharp-microelectrode intracellular recordings of granule cells revealed their participation in the emergent oscillation. During gamma activity the cells were depolarized from a membrane potential of −61 ± 11 mV by 13 ± 9 mV to −48 ± 9 mV, with the majority of cells (17 of 22) displaying rhythmic hyperpolarizing membrane potential fluctuations (decay time constant of 9.6 ± 2.6 ms) that were temporally correlated with the antiphasic extracellular field oscillation (Fig. 1*A3*). Suprathreshold depolarizations triggered action potential firing, being invariably in phase but at frequencies lower than the population oscillation (Fig. 1*A1*). Oscillatory activity was accompanied by a transient decrease (58 ± 8%) of input resistance.

To determine the concentration of extracellular potassium required to initiate gamma activity and to assess the degree of

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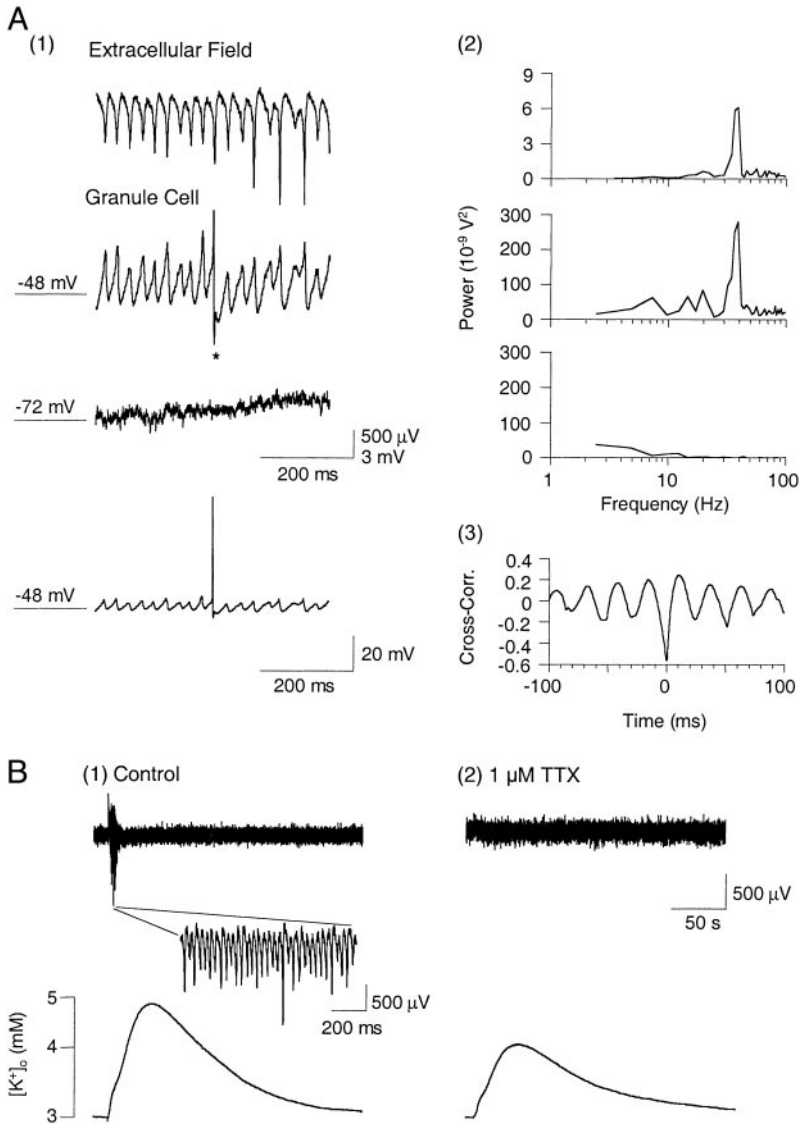

FIG. 1. Pressure ejections of high-molarity potassium solution elicit transient periods of gamma frequency oscillations. *A1*: example of extracellular field recording and concomitantly recorded granule cell following induction of oscillatory activity. At a depolarized membrane potential, rhythmic inhibitory postsynaptic potentials (IPSPs) are apparent, which disappear near the presumed IPSP reversal (−72 mV). Intracellular recording shows both rhythmic IPSPs and action potentials. * Truncated action potential. *A2*: corresponding power spectra of oscillatory activity from extracellular and intracellular recordings reveal a distinct peak at ~55 Hz. *A3*: cross-correlations of intracellular and extracellular traces show that granule cell postsynaptic potentials are phase-related to the extracellular field. *B*: recordings of extracellular potassium concentration changes accompanying oscillatory activity. *B1*: pressure ejection under control conditions caused $[K^+]_o$ to rise to 4.1 ± 1.5 mM. *B2*: application of tetrodotoxin (TTX) to block activity-dependent increases of $[K^+]_o$ resulted in a reduction to 63 ± 22% of the control response.

activity-dependent changes, an ion-sensitive electrode was positioned at a depth of approximately 100 μm in close proximity to the field electrode. Oscillations of representative amplitude and duration lead to a modest increase in $[K^+]_o$ of 1.4 ± 1.5 mM ($n = 9$; Fig. 1*B1*), comprising both exogenously applied K^+ and activity-dependent increases. To distinguish the relative contribution of both components, successive measurements were made, both after the induction of rhythmic network activity and following the bath application of 1 μM tetrodotoxin (TTX), which invariably abolished the oscillation. After normalizing $[K^+]_o$ levels in TTX, these data suggest that action potential–dependent network activity leads, on average, to a $[K^+]_o$ rise of 21 ± 34% ($n = 5$).

An assessment of the spatial extent of oscillatory activity along the transverse axis of the dentate gyrus was made using four extracellular field electrodes that were placed >100 μm apart into the granule cell layer (Fig. 2*A*). Cross-correlations of population activity at different locations showed that network activity was tightly synchronized, with phase lags being <1 ms across distances up to ~300 μm (Fig. 2*B; n* = 4).

Subsequently, glutamate and GABA receptor pharmacology was employed to determine the receptor and/or synaptic mechanisms underlying the generation of rhythmic network activity. Oscillations remained in the presence of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate receptor antagonist 6-nitro-7-sulphamoylbenzo(f)-quinoxaline-2,3-dione (NBQX) (20 μM) and the *N*-methyl-D-aspartate (NMDA) receptor antagonist D-2-amino-5-phosphonopentanoic acid (D-AP5) (50 μM; $n = 7$). The $GABA_A$ receptor antagonist bicuculline (10–20 μM; either added individually or following superfusion with NBQX and D-AP5) totally abolished oscillatory activity in 4 of 13 experiments (Fig. 3*A*). However, in the majority of experiments in which bicuculline was applied or in which there was conjoint application of antagonists of fast excitatory amino acid (NBQX, 20 μM; D-AP5, 50 μM) and GABAergic transmission (bicuculline, CGP55845, 1–5 μM; Fig. 3*B*), oscillatory network activity was diminished in peak amplitude but remained, albeit with a higher frequency (17 of 19 experiments; $n = 19$ slices; 86 ± 17 Hz vs. 67 ± 12 Hz in control; $P < 0.0001$). Likewise, oscillations of a significantly higher frequency (97 ± 35 Hz; $P < 0.0001$; $n = 16$) could also be evoked in calcium-free ACSF (Fig. 3*C*).

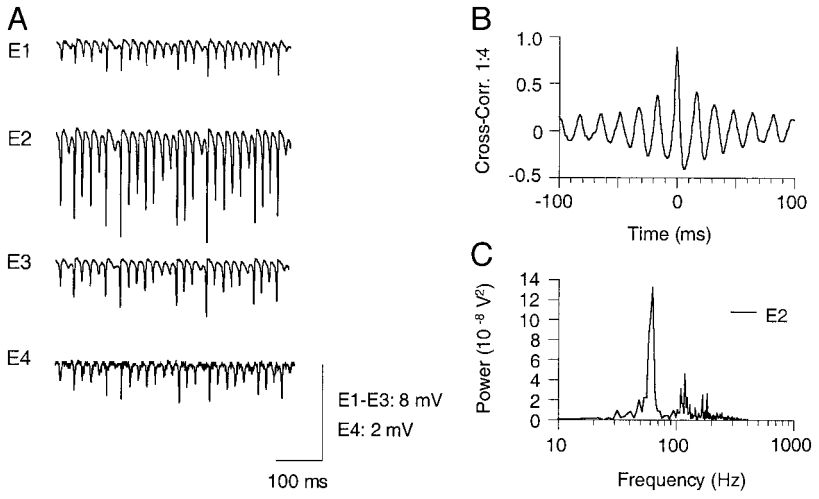

FIG. 2. Oscillatory network activity was tightly synchronized over distances >300 μm. *A*: population activity recorded at 4 sites (*E1–E4*) spaced >100 μm from each other in str. granulosum. *B*: cross-correlogram of activity recorded at sites 1:4 indicates a phase-lag of <1 ms. *C*: 1 corresponding power spectrum is shown.

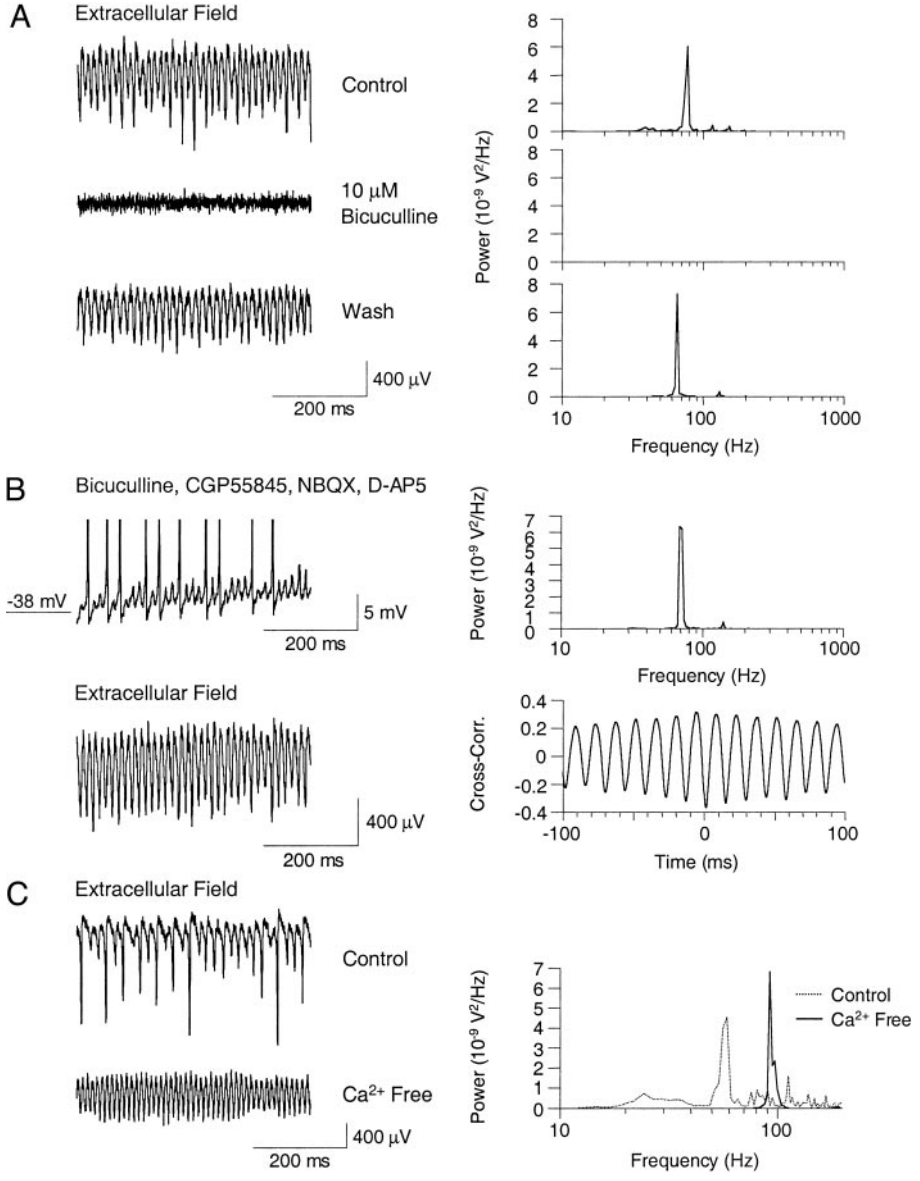

FIG. 3. Both synaptic and nonsynaptic factors underlie fast oscillatory activity. *A*: in 4 of 13 experiments, oscillations were abolished by bicuculline. *B*: in most instances extra- and intracellular (action potentials truncated) oscillatory activity remained, following the conjoint application of ionotropic glutamate and $GABA_A$ and $GABA_B$ receptors antagonists, with corresponding power spectra revealing distinct spectral peaks. Cross-correlograms indicate an anti-phasic relationship between extra- and intracellular activity. *C*: oscillatory activity remains after superfusion of calcium-free solution. However, corresponding power spectra reveal a significant increase in peak frequency of oscillations.

DISCUSSION

Here we provide physiological evidence showing that brief pressure application of a high-molarity potassium solution can elicit transient gamma-frequency oscillations in the dentate gyrus in vitro. In the absence of any phasic input, it is therefore reasonable to assume that the generation of rhythmic activity is an emergent property of the neuronal network. We suggest that the focal depolarization of granule cell dendrites in the outer molecular layer mimics the excitatory input provided by the entorhinal afferents, which appear to be largely responsible for inducing, but not necessarily entraining dentate oscillatory activity in vivo (Bragin et al. 1995). While transient gamma oscillations are phenomenologically similar to so-called "afterdischarge termination oscillations" (ATOs) that may follow a period of intense perforant path stimulation, the latter are accompanied by slowly propagating large-amplitude DC shifts and a dramatic decrease in interneuronal activity (Bragin et al. 1997). Likewise, potassium-induced spreading depression (SD) in vitro is also accompanied by a cessation of neuronal activity, a dramatic rise in extracellular potassium, and slowly propagating DC shifts (Herreras et al. 1994). Interestingly, however, the onset of spreading depression may be *preceded* by a transient burst of gamma-like activity (Herreras et al. 1994). In contrast to SD and ATOs, we are also able to evoke oscillatory activity without appreciable extracellular DC shifts (e.g., Fig. 1*B*). Moreover, dentate interneurons fire vigorously (Towers, unpublished data), as evidenced by hyperpolarizing phase-locked inhibitory postsynaptic potentials in granule cells and the pharmacological antagonism of $GABA_A$ receptors, which either resulted in a decrease or complete loss of oscillatory power. Finally, the block of $GABA_A$ receptors also lead to a frequency increase of the residual oscillation beyond a range (>80 Hz), where inhibitory mechanisms are believed to play a pivotal role in phasing oscillatory network activity (Whittington et al. 1995, 1997). It therefore appears that dentate gamma oscillations in vitro resemble experimental models of inhibition-based gamma rhythms that depend on tonically excited networks of mutually interconnected interneurons (Whittington et al. 1995, 1997).

Dentate fast rhythms also differ from pharmacological models of persistent gamma oscillatory activity that require the presence of AMPA/kainate receptors (Fisahn et al. 1998), whereas high $[K^+]_o$-induced gamma activity remained in the absence of fast glutamatergic excitation. It therefore appears that the synaptic mechanisms that sculpt dentate gamma activity resemble those of inhibition-based gamma rhythms that depend on tonically excited networks of mutually interconnected interneurons (Whittington et al. 1995, 1997).

Despite the important role of inhibitory mechanisms in governing oscillatory network activity in the gamma frequency range, the dentate neuronal network can generate high-frequency oscillatory activity that appears to be *entirely* dependent on nonsynaptic factors, not unlike dentate ATOs (Bragin et al. 1997). Regarding the underlying mechanisms, the low extracellular volume fraction of the dentate gyrus and common orientation of granule cells do indeed favor a contribution of electrical field effects (Snow and Dudek 1986). Likewise, experimental conditions may also facilitate synchronizing field effects during tetanically evoked oscillatory activity in the CA1 area (Bracci et al. 1999; Whittington et al. 2001). However, the overt absence of a significant degree of a net transmembrane depolarization, sporadic firing of granule cells, and a relatively modest drop in neuronal input resistance are more likely to favor a prominent contribution of other synchronizing factors, such as gap junction–mediated electrical neuronal coupling (Draguhn et al. 1998; Kosaka 1983; Venance et al. 2000). In summary, we therefore suggest that oscillatory activity in the dentate gyrus is due to a complex interplay of synaptic and nonsynaptic network mechanisms.

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