Fast Network Oscillations in the Rat Dentate Gyrus In Vitro

STEF 2. TOWERS, F. 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⁺]. 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.

M E T H O D S

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 NaH₂PO₄, 2 MgSO₄, 2 CaCl₂, 24 NaHCO₃, 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% O₂, 5% CO₂) and normal ACSF in which sucrose was replaced by equimolar (126 mM) NaCl. In calcium-free ACSF, CaCl₂ was omitted, and MgSO₄ was raised to 4 mM.

Picospritzer apparatus was used for pressure ejection of high-molarity (1.5 M) KCl, SO₄ through glass microelectrodes (tip diameter <2 µm) onto the outer third of stratum molecular (30–70 s; duration 5–100 ms). Extracellular potassium concentration [K⁺] 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.

R E S U L T S

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 KCl, SO₄) onto the outer third of stratum molecular. Extracellular field recordings in stratum granulosum revealed the occurrence of transient periods of oscillations (Fig. 1A1), 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 hyperpolarizing membrane potential fluctuations (decay time constant of 9.6 ± 2.6 ms) that were temporally correlated with the antiphase extracellular field oscillation (Fig. 1A3). Suprathreshold depolarizations triggered action potential firing, being invariably in phase but at frequencies lower than the population oscillation (Fig. 1A1). 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 oscillatory activity, three concentrations of potassium were used: 0.5, 1.5, and 2.5 M KCl, SO₄. The 0.5 M solution was used as a control (Fisahn et al. 1998). The concentration of extracellular potassium was 3 M KCl, SO₄ (1.5 M KCl, SO₄ in normal ACSF) or 1.5 (0.5 M KCl, SO₄ in normal ACSF) or 0.5 M (0.5 M KCl, SO₄ in normal ACSF) KCl, SO₄. The results were analyzed using analysis of variance (ANOVA) followed by post hoc analysis with Tukey’s honest-significant-difference test.

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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. 1B1), 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 mM 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. 2A). 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. 2B; $n=4$).

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$\text{A}$ 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. 3A). 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 GABAAergic transmission (bicuculline, CGP55845, 1–5 μM; Fig. 3B), 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. 3C).
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. 1994). In contrast to SD and ATOs, we are also able to evoke oscillatory activity, a dramatic rise in extracellular potassium, and slowly (SD) in vitro is also accompanied by a cessation of neuronal activity (Bragin et al. 1995). Likewise, potassium-induced spreading depression and a dramatic decrease in interneuronal activity (Bragin et al. 1995). It therefore appears that oscillatory activity in the dentate gyrus is due to a complex interplay of synaptic and nonsynaptic network mechanisms.

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REFERENCES