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Anaesthetic/amnesic agents disrupt beta frequency oscillations associated with potentiation of excitatory synaptic potentials in the rat hippocampal slice

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1 Anaesthetic agents produce disruption in cognitive function typified by reductions in sensory perception and memory formation. Oscillations within the EEG gamma and beta bands have been linked to sensory perception and memory and have been shown to be modified by anaesthetic agents.

2 Synchronous gamma oscillations generated by brief tetanic stimulation in two regions of hippocampal area CA1 in slices *in vitro* were seen to potentiate excitatory synaptic communication between the areas. This synaptic potentiation, was seen to contribute to a transition from gamma frequency (30–70 Hz) to beta frequency (12–30 Hz) oscillations.

3 Four drugs having anaesthetic/hypnotic and *amnesic* properties were tested on this synchronous gamma-induced beta oscillation. Thiopental 10–200 μM, Diazepam 0.05–1.0 μM, Morphine 10–200 μM, and Ketamine 10–200 μM were all added to the bathing medium. Each drug markedly disrupted the formation of beta oscillations in a manner consistent with their primary modes of action. Thiopental and morphine disrupted synchrony of gamma oscillations and prevented potentiation of recurrent excitatory potentials measured in *stratum oriens* (fEPSPs). Neither diazepam, nor ketamine produced such marked changes in synchrony at gamma frequencies or reduction in potentiation of fEPSPs. However, each disrupted expression of subsequent beta oscillation *via* changes in the magnitude of inhibitory network gamma oscillations and the duration and magnitude of tetanus-induced depolarization respectively.

4 The degree of disruption of fEPSP potentiation correlated quantitatively with the degree of disruption in synchrony between sites during gamma oscillations. The data indicate that synchronous gamma-induced beta oscillations represent a mode of expression of excitatory synaptic potentiation in the hippocampus, and that anaesthetic/amnesic agents can disrupt this process markedly.

Keywords: Anaesthetic; gamma oscillation; synchrony; beta oscillation; synaptic potentiation; memory

Abbreviations: EPSC, excitatory postsynaptic potential; fEPSP, field excitatory postsynaptic potential; NBQX, 6-nitro-7-sulphonamoylbenzo[f]quinoxaline-2,3-dione; GABA, gamma aminobutyric acid; GABA, decay constant for GABA receptor-mediated inhibitory synaptic potential; AHP, afterhyperpolarization; ACPD, (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid; ING, interneuron network gamma; PING, pyramidal-interneuron network gamma

Introduction

Oscillations in neuronal activity within the EEG gamma band (30–70 Hz) are a common feature of hippocampal activity. Experimentally they are generated by tonic depolarization mediated by cholinergic (Fisahn *et al*., 1998) or glutamatergic (Whittington *et al*., 1995) excitation and can be induced by stimuli such as a brief tetanic stimulus *in vitro* (Traub *et al*., 1996a) or sharp waves *in vivo* (Traub *et al*., 1996b). Their function has been proposed to be the provision of a temporal framework by which the hippocampus can compare and contrast afferent inputs from the array of input areas known *in vivo* (Chrobak & Buzsáki, 1998; Buzsáki, 1997). Gamma oscillations in general are associated with aspects of cognitive function such as exploratory behaviour (Bragin *et al*., 1995), and visual and auditory feature detection (Roelfsema *et al*., 1994; Joliot *et al*., 1994). In humans gamma activity is associated with selective attention (Tiitinen *et al*., 1993), Gestalt formation (Yordanova *et al*., 1997) and memory (De Pascalis & Ray, 1998).

At the small network level gamma oscillations in the hippocampus are generated in large part by populations of mutually interconnected inhibitory interneurons (Whittington *et al*., 1995). Tonic excitatory drive induces action potential generation in these populations and the emergent gamma rhythm is a consequence of the kinetics of mutual $GABA_A$ receptor-mediated synaptic inhibition (Traub *et al*., 1996b). Such oscillations appear to serve a number of functions including the provision of a framework for establishment of synchronous activity between two or more synaptically interconnected oscillating regions (Traub *et al*., 1996a). This synchrony provides coherence between pre- and postsynaptic events sufficient to cause potentiation of recurrent excitatory synaptic potentials (Whittington *et al*., 1997b).

This potentiation of synaptic excitation when two areas oscillate synchronously constitutes the subject of the present study. We have previously shown that synchronous gamma-induced potentiation of recurrent excitation contributes to the

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generation of a beta frequency oscillation in hippocampal slices (Whittington *et al*., 1997b; Traub *et al*., 1999). In this situation the procession of frequencies observed at the gross network level following a stimulus takes the form of a 100–300 ms period of gamma oscillation which transforms into a beta frequency over a period of 50–200 ms. The beta oscillation then continues for up to 2 s. Such gamma/beta switches are seen abruptly in sensory evoked potential studies in response to visual (Pantev, 1995) and auditory stimuli (Baldeweg, Gruzelier & Haenschel, personal communication). The resulting beta oscillation has been shown to be correlated with the long range synchronous activity of neocortical regions during visuomotor reflex generation (Roelfsema *et al*., 1997) and multimodal sensory processing (von Stein *et al*., 1999).

The present study uses the two-site model of generation of gamma and beta oscillations to study the effects of four anaesthetic/hypnotic drugs with a range of amnesic properties on both the ability of long-range synchronous gamma oscillations to potentiate excitatory synaptic transmission and the resulting generation of beta oscillations in the hippocampus *in vitro*. This model has been shown to be useful in the study of mechanisms of synchronization afforded by oscillogenesis (Traub *et al*., 1996a) a proposed mechanism underlying the binding phenomenon in cognitive function (Singer, 1999). Pilot data on the effects of morphine on beta oscillations has been published in abstract form (Faulkner *et al*., 1998a).

Note on terminology of beta oscillations

The term 'beta' refers to a frequency range of *c*. 12–30 Hz, but one must be careful in considering to what signal or signals the frequency range applies. Post-tetanic population oscillations are most often observed by recording population spikes (which reflect firing of multiple pyramidal cells), or by recording single pyramidal neurons. The mechanism underlying the oscillation, however, depends also on the firing patterns of the interneurons; without attention to the activities of both pyramidal cells and interneurons, drug actions are impossible to understand. We shall consider here two forms of beta oscillation. First, we have *Beta-e*/*Beta-i*, in which both excitatory pyramidal cells and inhibitory interneurons fire at beta frequencies, and approximately in phase; the mechanism appears to be a similar though slower version of gamma oscillations in which both pyramidal cells and interneurons participate (see Faulkner *et al*., 1998b). Second, we have *Beta-e*/*Gamma-i* (the subject of this paper), in which pyramidal cells fire at beta frequencies, but interneurons (in the small number of examples so far recorded) fire (in singlets, doublets or bursts) at gamma frequency. This is the sort of beta which often follows strong tetanization, particularly of two sites, and which is correlated with–and dependent upon–enhanced EPSPs in pyramidal cells (Whittington *et al*., 1997b; Traub *et al*., 1999). *Beta-e*/*Beta-i* is operationally distinguished from *Beta-e*/*Gamma-i* as follows: gamma-frequency voltage fluctuations are absent between population spikes and action potentials in *Beta-e*/*Beta-i*, but are present in *Beta-e*/*Gamma-i*.

Methods

Experimental methods

Transverse dorsal hippocampal slices (450 μm thick) were prepared from brains of male Sprague-Dawley rats, 250–274 g, after decapitation following cervical dislocation, and maintained at the interface of warm, wet 95% O_2/5% CO_2 and artificial cerebrospinal fluid (aCSF) containing (in mM): NaCl 135, $NaHCO_3$ 16, KCl 3, $CaCl_2$ 2, NaH_2PO_4 1.25, $MgCl_2$ 1, D-Glucose 10, equilibrated with 95% O_2/5% CO_2 pH 7.2 at 33–35°C.

A single stimulation protocol was used comprising brief tetanic stimuli (100 Hz, 200 ms, 12–50 V, 50 μs duration) at *twice* threshold necessary to evoke gamma oscillations, delivered simultaneously to the *stratum radiatum* at two recording sites at either end of the CA1 region (separation 1.5–2.5 mm) every 4 min throughout each experiment. Post tetanic oscillations were studied using two distinct recording protocols. (1) Field potentials (Band width 0.1 Hz–2 kHz) were recorded using glass micropipettes filled with 2 M NaCl (resistance 1–10 MΩ) at the level of *stratum pyramidale* (for population spikes) at each site simultaneously, or (2) from *stratum oriens*/*alveus* (for field excitatory post synaptic potentials) and *stratum pyramidale* within one site. Verification of the excitatory synaptic nature of potentials recorded in *stratum oriens* was carried out using pressure ejection of NBQX (6-nitro-7-sulphamoylbenzo[f]quinoxaline-2,3-dione, 20 μM in aCSF). In addition a number of intracellular recordings were taken from pyramidal cells using microelectrodes containing 4 M potassium acetate (30–90 MΩ) to monitor the amplitude and duration of the post-tetanic depolarization.

Gamma oscillations, synchronous between the two recording sites, were recorded in slices from 32 rats, and effects of the drugs thiopental, diazepam, morphine and ketamine (all from Sigma, U.K.) were quantified on the resulting *Beta-e*/*Gamma-i* oscillation. All drugs were prepared from stock solutions in dimethylsulphoxide (DMSO)/distilled water mixtures as appropriate and kept refrigerated. Final bath DMSO concentration did not exceed 0.1% v v^{-1}. Drugs were added to the perfusion solution at the following concentrations: Thiopental 10–200 μM, Diazepam 0.05–1.0 μM, Morphine 10–200 μM, and Ketamine 10–200 μM. Each slice acted as its own control for each drug tested and concentration-response relationships were performed cumulatively. A 1 h period of drug wash-out was allowed at the end of some experiments. Recovery from any quantified effects of each drug was seen in each case after this time. In addition, a series of five experiments were performed in which the slices were stimulated as above but no drug was added throughout the experiment to ensure that no time-dependent changes in oscillations were interfering with the observed drug effects.

For each drug, recordings were taken from a minimum of five slices, each from a different rat, with a minimum of three replicates per slice/drug concentration. Initial, control recordings were taken every 4 min for 1 h. Each concentration of drug was allowed 16 min to equilibrate in the bath before three recordings were taken (again separated by 4 min). Incidence, frequency, rhythmicity and phase relationships for beta frequency field potential oscillations were measured by performing auto- and cross correlation analyses of the post-tetanic oscillations (200 ms time window starting at the beginning of the *Beta-e*/*Gamma-i* phase of the oscillation). Data are expressed as mean$\pm$s.e.mean. Data obtained was normally distributed and statistical analysis was carried out using two-way parametric analysis of variance (ANOVA) and *t*-tests with degree of freedom adjusted for multiple comparisons (Bonferroni). Instantaneous frequency plots were derived from data analysis using automated detection of population spikes. Spikes were counted if their amplitude was greater than one standard deviation from the baseline noise. Instantaneous frequency was calculated for each period as the value ($t_{n+1}-t_n$)

where 't' is the absolute time during a recording that a population spike of number 'n' was detected.

Computer model methods

The program used for the simulation of oscillations was identical to that used in Traub *et al.* (1999), with these exceptions: in the present program, interneuron dendrites were electrically excitable in order to maintain consistency with the model and results presented in Whittington *et al.* (1998). As a result of this change, three other parameter changes needed to be compensated for the increased intrinsic excitability of the interneurons. First the tonic (metabotropic glutamate receptor-mediated) drive to interneurons was reduced by a factor of two. Second, pyramidal cell slow AHP conductance increased to 1.25× its standard value during beta, rather than 1.0× its standard value. Third, pyramidal/pyramidal excitatory postsynaptic currents (EPSCs) were increased to at most $2.85 \times t \times \exp(-t/2)$ *n*S (t = time in ms), during beta, rather $3.45 \times t \times \exp(-t/2)$ *n*S. These latter two minor parameter changes had the effect of stabilizing the beta phase under control conditions (Figure 2E).

For simulation of drug effects, additional changes were made in EPSC, τGABA and other parameter values: (1) τGABA in control = 10 ms. For thiopental τGABA = 25 ms. (Note: these values apply to outputs of basket cells and axo-axonic cells only, not to dendrite-contacting interneurons). GABA leak conductance in thiopental: 2.0 *n*S in pyramidal cells, 0.4 *n*S to interneurons. In addition, gamma-phase EPSCs are 40% of that used in control case and do not increase with time; (2) All $GABA_A$ conductances were increased by 50% for diazepam. EPSCs and all other parameters were as in control (i.e. 0.75 to $2.85 \times t \times \exp(-t/2)$ *n*S); (3) Morphine: All $GABA_A$ conductances ×0.6, pyramidal/pyramidal EPSCs constant at 40% of gamma-control (i.e. as in thiopental), AHP conductance goes from 0.25× 'normal' during gamma (as in control), to 0.5× 'normal' during *Beta-e/Gamma-i* (vs 1.25× 'normal' for control); (4) Ketamine: the tonic driving conductances, to both pyramidal cells and interneurons, were reduced by 60% compared with control. In addition, 200 ms after the beta-inducing increases in $g_{K(AHP)}$ and pyramidal/pyramidal EPSCs had been completed, the tonic conductances began to decrease to zero, linear in time, over a time interval of 600 ms.

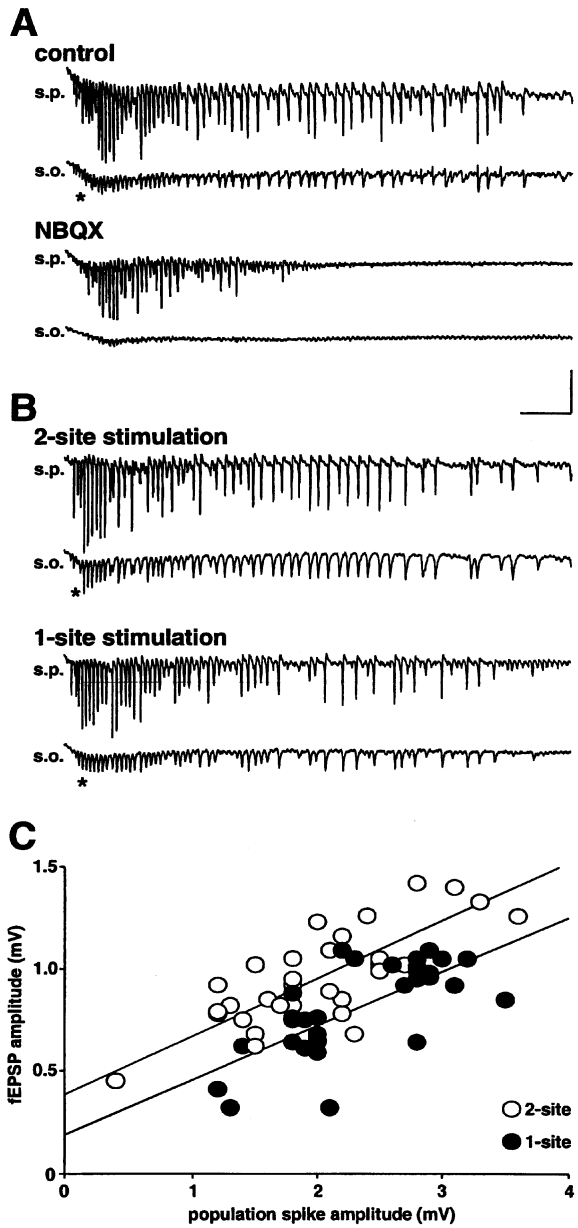

Figure 1 Gamma-induced beta oscillations are associated with recurrent glutamatergic fEPSPs. (A) Simultaneous tetanic stimuli (not shown), at twice threshold for inducing gamma oscillations, delivered to two sites in area CA1 produce a transition from gamma to stable beta oscillations. The initial gamma oscillations were associated with a potentiation of fEPSP amplitude in *stratum oriens* (s.o.) which persisted during the later beta component of the oscillation, seen in *stratum pyramidale* (s.p.) recordings. Expression of these fEPSPs was prevented by pressure ejection of 20 μM NBQX onto s.o. immediately after the tetanic stimulation. Prevention of fEPSP potentiation also prevented the transition to beta oscillation. (B) The majority of synaptic connections responsible for the fEPSPs were from excitatory neurons within the recording region. However, comparison of oscillations induced by 1-site (local) with 2-site (local and distal) tetani revealed a disrupted beta oscillation present concurrently with smaller fEPSPs (scale bars for A, and B, 5 mV, 200 ms). (C) Plot of population spike vs fEPSP amplitude for oscillations. Larger fEPSPs for a given population spike amplitude were associated with oscillations at two sites and a clear transition to beta frequency.

Results

Genesis of gamma-induced beta oscillations

Synchronous gamma oscillations progressed into a prolonged period of beta oscillation (about 10–25 Hz) when a stimulus equivalent to twice threshold for gamma was applied to two sites simultaneously. The beta period was synchronized between sites, rhythmic and stable for 1–2 s (depending on stimulus intensity). This beta oscillation was seen from the first tetanic stimulation in slices generating synchronous gamma and remained stable following repeated stimulations during the course of an experiment (*c.* 5 h). However, in some slices non-synchronous gamma oscillations were seen. In these latter slices beta oscillations were extremely difficult to elicit. The properties of these beta oscillations measured here (phase difference, rhythmicity, frequency and concurrent excitatory field potential recordings) did not alter during the course of each experimental day when no drugs were added to the bathing medium. This post-gamma period of beta activity takes the form of the *Beta-e/Gamma-i* oscillation as described in the introduction (Traub *et al.*, 1999). This is the only manifestation of beta frequency activity presented in this paper and will therefore simply be referred to as 'beta' for the remainder of this results section.

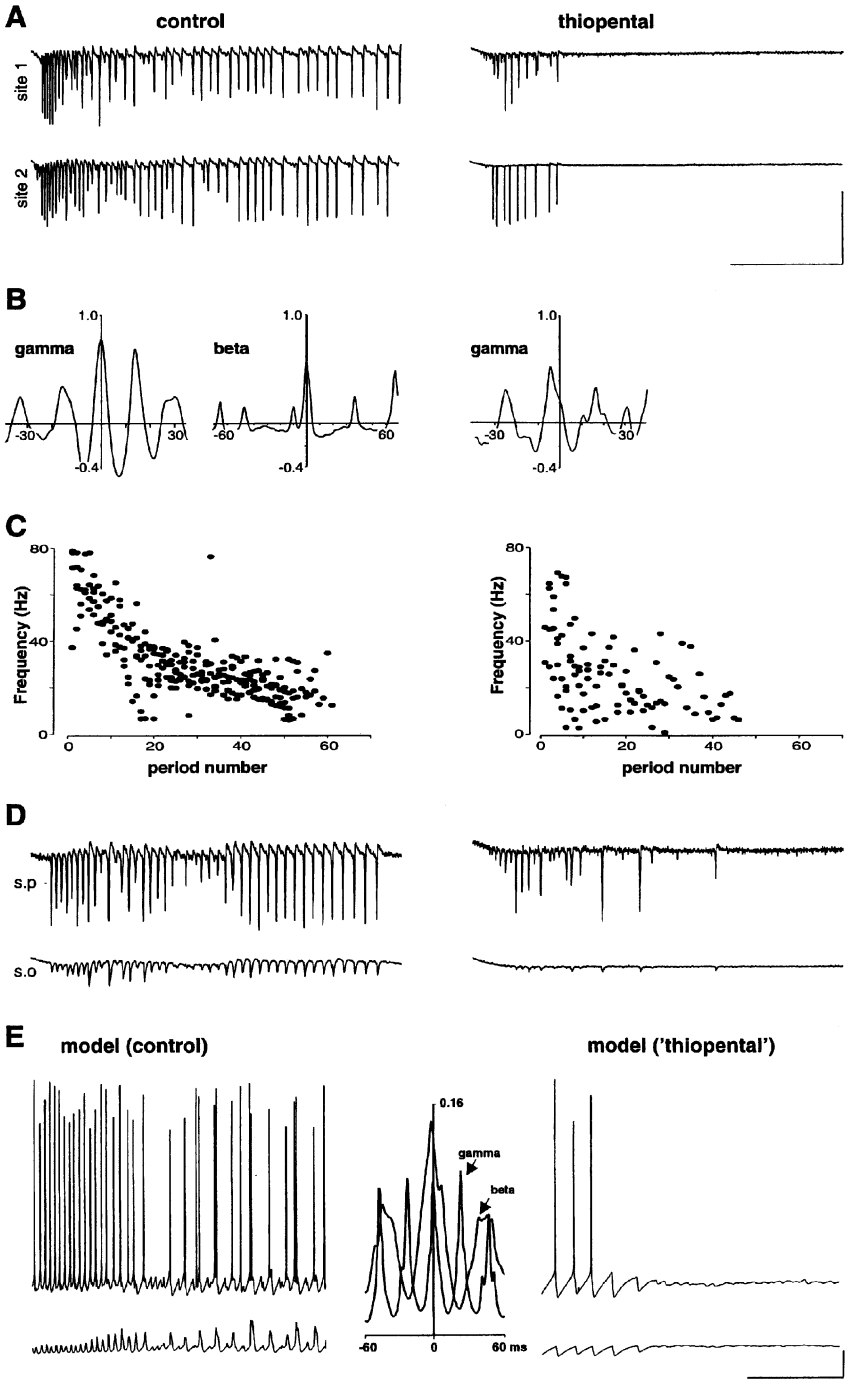

Figure 2 Effects of thiopental on beta oscillations. (A) Simultaneous 2-site tetani produced a post-tetanic field potential oscillation showing gamma followed by beta oscillations at both sites. The traces show the post-tetanic oscillation recorded in *stratum pyramidale* from the beginning (20–150 ms after the end of the tetanus). Population spikes occurred synchronously at both sites during both the gamma and beta sections. Bath application of 50 μM thiopental abolished the beta portion of the oscillation. Scale bars 5 mV, 500 ms. (B) Cross-correlation plots of post-tetanic oscillations for both the first 200 ms of the post-tetanic oscillation (gamma) and the last 200 ms (beta) showing synchrony within *c.*1 ms. Thiopental decreased synchrony within the gamma frequency range. No beta oscillation was seen following this non-synchronous gamma. (C) Instantaneous frequency plots show a clear transition from higher frequency gamma oscillation to a prolonged oscillation at beta frequency under control conditions. Thiopental abolished the procession from gamma to beta frequency oscillations, producing a slower initial oscillation with considerably reduced rhythmicity (graphs show pooled data $n=5$). (D) Concurrent recordings of population spikes from *stratum pyramidale* (s.p.) and fEPSPs from *stratum oriens/alveus* border (s.o.) revealed fEPSPs increasing during the initial synchronous gamma oscillation. Fifty μM thiopental significantly reduced the amplitude of the fEPSPs recorded from s.o. during this time. Scale bars as in (A). (E) Transitions from gamma to beta frequency spike firing in pyramidal cells and interneurons can be modelled using a ramped increase in AHP and recurrent EPSP amplitude (Whittington *et al.*, 1997b; Traub *et al.*, 1999). Data shows concurrently recorded outputs from two pyramidal cells using the adapted model described in the methods. Upper trace shows a single pyramidal cell response. Lower trace shows the response in another pyramidal cell artificially hyperpolarized to reveal EPSPs. Cross correlations demonstrate synchronous, rhythmic oscillations at both gamma and beta frequencies. The slowing of initial gamma activity and absence of beta response seen experimentally with thiopental were successfully modelled by adaptation of control conditions to include a larger $GABA_A$ receptor-mediated synaptic potential decay constant, a tonic GABA 'leak' conductance and absence of EPSP potentiation as seen experimentally. Traces shown are as in control (normal pyramidal cell and hyperpolarized pyramidal cell). Scale bars 10 mV 400 ms for model data.

The genesis of a beta oscillation in this manner has been previously shown to depend upon both the recovery of an AHP during the post tetanic response and the potentiation of excitatory recurrent connections between pyramidal cells in each stimulated group (Whittington *et al*., 1997a; Traub *et al*., 1999). The present data demonstrated that potentiation of these recurrent excitatory synaptic connections could be observed *via* the amplitude of field potentials (fEPSP) located in *stratum oriens*. The initial gamma oscillation was accompanied by an approximately period-by-period increase in fEPSP over 4–10 periods (Figure 1, asterisks). Following this, over a period of 50–200 ms, a transition from gamma field potential oscillations to a stable beta oscillation was observed. Blockade of the major component of fast glutamatergic synaptic excitation using pressure ejection of NBQX (20 μM) prevented both the potentiation of fEPSP amplitude during gamma oscillations and the genesis of a stable beta oscillation (Figure 1A). The origin of these fEPSPs was studied further by comparing single site with two site tetanic stimuli. A twice threshold tetanus at the single recording area generated a long oscillation with an erratic later component in which aperiodic population spikes were interspersed with a small amplitude filed potential oscillation continuing at gamma frequencies (Figure 1B). The same tetani delivered simultaneously to two sites produced the characteristic gamma/beta procession of oscillations (Figure 1B). Comparison of fEPSP amplitude with local population spike amplitude demonstrated that a large proportion of the potentiated fEPSPs appeared to come either directly from the site distal to the region recorded, or indirectly *via* the influence of the distal rhythm on the local site oscillation (Figure 1C). Comparison of fEPSP/population spike amplitude ratio for 2-site compared to 1-site stimulation revealed a mean 30% reduction in the amplitude of fEPSPs for a given population spike size (0.51 ± 0.15 2-site, 0.35 ± 0.07 1-site. $P < 0.001$, unpaired *t*-test).

Effects of thiopental on beta oscillations

Bath application of thiopental caused a concentration dependent attenuation of the late beta portion of the post tetanic oscillation at each site (Figure 2A). Thiopental concentrations of 50 μM and above abolished beta oscillation (Figure 7A). At lower concentrations, cross correlation analysis showed a concentration-dependent increase in the phase difference between sites during the gamma (Figure 1B) and the beta components of the post tetanic oscillation (Figure 7A). In the control condition beta oscillations had a phase difference between the two sites of only 1.4 ± 0.4 ms. This increased to 2.0 ± 0.6 ms for 10 μM, and 2.7 ± 0.7 ms for 20 μM. Higher concentrations of thiopental produced no measurable late component. The reduction in the duration of the overall post-tetanic field response was not attributable to a reduction in the tetanus-induced depolarization underlying the oscillation. In control and thiopental conditions the depolarization was 2.8 s long (Figure 6A). In addition, the amplitude of the underlying depolarization was not significantly altered by 20 μM thiopental during the post-tetanic beta period (control 20 ± 4 mV, thiopental 22 ± 6 mV, $P > 0.05$).

Instantaneous frequency plots for 50 μM thiopental showed a change in the overall pattern and frequency of the oscillation (Figure 2C). In the control condition an initial period of fast oscillation (40–80 Hz) was followed by a transition to a consistent 15–25 Hz (beta) oscillation. Thiopental resulted in a reduction in the overall frequency of the oscillation and a total loss of the steady state late beta frequency. The remaining oscillation showed a more erratic periodicity, which decayed to beta frequency within the first 4–8 periods and a gradual fade over time. In 3/5 slices the initial 2–4 periods of post-tetanic oscillation had a very high frequency (*c*. 70–80 Hz) which did not reduce even at the highest thiopental concentration (Figure 2C), suggesting that no GABAergic involvement in this brief initial component of the post-tetanic response was present.

Recordings from the *stratum oriens-alveus* border show an almost complete elimination of the fEPSPs despite the continued presence of large population spike activity (Figure 2D). In the control slices fEPSPs had a mean amplitude of 1.2 ± 0.2 mV, and were maintained throughout the beta oscillation. Fifty μM thiopental reduced fEPSP mean amplitude by 83% to 0.2 ± 0.03 mV.

These effects of thiopental on gamma-induced beta oscillations were reliably modelled by increasing $GABA_A$ receptor-mediated IPSP decay constant and including a small tonic GABA 'leak' current in the model (Figure 2E). Simulation runs in these conditions produced a brief, slow frequency train of spikes which terminated on recovery of the AHP, despite continued depolarizing drive to the model population of neurons.

Effects of diazepam on beta oscillations

Diazepam produced less marked effects on beta oscillations than thiopental. Neither the beta portion of the oscillation, nor the gamma oscillation, was affected to any great extent (Figure 3A). The period of disrupted oscillatory population spike activity present in control slices between the end of the gamma oscillation and the beginning of the later beta component was, however, considerably prolonged in the presence of diazepam (>250 nM). Transition from gamma to beta oscillations in controls took place over 50–200 ms. This transitional period lasted 0.5–1.2 s at the above diazepam concentrations. Control gamma oscillations transformed into beta oscillations between periods 8 and 20 of the post-tetanic response. This transformation occurred closer to period 30 in the pooled data for a diazepam concentration of 500 nM (Figure 3C). This erratic nature of spiking can be seen in intracellular recordings too (Figure 6B). Once beta oscillations were established the frequency was less than that seen in control conditions (Figure 3B,C). This effect was significant for concentrations of 0.5 μM and above. (Control 24.6 ± 0.9 Hz, and 18.8 ± 0.9 Hz for 0.5 μM, $P < 0.05$). These slower beta oscillations also had a significant phase difference between sites at diazepam concentrations over 100 nM ($P < 0.05$, Figure 7B). These changes were not associated with a significant difference in amplitude of post-tetanic depolarization during the beta control (Control 15 ± 2 mV, diazepam 1 μM 15 ± 4 mV, $P > 0.05$, Figure 6B). As with the thiopental data, a brief train of very fast population spikes (*c*. 70–80 Hz) was seen prior to established gamma activity, immediately post tetanus. This brief, fast train was, again, insensitive to the GABAergic agent diazepam.

Diazepam did not significantly affect the mean amplitude of fEPSPs recorded from *stratum oriens* (control, 1.2 ± 0.1, diazepam 0.5 μM, 1.1 ± 0.1, Figure 3D). However, a significant reduction in the duration of fEPSPs during the beta period of oscillation was observed (control duration 31 ± 1 ms, diazepam 500 nM, 22 ± 1 ms, Figure 3E). Most of the above observed effects of diazepam in experiments were seen in simulations using an increase in the amplitude of the $GABA_A$ synaptic current to model the primary effects of diazepam (Figure 3E).

The effects of diazepam were shared by the benzodiazepine temazepam (data not shown). A reduction in frequency was seen over the concentration range 0.05–1.0 μM along with a longer transition time to beta.

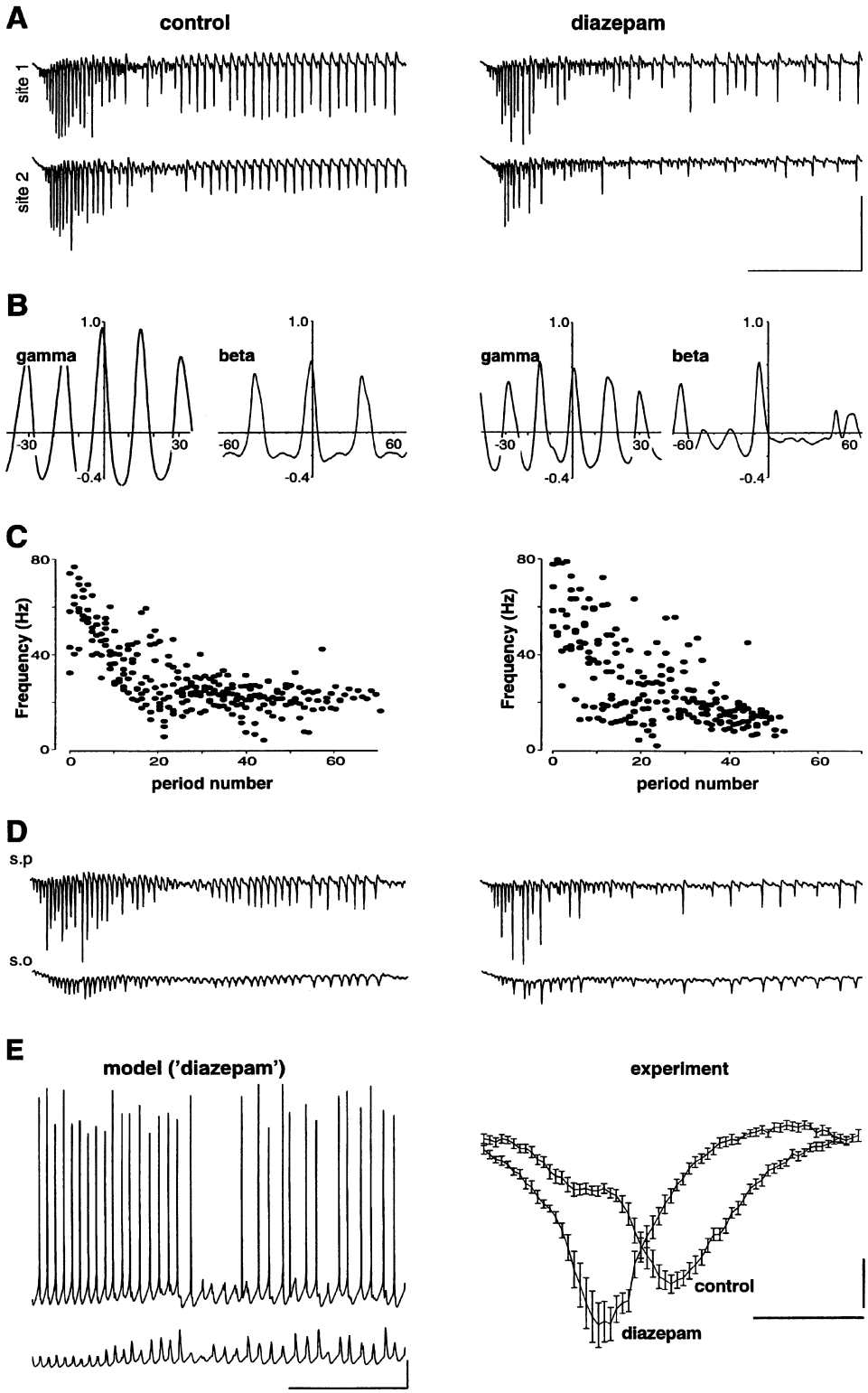

Figure 3 Effects of diazepam on beta oscillations. (A) Post-tetanic field potential oscillations in the gamma and beta frequency bands were evoked at both sites by simultaneous 2-site tetani. Population spikes occurred synchronously at both sites during both the gamma and beta periods. Bath application of diazepam, 0.5 μM, resulted in only a small attenuation of the oscillation, with definable, if slower, gamma and beta oscillation periods. Beta oscillations became more erratic. Scale bars 5 mV, 500 ms. (B) Cross-correlation plots of the post-tetanic oscillation (as in Figure 2) show synchrony within *c*.1 ms for both gamma and beta oscillation periods under control conditions. 0.5 μM diazepam had no effects on the phase difference of the gamma oscillation, but it did reduce the synchrony at beta frequencies. (C) Under control conditions instantaneous frequency plots show a clear switch from high frequency gamma oscillation to a stable beta frequency oscillation. Diazepam did not reduce the total duration of the oscillation, however it did cause a reduction in the frequency of both the gamma and beta portions thus reducing the total period number (abscissa) (graph shows pooled data $n=5$). (D) Recordings of fEPSPs showed gamma followed by beta frequency oscillations concurrent with population spikes. 0.5 μM diazepam did not reduce the amplitude of the fEPSPs. Scale bars as in (A). (E) The effects of diazepam on beta oscillations were modelled by increasing IPSC conductance to 150% of control values. This manipulation also reliably reproduced the increase in amplitude and decrease in duration of EPSP seen experimentally. Traces shown and scale bars as in Figure 2E for model data, and 0.1 mV, 10 ms for field EPSP experimental data.

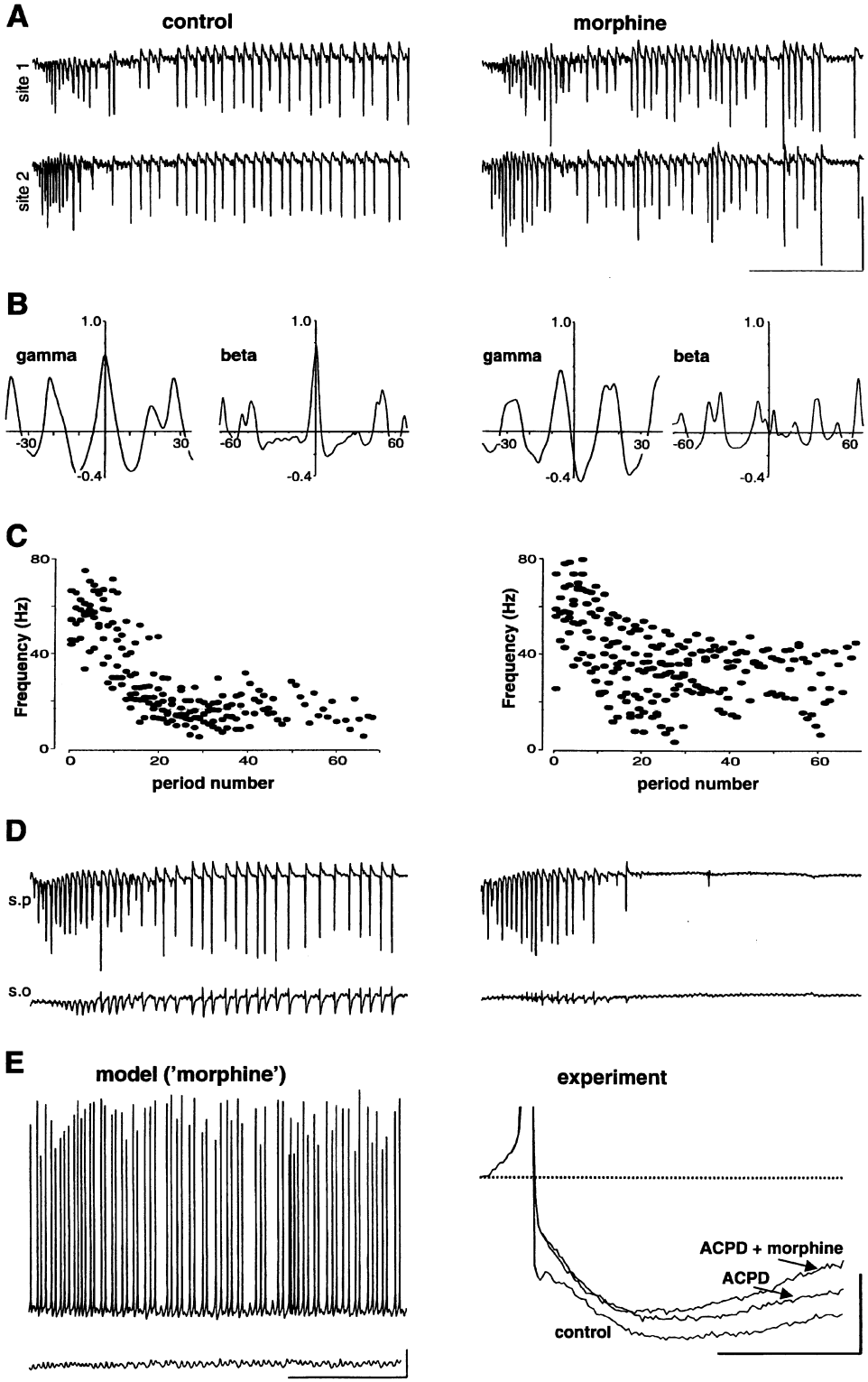

Figure 4 Effects of morphine on beta oscillations. (A) Gamma followed by beta frequency post-tetanic field potential oscillations were generated as in previous figures. Bath application of 50 μM morphine disrupted the transition to beta frequency oscillations, producing periods of beta activity punctuated by a return to gamma oscillations. Scale bars 5 mV, 500 ms. (B) Cross-correlation plots of the control post-tetanic oscillation show synchrony within *c*.1 ms for gamma and beta oscillations. Morphine produced large decreases in synchrony within the initial gamma periods and abolished synchrony during the later 'beta' components of the oscillation. (C) Under control conditions instantaneous frequency plots show a clear switch from gamma oscillation to a stable beta frequency oscillation. Morphine did not reduce the total duration of the oscillation. However it did disrupt rhythmicity in the later part of the oscillation producing a very variable range of period durations (graph shows pooled data $n=5$). (D) Recordings of fEPSPs in the presence of 50 μM morphine showed a marked reduction in their amplitude. Scale bars as in (A). (E) The effects of morphine were reproduced in the model by failure of EPSP potentiation, a reduction in the degree of recovery of the post-spike AHP and a 40% reduction in $GABA_A$ conductances. Synergistic effects of morphine and metabotropic glutamate receptor activation on AHPs were quantified experimentally by recording spontaneous spikes from depolarized pyramidal cells (-62 to -65 mV) in the presence of ACPD (20 μM) or ACPD and morphine (50 μM). Traces shown and scale bars as in Figure 2E for model data, and 2 mV, 20 ms for AHP experimental data.

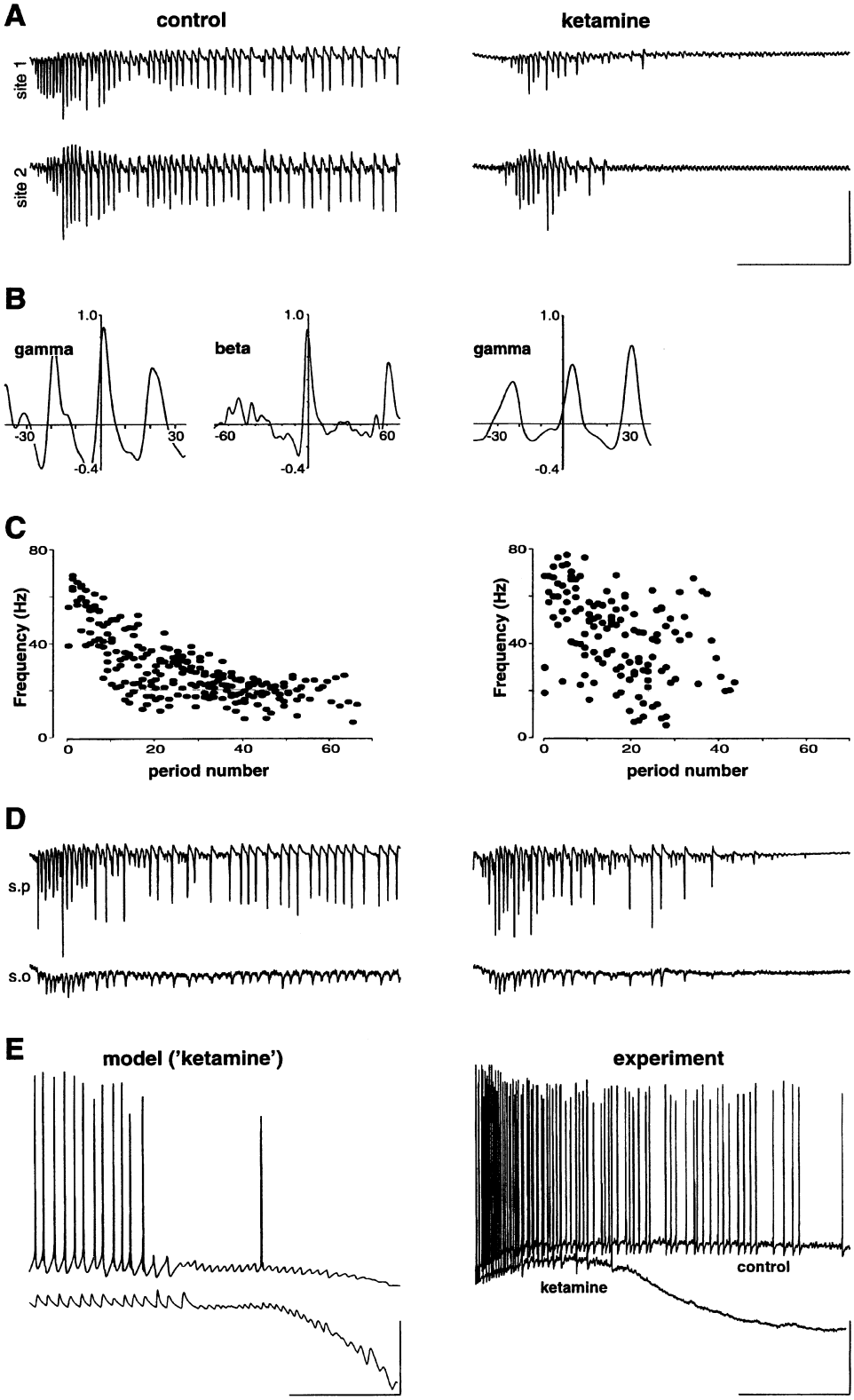

Figure 5 Effects of ketamine on beta oscillations. (A) Gamma/beta oscillations were generated as in previous figures. Bath application of 50 μM ketamine abolished the beta portion of the oscillation. Scale bars 5 mV, 500 ms. (B) Cross-correlation plots of post-tetanic oscillations demonstrated that ketamine produced only a small decrease in synchrony within the gamma frequency range but still completely abolished beta oscillations. (C) Instantaneous frequency plots show a clear progression from high frequency gamma oscillation to a prolonged oscillation at beta frequencies under control conditions. Ketamine greatly reduced the total duration of the oscillation by abolishing the beta frequency oscillation leaving only an erratic gamma oscillation with widespread distribution of frequencies (graph shows pooled data $n=5$). (D) fEPSPs were not affected by bath application of 20 μM ketamine. Scale bars as in (A). (E) Effects of ketamine were modelled by a 60% reduction in pyramidal cell and interneuron driving force beginning 1.4 s into the oscillation, followed by a further time-dependent reduction beginning 1.4 ms into the oscillation. A 'standard' and 'hyperpolarized' pyramidal neuron are shown, as in previous simulation figures. Scale bars for model data 500 ms 30 mV. Experiment data shows two oscillations from the same pyramidal cell before (control) and after addition of 50 μM ketamine. Scale bars 1 s, 25 mV. (E) Effects of ketamine were modelled by a 60% reduction in both pyramidal cell and interneuron driving force.

Effects of morphine on beta oscillations

Morphine, at concentrations >20 μM, produced effects on the later portion of the post-tetanic oscillation which differed from the other drugs tested. A small increase in the frequency of the post-tetanic gamma oscillation was accompanied by an increase in the phase difference between sites as previously reported (Faulkner *et al*., 1998b). Transition to a later beta oscillation was either absent (at higher concentrations) or consisted of brief periods of beta oscillation punctuated by a return to gamma frequencies (Figure 4). At concentrations of 50 μM and above beta activity was either abolished or replaced by low amplitude field potential oscillations which continued at gamma frequency. At these concentrations cross correlation analyses revealed a large phase difference between sites or absence of synchrony at beta frequencies altogether (Figures 4B and 7C). In control conditions the phase difference was 1.8 ± 0.8 ms, this increased to 6.1 ± 1.0 ms for 50 μM ($P < 0.05$). Cross correlations also revealed that multiple split peaks were common, suggestive of a loss of rhythmicity. The persistence of gamma oscillations could also be seen in intracellular recordings from pyramidal cells. The change in

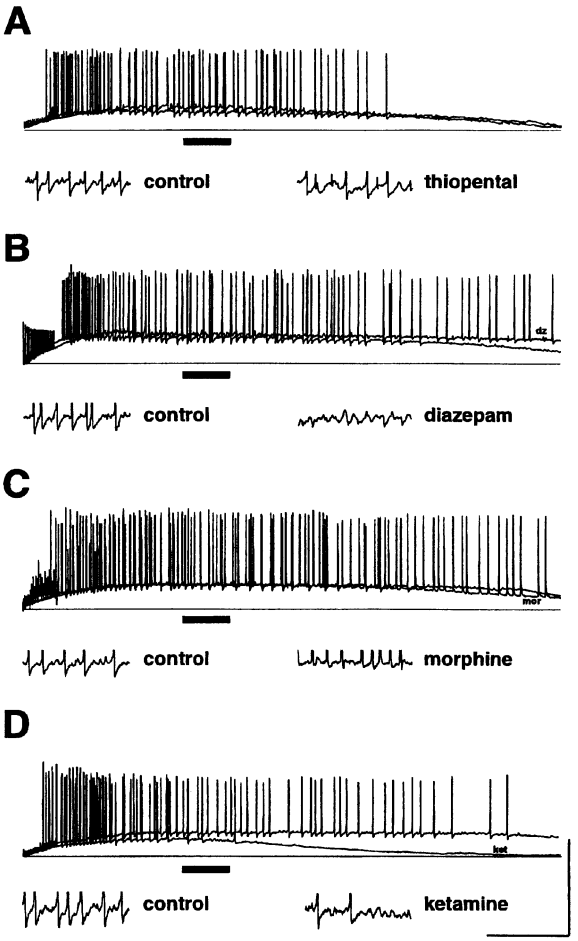

Figure 6 Pattern of change of intracellular pyramidal cell responses. Traces show example of the pyramidal cell response to the tetanus used. Upper traces are superimposed 3 s epochs of the entire response with control and drug-present recordings superimposed to show changes in depolarization. Lower expanded traces are from the above recordings during the period marked with a dark bar (mid beta). (A) Effects of 20 μM thiopental on depolarization and oscillation. Little change in depolarization is seen but, where present the beta oscillation was considerably slowed. (B) Effects of 1 μM diazepam. The mid beta period in control recordings was replaced by a predominantly subthreshold gamma oscillation (*cf*. Figure 3D). (C) Fifty μM morphine attenuated post-spike afterhyperpolarizations and caused a persistence of the initial gamma rhythm. (D) Ketamine attenuated the underlying depolarization. Stable, mid beta control oscillations were replaced by sporadic spiking superimposed on a gamma frequency membrane potential oscillation. Scale bars (upper traces) 80 mV, 500 ms, (lower traces) 20 mV, 200 ms.

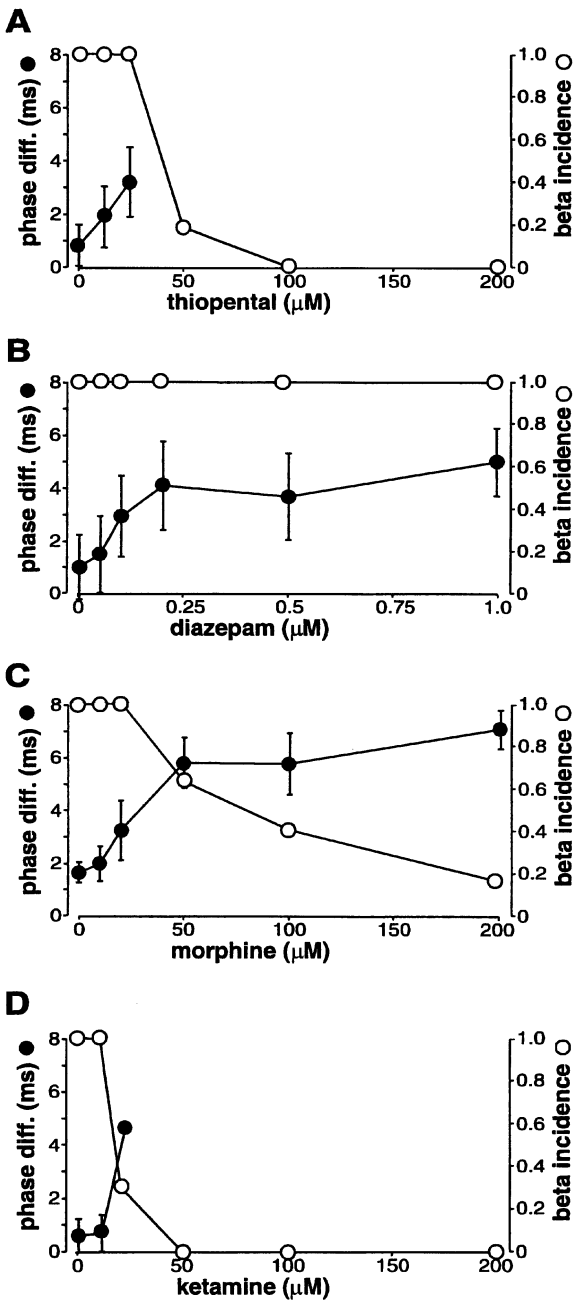

Figure 7 Sedative/anaesthetic agents disrupt beta synchrony and incidence of beta oscillations. Phase difference is expressed as mean $\pm$ s.e.mean ($n = 5$), incidence as the proportion of slices (out of five) showing gamma-induced beta activity at any one concentration of drug. (A) Thiopental concentration-dependently disrupted synchrony at beta frequencies before dramatically attenuating incidence at concentrations greater than 20 μM. (B) In contrast diazepam had no effect on the incidence of beta oscillations but did markedly disrupt synchrony at beta frequencies in a concentration-dependent manner. (C) Morphine also disrupted synchrony at beta frequencies and also affected incidence at concentrations >20 μM. (D) Ketamine produced a similar degree of disruption of beta synchrony and reduced the incidence of beta oscillations at concentrations over 10 μM.

firing patterns seen did not correspond to any significant change in depolarization amplitude (control 20 ± 5 mV, morphine 50 μM 17 ± 5 mV, $P > 0.05$, Figure 7C).

Plots of instantaneous frequency from the pooled data in control conditions and in the presence of 50 μM morphine clearly showed the disruption in transition to beta frequencies. The majority of the post-tetanic oscillation continued at gamma frequencies with occasional frequency halving down to beta frequency oscillations (Figure 4C). Extracellular recordings of fEPSPs demonstrated a lack of potentiation of amplitude during the initial disrupted gamma component of the oscillation. Peak mean fEPSP amplitudes were reduced by 58% to 0.5 ± 0.1 mV from control levels of 1.2 ± 0.1 mV (Figure 4D).

This continuation of the post-tetanic gamma oscillation was modelled by the combination of reduced synaptic inhibition and reduced overall AHP amplitude. Since the initial reduction in AHP amplitude, prior to recovery, has been linked to tetanically-induced metabotropic glutamate receptor activation we tested for possible synergistic effects of the metabotropic glutamate agonist ACPD and morphine. Bath application of ACPD, 20 μM, alone reduced the amplitude of the early AHP by 50% and significantly reduced the duration of the late component (control duration 120 ± 10 ms, ACPD duration 100 ± 10 ms, $P < 0.05$ ($n = 10$), Figure 4E). Addition of morphine to the bathing medium in the presence of ACPD produced no further reduction in duration of early or late AHP but significantly reduced the amplitude of the late component (ACPD amplitude 3.8 ± 0.3 mV, ACPD + morphine 2.9 ± 0.3 mV, $P < 0.05$ ($n = 10$)).

Effects of ketamine on beta oscillations

Ketamine abolished the later, beta frequency component of the post-tetanic oscillation (Figure 5A). The initial gamma component either ended abruptly after *c*.200 ms, or decayed slowly over *c*.500 ms in both frequency and population spike amplitude. This phenomenon was similar to the oscillations produced by tetanic stimuli of low intensity. The effect was concentration dependent, with little effect seen at 10 μM, eradication of beta in four out of five slices at 20 μM, and complete absence of beta at higher concentrations (Figure 6D). The initial gamma component was little effected by ketamine, with little change in frequency and a slight increase in phase difference (Figure 5B). Cross correlation analysis on the one instance where beta was preserved at a dose of 20 μM revealed a large increase in phase difference to 4.2 ms from control values of 1.1 ± 0.5 ms (Figure 5B). Ketamine also produced a high degree of variability in instantaneous frequency plots (Figure 5C), with longer oscillations terminating within the broad frequency range of 10–65 Hz. Subthreshold gamma oscillations can be seen towards the end of these longer oscillations (Figure 5D) which result in only occasional population spike generation. Despite the effective attenuation of the beta oscillation, fEPSPs are still present and are not reduced from control levels (control 1.2 ± 0.1 mV, ketamine 20 μM, 1.3 ± 0.1 mV, Figure 5D). This attenuation of the oscillation prior to formation of a beta frequency oscillation was attributable to a large reduction in the duration of the depolarizing driving force induced by the tetanic stimulation. Control depolarization duration was 2.8 ± 0.0 s, this was reduced to 2.0 ± 0.1 s in the presence of 50 μM ketamine. More importantly, during the period of beta activity in controls the amplitude of the underlying depolarization was reduced from 17 ± 4 mV to 12 ± 2 mV in the presence of 50 μM ketamine ($P < 0.05$, Figure 7D). A decay in driving force to both pyramidal cells and interneurons in the computer model demonstrated the same ablation of beta oscillations in the computer simulations (Figure 5E).

Discussion

These data demonstrate that a number of hypnotic/anaesthetic agents with amnesic properties possess the ability to disrupt beta oscillations generated by synchronous gamma activity in hippocampal area CA1 (*Beta-e*/*Gamma-i*). In each case the known primary mechanism of action of each drug is very different and this is reflected in the pattern of disruption of *Beta-e*/*Gamma-i* activity observed. The effects of these drugs on inhibitory network induced fast oscillations are summarized in Table 1.

From previous studies we have established that *Beta-e*/*Gamma-i* activity is generated in this manner as a consequence of a number of interacting phenomena in the CA1 network

Table 1 Summary of drug effects on interneuron network driven fast oscillations

Drug	*ING*	*PING*	Beta-e/Gamma-i
Diazepam	50% ↓ frequency 20%	↓ frequency	reduced 2-site sync. faster EPSP timecourse
Thiopental	>50% ↓ frequency	>50% ↓ frequency reduce incidence reduce 2-site sync. prevent EPSP potentiation	abolish oscillation
Morphine	Increase max frequency period/period variability	Irregular rhythm reduce 2-site sync. interneuron bursts prevent EPSP potentiation	persistence of gamma reduce 2-site sync.
Ketamine	*	reduce duration reduce 2-site sync.	reduce incidence reduced 2-site sync. (when beta present)

ING = interneuron network gamma, recorded as GABAA receptor mediated IPSCs in the presence of ionotropic glutamate receptor blockers (Whittington *et al*., 1996). PING = pyramid/interneuron network gamma, the same underlying phenomenon as ING but with marked, superthreshold pyramidal cell involvement (Traub *et al*., 1996a; Faulkner *et al*., 1998a; Whittington *et al*., 1998). *Beta-e*/*Gamma-i* = persistent gamma frequency interneuron network gamma with synchronous pyramidal cell involvement on every second or third period of the rhythm (i.e. beta frequencies). Generated by AHP and EPSP potentiation (Whittington *et al*., 1997b; Traub *et al*., 1999). *Ketamine has no effect on ING as experiments were performed in the presence of NMDA receptor blockers. Sync. = synchrony.

(Whittington *et al*., 1997b; Traub *et al*., 1999):

(a) A prolonged depolarization of both excitatory and inhibitory neurons within each area of CA1.
(b) A persistent sub-threshold gamma oscillation in pyramidal cells generated by mutually inhibitory networks of interconnected interneurons (Whittington *et al*., 1995).
(c) Synchronous firing of action potentials in pyramidal cells at each site controlled by this inhibitory gamma oscillation (Traub *et al*., 1996a,b).
(d) Potentiation of recurrent excitatory synaptic connection between each oscillating region (Whittington *et al*., 1997b; Traub *et al*., 1999).
(e) Development of a slow hyperpolarizing or shunting conductance in pyramidal cells, as could occur with recovery of an AHP that had been suppressed by metabotropic receptor activation (Charpak *et al*., 1990; Guérineau *et al*., 1994) during gamma activity.

Thus the *Beta-e*/*Gamma-i* oscillations resulting from synchronous gamma activity, presented here represent a phenomenon where populations of principal pyramidal cells generate synchronous action potentials at sub-harmonics of the underlying inhibitory gamma oscillation ('missed beats'). Beats are missed as a consequence of the time course of the potentiated recurrent excitatory synaptic connections and the AHP (see example traces in Figure 6). Data demonstrate that up to 30% of the observed excitatory potentials in pyramidal cells originate from the distal site, and that, with all other factors remaining constant, absence of this distal contribution destabilizes the emergent *Beta-e*/*Gamma-i* rhythm (Figure 1).

The demonstration that the agents tested in the present study disrupt *Beta-e*/*Gamma-i* oscillations may be seen as contrary to the observed response to anaesthetics and hypnotics as measured by surface EEG electrodes (e.g. see Pichlmayr & Lips, 1983). In this case administration of benzodiazepines and general anaesthetics (not dissociative) produce a 'beta buzz'. Experimentally, such beta oscillations can be produced by pressure ejection of glutamate or specific metabotropic glutamate agonists onto a hippocampal slice (Traub *et al*., 1996b; Whittington *et al*., 1996), or by bath application of carbachol (Fisahn *et al*., 1998) in the presence of some anaesthetic agents. In each case the observed beta rhythm is seen as a slower pyramidal-interneuron network oscillation determined by the larger (benzodiazepines) and/or longer (barbiturates) GABAergic synaptic potentials in pyramidal cells and interneurons (i.e. *Beta-e*/*Beta-i*). In this case no 'missed beats' are seen and there is no underlying inhibitory gamma frequency oscillation (Faulkner *et al*., 1998b; Whittington *et al*., 1999). Mechanistically, then, these drug-induced *Beta-e*/*Beta-i* oscillations can be described as 'slow gamma' oscillations as the same fundamental mechanisms apply. The *Beta-e*/*Gamma-i* oscillations seen in the present study differ markedly from this (see Whittington *et al*., 1999, Figure 5). They possess an underlying inhibitory gamma component but also show strong involvement of recurrent excitatory synaptic activity.

As stated above, each drug tested possessed a different profile of disruption of synchronous *Beta-e*/*Gamma-i* oscillations. In each case, however, the disruption seen was explained on the basis of the known primary mode of action within the above framework. Thiopental, like other anaesthetic barbiturates, increases the decay constant of $GABA_A$ receptor-mediated synaptic inhibition (Barker & McBurney, 1979) and produces an agonist-insensitive GABAergic leak current (Rho *et al*., 1996). Both of these effects combined to decrease the oscillation frequency and disrupt the synchrony and rhythmicity of the initial gamma oscillation (Faulkner *et al*., 1998b). *Beta-e*/*Gamma-i* is generated, at least in part, by the potentiation of excitatory synaptic connections on a period-by-period basis. Synchronous oscillations provide the precise timing required for coherence of postsynaptic action potentials in pyramidal cells and the onset of synaptic excitation. Such timing has been shown to lead to EPSP potentiation in non-oscillating systems (Magee & Johnston, 1997; Markram *et al*., 1997). As thiopental disrupts this timing it also disrupts the potentiation of EPSPs (Figure 2D) and thus the generation of *Beta-e*/*Gamma-i* oscillations.

Similar effects on synchrony during the initial gamma oscillation are seen with morphine at high concentrations (Figure 4B), owing to an effective uncoupling of inhibitory neurons from each other and from pyramidal cells (Whittington *et al*., 1998) and a reduction in post-spike AHP amplitude (Figure 4E). The continuation of gamma activity during the later stages of the post-tetanic oscillation contrasts the observation with thiopental. However, no leak current is generated by morphine and the net effect on networks in hippocampal area CA1 is that of excitation *via dis*inhibition (Madison & Nicoll, 1988) and not inhibition as in the case of thiopental.

Diazepam did not abolish *Beta-e*/*Gamma-i*. Instead a prolongation of the time taken to establish coherent *Beta-e*/*Gamma-i* was seen along with a reduction in between-site synchrony at beta frequencies (Figure 7B). Diazepam causes a large potentiation in macroscopic $GABA_A$ receptor-mediated currents by increasing agonist-induced opening probability (Mody *et al*., 1994), but does not induce a GABA leak current, nor does it affect synchrony, EPSP potentiation, or the duration of the depolarizing drive (Faulkner *et al*., 1998b).

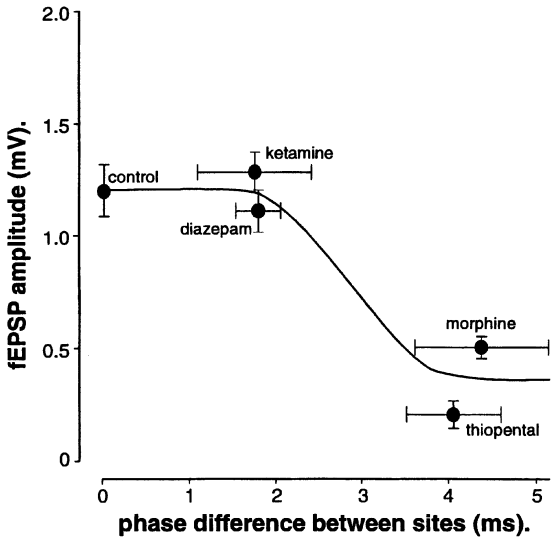

Figure 8 Relationship between phase difference during initial gamma oscillation and degree of fEPSP potentiation. In the control condition gamma oscillations and subsequent beta frequency oscillations can occur with near zero millisecond synchrony. Under these conditions, recordings from the *stratum oriens* reveal fEPSPs with an amplitude of 1.2 ± 0.1 mV. Twenty μM ketamine and 0.5 μM diazepam have only small effects on the phase difference of the gamma oscillation between sites (<2 ms), and do not reduce the amplitude of the fEPSP. However 100 μM morphine and 50 μM thiopental increase the phase difference between the two sites to >3 ms and produce a decrease in fEPSP amplitude generated by gamma oscillations to <0.5 mV.

However, expression of excitatory postsynaptic potentials was affected by diazepam (Figure 3E). This decrease in the duration of excitatory synaptic events (also seen in the model) was probably a byproduct of the larger inhibitory synaptic potentials and may interfere with the ability of EPSPs to stabilise missed beats into a coherent *Beta-e*/*Gamma-i* oscillation.

Diazepam produced little quantifiable change in synchrony during the initial gamma oscillation and ketamine, too, had little effect. In the case of these two agents no disruption of synchronous gamma-induced EPSPs was seen. Plotting the relative effects on synchrony and EPSP amplitude for each of these drugs at concentration relative to hypnosis/anaesthesia reveals a strong relationship between the two measurements (Figure 8). Phase differences of up to 2 ms between sites appear to provide adequate timing of pre- and postsynaptic events to facilitate EPSP potentiation. However, if the degree of synchrony falls further, to give phase differences above 3 ms, then the mechanism of EPSP potentiation fails and no *Beta-e*/*Gamma-i* oscillation is seen.

Unlike each of the above agents, the disruptive effect of ketamine on *Beta-e*/*Gamma-i* oscillations appears to be related directly to its ability to decrease the post-tetanic depolarization, presumably *via* its actions as a non-competitive antagonist at the NMDA subtype of glutamate receptor (Anis *et al*., 1983). We have previously shown that the underlying depolarization that drives the tetanus-induced oscillations includes both NMDA receptor-mediated and metabotropic glutamate receptor-mediated excitation (Whittington *et al*., 1997b). Removal of the NMDA component dramatically shortens the duration of this depolarization and prevents the expression of the later *Beta-e*/*Gamma-i* oscillation (despite the initial potentiation of EPSPs) simply by removing the network driving force at this time. This was not the case for the dramatic shortening of post-tetanic field oscillations in the presence of thiopental. In this case, unlike ketamine, the tetanus-induced depolarizing drive to the network persisted. Cessation of population activity was modelled using increased decay constants for inhibitory synaptic activity and a GABAergic leak conductance (Figure 2E). These two factors combine with the recovery of the AHP to abolish action potential generation in most cells within the population.

The lack of observed effects on the amplitude of the post-tetanic depolarization for drugs such as morphine and thiopental strongly argues against an action on depolarizing GABA responses mediating the observed effects of these drugs. However, experiments on younger animals at higher bicarbonate concentrations have demonstrated a strong depolarizing GABA component to the post-tetanic response (Kaila *et al*., 1997). The pattern of pyramidal cell spiking during depolarizing GABA-mediated events has been shown to have a component within the gamma frequency range (Bracci *et al*., 1999). These authors demonstrated little, if any, synaptic component to their post-tetanic activity but do suggest that the effects of glutamatergic agents on post-tetanic depolarizations seen previously (e.g. see Whittington *et al*., 1997b) may be due to a decrease in GABA release *via* control of the excitability of interneurons.

Enhancement of excitatory synaptic potentials in the hippocampus has proved to be a robust model of learning and memory (Bliss & Collingridge, 1993). Potentiation of EPSPs within the framework of interconnected oscillating areas affords an additional dimension of selectivity. The oscillations themselves are capable of filtering incoming afferent information on the basis of relative timing (Burchell *et al*., 1998) and, on the basis of this temporal coding, provide a pattern of regional intercommunication which, through EPSP potentiation, will be favoured on representation of the original set of stimuli (Hebb, 1949). The anaesthetic/hypnotic agents tested here all possess amnesic properties to some extent at the concentrations used. The GABAergic agents thiopental and diazepam markedly disrupt performance in certain memory paradigms (Osborn *et al*., 1967; Fang *et al*., 1987). Ketamine disrupts working and sematic memory function (Adler *et al*., 1998) and morphine inhibits simple learned responses and the concomitant increase in hippocampal neuronal activity (Mauk *et al*., 1982, but see also Classen & Mondadori, 1984).

The present study reinforces the hypothesis that synchronous, gamma-induced beta oscillations constitute a fundamental behaviour of networks of hippocampal neurons. Each of the drugs tested possess some amnesic properties and each drug disrupted the generation of this type of beta oscillation. As this beta activity may possibly represent a mode of expression for the potentiation of excitatory neurotransmission between co-activated neuronal networks it may therefore constitute a correlate of short term memory function within the hippocampus.

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