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Disruption of synchronous gamma oscillations in the rat hippocampal slice: A common mechanism of anaesthetic drug action

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1 At the molecular level much progress has been made towards elucidating the mechanisms of action of general and dissociative anaesthetics. However, little is known about how these molecular actions may lead to disruption of cognitive function.

2 A promising physiological correlate of cognitive function is the ability of spatially separate areas of the brain to synchronize firing patterns *via* mutual inhibitory, gamma-frequency (20–80 Hz) electrical oscillations. Here we examine the effects of five different anaesthetic/hypnotic agents with different primary mechanisms of action on these oscillations in the hippocampus.

3 Gamma oscillations were elicited simultaneously at two sites at either end of area CA1 by tetanic stimulation. Such oscillations are synchronous between these areas even when separated by up to c. 4 mm in control conditions.

4 Agents which act directly on $GABA_A$ receptor-mediated inhibition had different effects on synchronous gamma oscillations. Thiopental (10–200 μm) markedly disrupted the oscillation and resulting synchrony whereas the benzodiazepines diazepam and temazepam (0.05–1.0 μM) had little effect.

5 The opiate morphine (10–200 μM) and dissociative agent ketamine (10–100 μM) had a different profile of effects on gamma oscillations. However, as with thiopental, both agents markedly disrupted between site synchrony. These three agents demonstrated this effect at aqueous concentrations relevant to anaesthetic ED_{50}.

6 Using the hippocampus as a model neuronal network we propose that, despite differing primary mechanisms of action, anaesthetics may disrupt cognitive function by interfering with the mechanism of generation of synchronous firing patterns between spatially separate areas of the brain.

Keywords: Anaesthetics; thiopental; ketamine; morphine; diazepam; gamma; synchrony; hippocampus

Introduction

At the reductionist level general anaesthetic agents are known to affect a wide range of specific neuronal control mechanisms. Voltage-gated ion channels (Haydon & Urban, 1986; Herrington *et al*., 1991) and ligand-gated ion channels have been shown to be sensitive to general anaesthetics across a wide range of concentrations. In particular a 'superfamily' of receptor/ionophores sensitive to general anaesthetics at or below clinically relevant concentrations has been identified. These include the nicotinic cholinergic and glycine receptors, the 5-HT_3 receptor and the $GABA_A$ receptor (Franks & Lieb, 1996). In addition, a number of atypical anaesthetic agents and adjuvants also affect other neurotransmitter receptors such as the a_2 adrenoceptor (xylazine) and the NMDA subtype of glutamate receptor (ketamine).

This divergent nature of primary sites of action for general anaesthetic agents argues against a single molecular mechanism responsible for the whole-brain effects of these agents. The intravenous agents thiopental and propofol share many effects with inhalational agents on the $GABA_A$ receptor but have different clinical profiles. The dissociative anaesthetic ketamine appears to have no effect on the GABAergic system (but see Little, 1982). However, at a more holistic level, both $GABA_A$ receptor function and NMDA receptor function act together to play a role in generating gamma oscillations, and subtly different changes in $GABA_A$ receptor function have differing effects on these oscillations (Whittington *et al*., 1995, 1997).

Gamma oscillations occur fairly ubiquitously in the neocortex, hippocampus, thalamus and related structures. In any one area they appear to be generated by at least one of two primary mechanisms; the entrainment of populations of mutually interconnected inhibitory interneurons (Whittington *et al*., 1995; Traub *et al*., 1996a,b), and the activity of intrinsic gamma oscillating neurons (Llinás *et al*., 1991; Gray & McCormick, 1996). The latter mechanism appears not to be active in the hippocampus, where inhibitory network oscillations, driven by tonic excitatory drive and shaped by $GABA_A$ receptor-mediated inhibition, can entrain and synchronize excitatory cells to a gamma frequency rhythm (Whittington *et al*., 1995, 1997). Evidence of a role for these oscillations in cognitive function comes from a number of key observations. For example, gamma activity is associated with exploratory behaviour in the hippocampus (Bragin *et al*., 1995) and in the neocortex gamma activity appears to play a critical role in both visual and auditory feature detection (Roelfsema *et al*., 1994; Joliot *et al*., 1994).

At a neuronal network level, gamma oscillations appear to provide a temporal framework which can be used by populations of neurons to precisely control the timing of action potential generation. Within a single neuronal population gamma oscillations produce a pattern of activity which is synchronous both between excitatory neurons within the area and also between excitatory and inhibitory interneurons within the area (Whittington *et al*., 1997). If two oscillating areas each have their interneuron populations influenced by excitatory synaptic connections from the other site then this leads to a

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self-organized synchrony of action potential generation between sites despite long axon conduction delays (Traub *et al*., 1996b; Whittington *et al*., 1997). This synchronization of neuronal population outputs has been proposed to represent a mechanism underlying the 'binding' phenomenon whereby separate areas of the brain, coding for individual features of a perceived object, temporally orchestrate their outputs to give an impression of the object as a whole (see Gray, 1994; Jefferys *et al*., 1996 for reviews).

General anaesthetics can disrupt gamma oscillations associated with cognition. A specific reduction in cortical EEG gamma activity has been demonstrated for inhalational agents (Plourde & Picton, 1990), opiates (Plourde & Boylan, 1991), propofol and thiopental (Plourde & Picton, 1990; Plourde, 1996) and the hypnotic agent diazepam (Pichlmayr and Lips, 1983). This effect is not seen for dissociative anaesthetics (Plourde *et al*., 1997). However, detailed analysis of gamma oscillations induced in hippocampal slices *in vitro* have shown that enhancement of $GABA_A$ergic transmission (with thiopental or propofol), reduction in $GABA_A$ergic transmission (with morphine), or blockade of NMDA receptor-mediated excitation (with R-CPP, 3-((R)-2-Carboxypiperazin-4-yl)-propyl-1-phosphonic acid) all detrimentally affect the generation and maintenance of gamma oscillations (Whittington *et al*., 1996, 1997, 1998).

Here we examine the effects of four hypnotic/anaesthetic agents, with very different primary modes of action, on synchrony induced by inhibitory gamma oscillations. The effects of thiopental, diazepam, morphine and ketamine were examined in detail in order to test the hypothesis that disruption of this mode of neuronal network communication constitutes a unifying mechanism of general anaesthetic action.

Some preliminary observations on the effects of morphine have been previously published (Whittington *et al*., 1998).

Methods

Experimental methods

Transverse, 450 μm thick, dorsal hippocampal slices were prepared from brains of male Sprague-Dawley rats, 250–274 g, after decapitation following cervical dislocation. Slices were 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 7.4 at 35°C.

Brief tetanic stimuli (100 Hz, 200 ms, 8–25 V, 50 μs duration) were delivered simultaneously to the *stratum oriens* at two recording sites at either end of the CA1 region (separation 1.5–3 mm) every 4 min throughout each experiment. Field potentials were recorded at the level of *stratum pyramidale* at each site simultaneously using glass micropipettes filled with 2 M NaCl (resistance 0.5–2 MΩ).

Intracellular recordings of oscillatory activity in pyramidal cells were also made with glass microelectrodes filled with 2 M potassium methylsulphate or potassium acetate (30–80 MΩ). All recordings of membrane potential were made in bridge mode. Both intra- and extracellular recordings were digitized at 2 kHz using a CED A/D converter (Cambridge, U.K.) and stored on disk for off-line analysis using Spike2 software (CED, Cambridge, U.K.).

Thiopental, diazepam, temazepam, morphine, and ketamine, (all from Sigma, U.K.) were prepared as stock solutions in dimethylsulphoxide (DMSO)/distilled water mixtures as appropriate and kept refrigerated. Final bath DMSO concentration did not exceed 0.1% v/v. Drugs were added to the perfusion solution at the following concentrations: Thiopental 10–200 μM, Diazepam and temazepam 0.05–1.0 μM, Morphine 10–200 μM, and Ketamine 10–100 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 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.

Four recordings were taken from five slices for each drug except temazepam (two 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). Frequency, rhythmicity and phase relationships for field potential oscillations were measured by performing auto- and cross correlation analyses of the post-tetanic oscillations (100–200 ms time window starting at the beginning of the oscillation). Data are expressed as means $\pm$ s.e.mean. Data obtained were normally distributed and statistical analysis was carried out using 2-way parametric analysis of variance (ANOVA) and *t*-tests with degree of freedom adjusted for multiple comparisons (Bonferroni).

Simulation methods

A detailed description of the model used is available in Whittington *et al*. (1998). The model is a much developed version of that used in Traub *et al*. (1996a,b) and Whittington *et al*. (1997), the latter containing a diagram of the network topology. In brief, aspects pertinent to the present study are:

Network topology Rather than using a model with five discrete groups of cells (Traub *et al*., 1996a,b), we sought to use a more realistic connectivity. We constructed two overlying arrays, each 30×20, of pyramidal cells and interneurons, respectively. This was intended to represent the entire CA1 region. The lattice spacing in the array is taken to be 150 μm, so that the array is about 4.5×3 mm. Cells are 'wired up', so that each neuron receives 50 excitatory inputs, coming from a 5×10 band of pyramidal cells overlying the neuron; and it receives 49 inhibitory inputs, coming from a 7×7 square of interneurons overlying the cell. The input patterns were the same for pyramidal cells and interneurons. Next, a 'slice' was cut, consisting of the lower 6×20 part of the array. The resulting 120 pyramidal cells and 120 interneurons were used for the simulations. Note that this network is 'distributed' in the sense that cells at the two ends can only communicate with each other via synaptically intercalated neurons.

Axon conduction delays In the model were calculated from the Euclidean distance between pre- and postsynaptic cells, assuming a conduction velocity of 0.2 m/s, for axons of pyramidal cells and of interneurons.

Unitary GABAergic conductances $GABA_A$ receptor-mediated conductances were simulated with units of nS for conductance and ms for t = time as follows: interneuron to pyramidal cell, $1.3 \times \exp(-t/z)$; interneuron to interneuron, $0.5 \times \exp(-t/z)$, where $z = 10$ ms for control oscillations and 40 ms for

simulations of the effects of thiopental. Thiopental-induced GABA 'leak' current was modelled as a tonic $GABA_A$ conductance of 1.0 nS onto pyramidal cells and 0.2 nS onto interneurons.

Oscillatory activity was induced by providing a tonic excitatory drive to both excitatory pyramidal cells (120–140 nS, delivered to apical dendrites) and to inhibitory interneurons (2.0 to 2.25 nS, to somata).

Results

Simultaneous, paired tetanic stimuli reliably (>80% of slices) generate periods of post-tetanic gamma activity which can synchronise across the hippocampal CA1 region. Oscillations last from 0.8–2.5 s (depending on stimulus intensity) and fade in amplitude and frequency as they progress (see Whittington *et al.*, 1997). The properties of these gamma oscillations

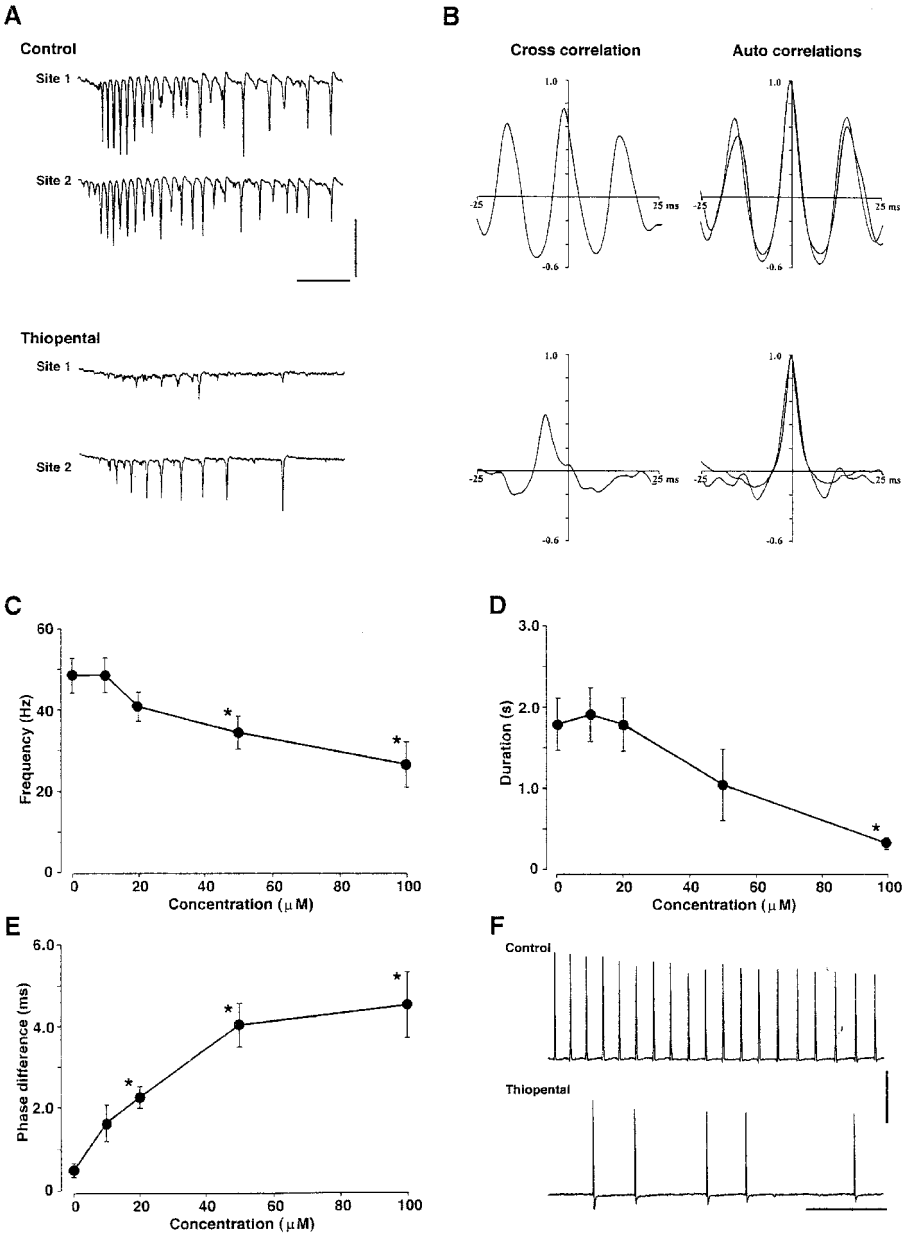

Figure 1 Effects of thiopental on synchronous gamma oscillations. (A). Simultaneous 2-site tetani produced a post-tetanic field potential oscillation at both sites. Traces show first 0.5 s of the post-tetanic oscillation from the beginning (20–150 ms after the end of the tetanus). Population spikes occurred synchronously at both sites. Bath application of 50 μM thiopental reduced the incidence of post-tetanic oscillations and reduced oscillation frequency by c. 50%. Scale bars 1 mV, 100 ms. (B). Cross- and Autocorrelation plots of post-tetanic oscillations at site 1 and 2 showing degree of synchrony (c.1 ms) and rhythmicity. Thiopental decreased rhythmicity within the gamma frequency range and introduced large phase differences between sites. (C). Thiopental decreased mean oscillation frequency at concentrations above 10 μM. Significant decreases were seen with concentrations over 20 μM (D). Thiopental decreased the duration of oscillation in a concentration dependent manner ($^*P<0.05$). E. Thiopental significantly increased betweensite phase difference (ie. decreases synchrony) at concentrations above 10 μM. Higher concentrations increased phase difference by up to 10 fold. $^*P<0.05$, 2-way ANOVA. All data in (C–E) expressed as mean$\pm$s.e.mean ($n=5$). (F). Example intracellular recordings from pyramidal cells at one stimulated site. Traces taken from the initial post-tetanic oscillation demonstrating rhythmic trains of action potentials at gamma frequency in controls. 50 μM Thiopental dramatically reduced the incidence, rhythmicity and frequency of action potentials. Membrane potentials during the oscillation (ie during the post-tetanic depolarization, see Whittington *et al.*, 1997) were -54 mV and -52 mV respectively. Scale bars 40 mV, 100 ms.

measured here (duration, phase difference and frequency) did not alter during the course of each experimental day when no drugs were added to the bathing medium.

Effects of thiopental on gamma oscillations

Bath application of thiopental caused a concentration dependent disruption of the pattern of oscillation at each site (Figure 1A). Cross correlation analysis showed a concentration dependent increase in the phase difference between sites (Figure 1B), demonstrative of a reduction of oscillation-induced synchrony between the two areas. In the control condition gamma oscillations at the two sites had a phase difference of only 0.5 ± 0.2 ms. This increased to 4.1 ± 0.5 ms for 50 μM, and 4.6 ± 0.8 ms for 100 μM and was statistically significant for concentrations over 10 μM ($P < 0.05$, Figure 1E).

Auto correlation analysis (see Figure 1B), revealed a significant 27% decrease in the frequency of the gamma oscillation from control 48.6 ± 4.0 Hz, to 50 μM, 35.6 ± 3.7 Hz ($P < 0.05$, Figure 1C). Furthermore, comparison of the auto-correlations for the effects of thiopental at each site showed a significant frequency mismatch between the areas at higher thiopental concentrations ($P < 0.05$). i.e. the effects of thiopental on frequency were highly variable within individual slices. These changes were accompanied by a significant decrease in the duration of the post-tetanic oscillation ($P < 0.05$, Figure 1D). At 200 μM, in four out of five cases, the oscillation was totally abolished with only occasional population spikes remaining. Intracellular recordings from pyramidal cells, (Figure 1F), show a reduced frequency of action potential firing to as low as c. 20 Hz, together with an erratic firing pattern, mirroring the population effects of thiopental.

Thiopental shares a number of primary mechanisms of action with propofol and inhalational anaesthetic agents, namely the prolongation of inhibitory postsynaptic events and the generation of a tonic GABA 'leak' current (Maciver & Kendig, 1991; Hales & Lambert, 1991; Whittington *et al.*, 1996). We examined the contribution of these effects to the above disruption of synchronous gamma oscillations by modelling each in-turn using a computer simulation of the hippocampal CA1 region.

Increasing the decay constant of $GABA_A$ receptor-mediated synaptic currents from 10–40 ms produced a slower oscillation which was, however, still highly rhythmic and demonstrated synchrony between sites (Figure 2B). Control frequency was

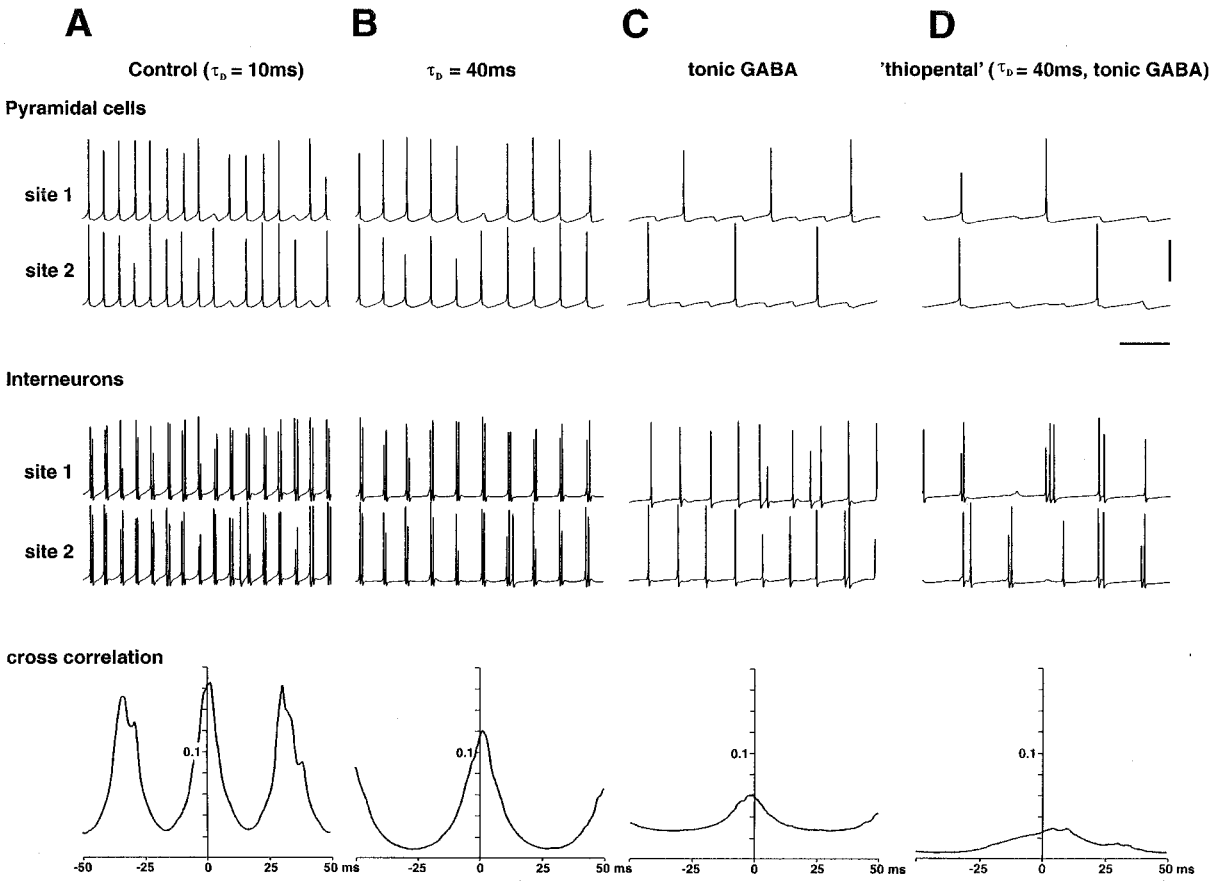

Figure 2 Computer simulation of the effects of thiopental on 2-site gamma oscillations. Simulation methods are described in the main text. Traces illustrate the activity of a single pyramidal cell at each site and a single GABAergic inhibitory interneuron at each site. Each set of four traces was taken concurrently. i.e. absolute timing of action potentials during each example is comparable both between sites and between pyramidal cells and interneurons. Cross correlation analyses of logically averaged *pyramidal cell* oscillatory activity are illustrated for each simulation condition. (A) Control, gamma frequency oscillations were modelled using a decay constant for $GABA_A$ receptor-mediated inhibitory synaptic events of 10 ms. (B) Effects of 50 μM thiopental were initially modelled purely as a 4-fold increase in the decay constant for inhibitory synaptic events (see Whittington *et al.*, 1996). Oscillation frequency was decreased to c. 60% of control values but rhythmicity and synchrony were preserved. (C) Addition of a tonic GABA conductance to both pyramidal cells (1 nS) and interneurons (0.2 nS) also caused a dramatic drop in frequency of oscillation but, again, synchrony was preserved. (D) Simulation of thiopental using both prolonged $GABA_A$ decay constant (as in B) and a tonic GABA current of 1 nS to pyramidal cells and 0.2 nS to interneurons. Decreased pyramidal cell firing exposed prolonged inhibitory waves and disrupted rhythmicity (cf.) D and synchrony. Scale bars 40 mV, 100 ms.

32 Hz and this fell to <20 Hz with the longer decay constant. The degree of synchrony, defined as position of central cross correlation peak, was c. 1 ms in both cases. Introduction of a small $GABA_A$ receptor-mediated 'leak' current also reduced frequency to <20 Hz but again the phase angle between sites was c.1 ms i.e. synchrony was preserved (Figure 2C). If both decay constant and tonic GABA conductance were introduced into the model then a far more dramatic effect was observed. Pyramidal cell firing was further reduced and synchrony and rhythmicity were practically abolished (phase angle between pyramidal cells was 5–10 ms, Figure 2D). The combination of these two changes produced model behaviour which closely mimicked the experimental observations in the presence of 50 μM thiopental.

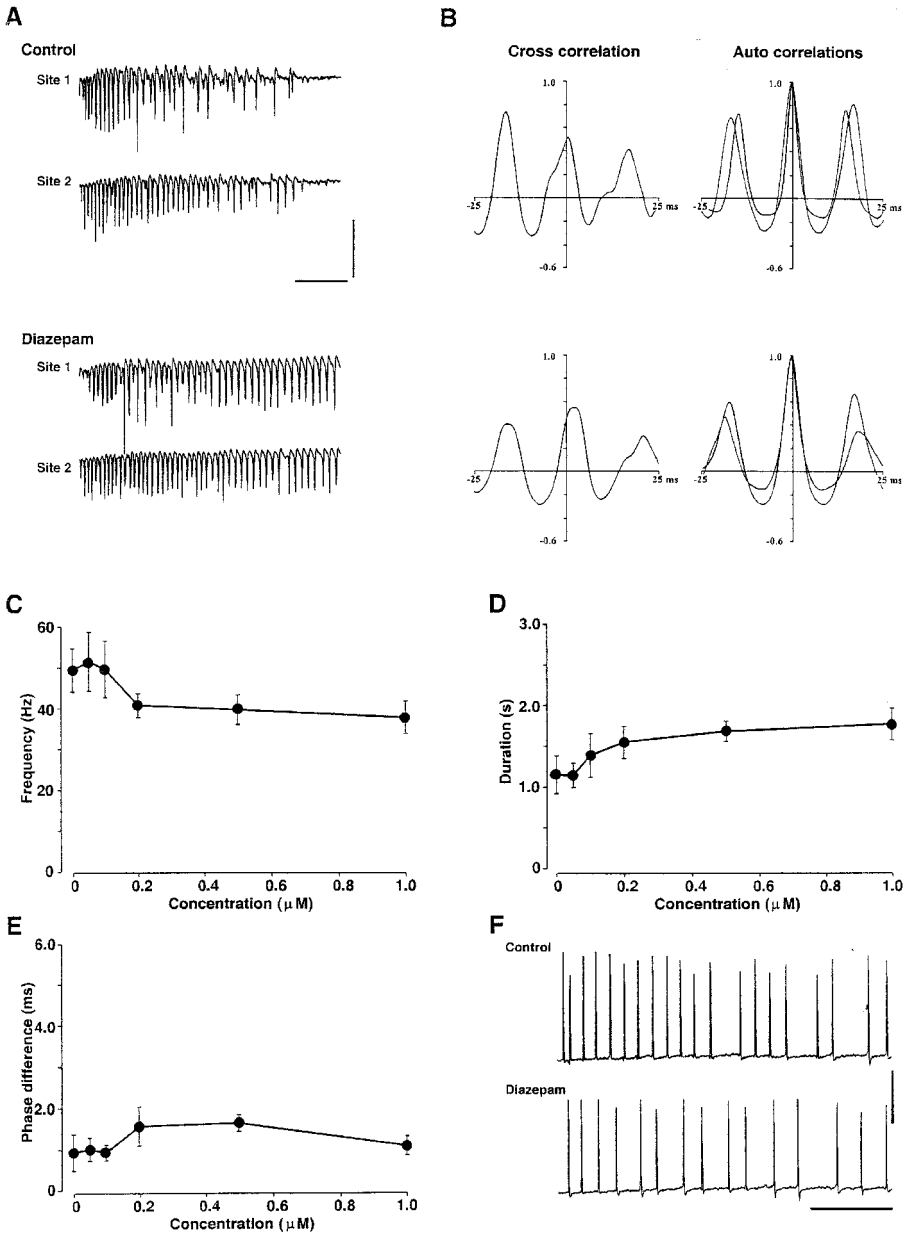

Figure 3 Effects of diazepam on synchronous gamma oscillations. (A) Simultaneous 2-site tetani produced a post-tetanic field potential oscillation at both sites. Example oscillations illustrate first 1 s from start. Population spikes occurred synchronously at both sites. Bath application of 0.5 μM diazepam had no effect on the initial component of the synchronous 2-site oscillation but prolonged the duration of the overall post-tetanic oscillation. Scale bars 1 mV, 200 ms–NB. For comparison these traces are twice the length of those in Figure 1A. (B) Cross- and Autocorrelation plots of post-tetanic oscillations at site 1 and 2 showing degree of synchrony (c.1 ms) and rhythmicity. Diazepam had no effect on rhythmicity within the gamma frequency range and had no effect on phase differences between sites. i.e. Synchrony was preserved. (C) Diazepam showed a small, concentration dependent decrease in mean oscillation frequency at concentrations above 0.1 μM No significant decreases were seen when comparing each concentration used with control values ($P>0.05$). However, 2-way analysis of variance revealed a significant general effect on frequency ($P<0.001$). (D) Effects of diazepam on duration of oscillation. A concentration-dependent effect was seen with a maximal increase in duration of c. 150%. 2-way analysis of variance revealed that this general effect was significant ($P<0.001$). (E) Diazepam had no significant effect on between-site phase difference (ie. synchrony) at any concentration used ($P>0.05$). All data for (C–E) are expressed as mean ± s.e.mean ($n=5$). (F) Example intracellular recordings from pyramidal cells at one stimulated site. Traces taken from the initial post-tetanic oscillation demonstrating rhythmic trains of action potentials at gamma frequency in controls. 0.5 μM diazepam had a slight effect on frequency of action potentials within an oscillation. Membrane potentials during the oscillation were −54 mV and −53 mV respectively. Scale bars 40 mV, 100 ms.

Effects of diazepam on gamma oscillations

Like thiopental, diazepam potentiates $GABA_A$ receptor-mediated inhibition. However, the effects of this drug on gamma oscillations was much less marked. Other than small changes in some oscillation parameters no overt effect of the drug was seen (Figure 3A). Cross correlation analysis showed no significant change in the phase difference between sites at any concentration tested ($P>0.05$, Figure 3B, E). There was a statistically significant decrease in the frequency of the oscillation when the overall effect of the drug was analysed using ANOVA ($P<0.05$, Figure 3C). Control values were 49.6 ± 4.5 Hz, and 42.3 ± 2.5 Hz for 0.5 μM. No frequency mismatch effects were seen at any diazepam concentration studied ($P>0.05$, data not shown). These effects were accompanied by a small but significant increase in the overall duration of the oscillation ($P<0.05$, Figure 3D). Intracellular recordings also showed the maintenance of a rhythmic

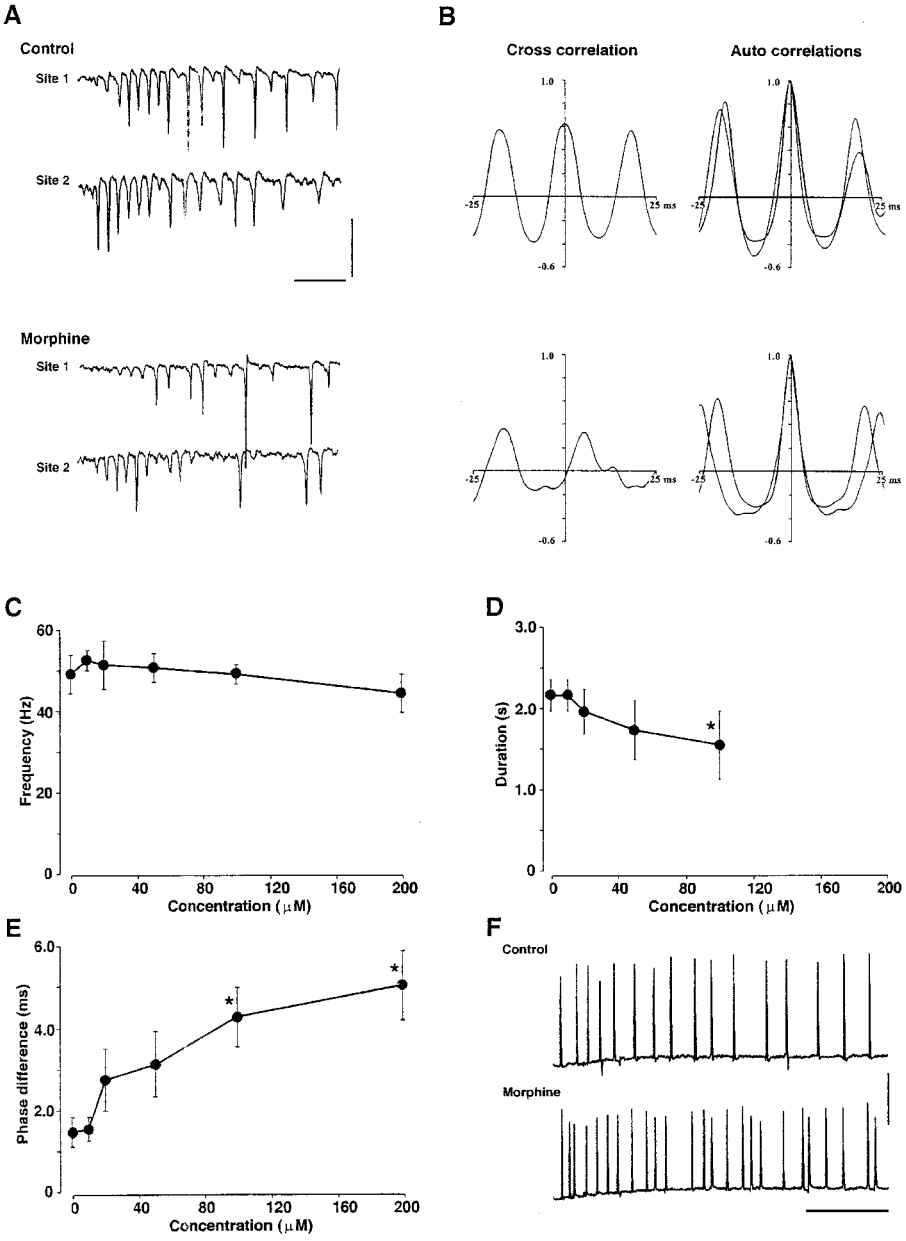

Figure 4 Effects of morphine on synchronous gamma oscillations. (A) Simultaneous 2-site tetani produced a synchronous post-tetanic field potential oscillation at both sites. Traces show first 0.5 s of oscillation. Bath application of 50 μM morphine caused oscillations to become far more erratic and disrupted between-site synchrony. Scale bars 1 mV, 100 ms. (B) Cross- and Autocorrelation plots of post-tetanic oscillations at site 1 and 2 showing degree of synchrony (<1 ms in controls) and rhythmicity. Morphine reduced rhythmicity within the gamma frequency range and had a marked detrimental effect on the pattern of synchrony between sites. (C) Morphine had no significant effect on the overall frequency of post-tetanic oscillation at all concentrations applied (10–200 μM, $P>0.05$). (D) Morphine significantly decreased the duration of post-tetanic oscillation at a bath concentration of 100 μM (*$P<0.05$). (E) Morphine showed a concentration dependent increase in mean between-site phase difference (ie. decreases synchrony) at concentrations above 10 μM. With bath concentrations over 50 μM this disruption of synchrony was significant (*$P<0.05$). All data expressed as mean$\pm$s.e.mean ($n=5$). (F) Example intracellular recordings from pyramidal cells at one stimulated site. Traces taken from the initial post-tetanic oscillation demonstrating rhythmic trains of action potentials at gamma frequency in controls. Fifty μM morphine increased the incidence and frequency of action potentials and reduced rhythmicity. Membrane potentials during oscillation were -52 mV and -49 mV respectively. Scale bars 40 mV, 100 ms.

coherent oscillation, but a c. 20% decrease in oscillatory frequency after bath administration of 0.5 μM diazepam (Figure 3F). The effects of diazepam were modelled closely in computer simulations by an increase in amplitude of $GABA_A$ receptor mediated synaptic currents (data not shown), with rhythmicity and synchrony of the gamma oscillation being preserved.

The effects of diazepam were shared by the benzodiapine temazepam (data not shown). A reduction in frequency was seen with temazepam concentrations above 0.5 μM but rhythmicity and synchrony were preserved.

Effects of morphine on gamma oscillations

μ-opiate agonists or partial agonists are capable of inducing anaesthesia at high concentrations but their primary effects on archicortical and neocortical brain regions is to reduce overall levels of GABAergic inhibition and reduce excitability of inhibitory interneurons (Madison & Nicoll, 1988). The overall effects of morphine on gamma oscillations was different to that seen for thiopental (compare Figures 1A, 4A) – reflecting the diametrically opposing primary effects on $GABA_A$ergic inhibition. These effects of morphine are mediated by actions at the μ-opiate receptor (Whittington *et al*., 1998). Unlike thiopental, morphine produced a post-tetanic oscillation with comparable frequency to controls, but with a visibly more erratic nature. However, like thiopental, cross correlation analysis revealed that morphine reduced the synchrony of gamma oscillations (Figure 4B, E). A small increase in the mean phase difference was seen at concentrations as low as 20 μM (Figure 4E). Significant increases in phase difference were seen at concentrations above 50 μM, reflecting a loss of synchrony between the two areas (control 1.7 ± 0.2 ms, 100 μM morphine 4.4 ± 0.8 ms, $P < 0.05$).

Morphine also produced a concentration dependent effect on the duration of the post tetanic oscillation (Figure 4D), with a small but significant reduction in the total length of oscillation at a concentration of 100 μM ($P < 0.05$). Auto-correlation analysis revealed no significant change in the frequency of oscillation at any concentration used (Figure 4C, $P > 0.05$), however, smaller split peaks were common on cross correlation plots. Morphine also increased the mean mismatch in frequencies between sites (control 1.0 ± 1.0 Hz, morphine, 20 μM 6 ± 2 Hz) but not to a significant extent in the present study ($P > 0.05$). Intracellular recordings also show a very erratic rhythm after bath application of morphine, with bursts of faster activity (Figure 4F).

Effects of ketamine on gamma oscillations

Ketamine produced effects on both the synchrony of gamma oscillations and the duration, (Figure 5). Cross-correlation analysis showed a concentration-dependent reduction in between-site synchrony (Figure 5E). A 3-fold increase in phase difference was seen with 100 μM ketamine. However, at concentrations above 20 μM a large increase in the variability of the between-site phase relationship was seen during individual experiments. This far more erratic phase relationship between sites was reflected in the magnitude of the variance of the pooled data (Figure 5E, insert). Overall there was a significant increase in the phase difference for 100 μM ketamine ($P < 0.05$) indicative of a loss of synchrony. Statistical analysis of mean phase differences produced by lower ketamine concentrations was compromised by the large increase in variance of the data produced by the drug. Auto correlation analysis revealed no significant change in common frequency at any concentration examined and did not produce a frequency mismatch (Figure 5C, $P > 0.05$). Ketamine also markedly decreased the duration of the oscillation, as can be clearly seen in examples of intracellular potential and extracellular field recordings (Figure 5A, F). This effect was concentration dependent and statistically significant (Figure 5D, $P < 0.05$ for concentrations of 20 μM and above).

Discussion

We have previously demonstrated that some general anaesthetic agents have a marked effect on gamma oscillations elicited in pharmacologically isolated inhibitory networks (Whittington *et al*., 1996). Here we examined effects of anaesthetics and the benzodiazepines diazepam and temazepam on the intact hippocampal network. Tetanic stimulation reliably evoked gamma frequency oscillations in the hippocampal CA1 region. Rhythmic, 36 – 60 Hz oscillations at two sites occurred and led to a highly synchronous pattern of field potential generation under control conditions. This synchronous activity was significantly disrupted by the general/dissociative anaesthetics studied, but unaffected by the hypnotic agent diazepam.

Other than the effects on synchrony each of the agents tested had a different overall profile of effects on gamma oscillations. Thiopental decreased the overall frequency and duration of the post tetanic oscillation. The decrease in frequency is consistent with the effects of barbiturates on the amplitude and decay constant of the $GABA_A$ receptor mediated inhibitory synaptic currents. This is a post synaptic effect mediated by an increase in the time individual $GABA_A$ receptor/chloride ionophores spend in the open state (Barker & McBurney, 1979). The frequency of the gamma rhythm is dictated by the decay constant of mutual inhibitory post synaptic currents onto inhibitory interneurons and pyramidal cells (Traub *et al*., 1996b), with longer inhibitory synaptic events prolonging the period of each oscillation. This effect alone was not sufficient to explain the marked disruption of synchrony. In addition, the ability of barbiturates and other general anaesthetics to induce a GABAergic leak current, when modelled, also failed to disrupt synchrony. This current has been reported for inhalational agents, barbiturates and propofol (MacIver & Kendig, 1991; Peters *et al*., 1988; Hara *et al*., 1993) and may be a consequence of either agonist-insensitive ionophore opening (Rho *et al*., 1996), temporal summation of prolonged miniature inhibitory post synaptic events, or both. Instead, the computer model revealed that the most detrimental effect of barbiturates on the synchronous oscillation was mediated by the synergistic effects of both this leak current and the prolonged decay constant (Figure 2D). The decrease in the duration of the field potential oscillation was not accompanied by a decrease in the duration of the underlying membrane potential depolarization measured using intracellular recording (data not shown) suggesting that the disrupted synchrony was of sufficient magnitude at higher thiopental concentrations to interfere with population spike generation.

Diazepam and temazepam, like thiopental, interact with post synaptic $GABA_A$ receptors. However, though there is an increase in the decay constant of inhibitory synaptic currents, the predominant effect is an increase in amplitude of these events. This effect is mediated by an increase in the probability of agonist-induced opening of chloride ionophores (Haefely, 1984; Mody *et al*., 1994) and an increase in channel conductance (Eghball *et al*., 1997). An increase in $GABA_A$

receptor-mediated synaptic currents on inhibitory interneurons causes a small decrease in the frequency of gamma oscillations (Traub *et al*., 1996a), but does not affect synchrony or rhythmicity as demonstrated here. Benzodiazepines do not induce a tonic leak current (Whittington *et al*., 1996) and so have only marginal effects on post tetanic oscillations. This observation was born out by the computer model, where even large increases in amplitude and/or decay constant of $GABA_A$ receptor-mediated synaptic events had little effect on the gamma rhythm (see Figure 2B). Comparison of the effects of these drugs at the biophysical and network levels provides insight into the mechanism of disruption of GABAergic transmission pertinent to the effects of general anaesthetic agents. We suggest that the important effects of general anaesthetics that act on the $GABA_A$ receptor/ionophore is not just the prolongation of GABA-induced channel opening, but

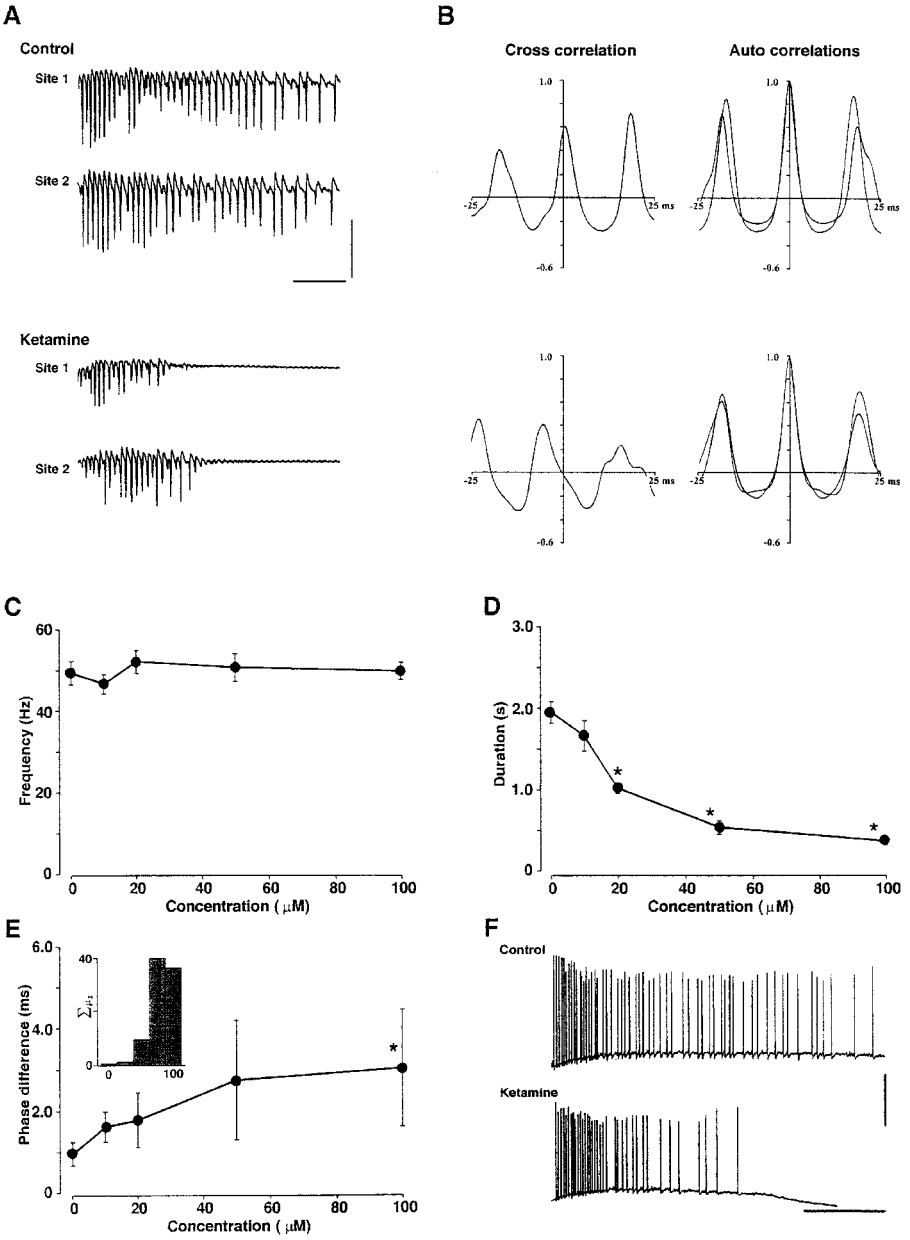

Figure 5 Effects of ketamine on synchronous gamma oscillations. (A) Simultaneous 2-site tetani produced a synchronous post-tetanic field potential oscillation at both sites. Traces show first 1 s of oscillation. Bath application of 50 μM ketamine dramatically reduced the duration of post-tetanic oscillations and caused the erratic appearance of phase differences between sites. Scale bars 1 mV, 200 ms. (B) Cross- and Autocorrelation plots of post-tetanic oscillations at site 1 and 2 showing degree of synchrony (<1 ms in controls) and rhythmicity. Ketamine had little effect on rhythmicity but had a detrimental effect on the pattern of synchrony between sites. (C) Ketamine had no significant effect on the overall frequency of post-tetanic oscillation at all concentrations applied (10–200 μM, $P>0.05$). (D) Ketamine significantly decreased the duration of post-tetanic oscillation at bath concentrations above 10 μM ($^*P<0.05$). (E) Ketamine showed a concentration dependent increase in mean between-site phase difference (ie. decreases synchrony). This was also accompanied by a large increase in the variance of phase differences pooled both within individual hippocampal slices and across different slices (insert). Variance expressed as the sum of the squares of the differences of individual phase angles from the mean ($\Sigma\mu_2$) for each concentration of ketamine. With bath concentrations over 50 μM the disruption of synchrony was significant ($^*P<0.05$). All data expressed as mean$\pm$s.e.mean ($n=5$). (F) Example intracellular recordings from pyramidal cells at one stimulated site. Traces taken from the initial post-tetanic oscillation demonstrating rhythmic trains of action potentials at gamma frequency in controls. Fifty μM ketamine decreased the overall duration of the post-tetanic oscillation. Peak depolarization of membrane potentials for traces illustrated were -50 mV and -54 mV respectively. Scale bars 40 mV, 750 ms.

the coexistence of this effect with the generation of a tonic leak current independent of background levels of synaptic transmission.

In contrast to the above two GABAergic agents morphine has the opposite effects on this aspect of inhibitory neurotransmission. Acting via μ-opioid receptors, morphine has two complementary effects, both of which decrease the level of GABA-mediated inhibition. Presynaptically, a decrease in the release of GABA is seen via a G-protein mediated mechanism (Capogna *et al*., 1993). In addition, a post synaptic effect resulting in hyperpolarization of inhibitory interneurons has also been reported (Madison & Nicoll, 1988). The overall consequence of these two effects on gamma oscillations is to increase the fundamental frequency of oscillation of the inhibitory network and functionally uncouple excitatory pyramidal cells and interneurons from their inhibitory neighbours. This results in an inability of individual excitatory neurons to follow the faster rhythm and disrupts synchrony through induction of burst firing in disinhibited interneurons via multiple, mis-timed excitatory post synaptic potentials. This effect was antagonised by cyprodime, but not naltrindole or nor-binaltorphimine indicating a μ-opioid receptor-mediated effect (Whittington *et al*., 1998).

Ketamine has no direct effects on $GABA_A$ receptor-mediated synaptic inhibition at clinically relevant concentrations. However, though these $GABA_A$ receptor-mediated events provide the fundamental temporal framework for the oscillation, no oscillation can be generated unless populations of pyramidal cells and interneurons are tonically activated. Tetanic stimulation provides this tonic activation in the form of a post tetanic depolarisation mediated by activation of a number of receptor subtypes. Both NMDA and metabotropic subtypes of glutamate receptor, in addition to a muscarinic cholinergic component are involved (Whittington *et al*., 1997). Selective blockade of NMDA receptors with the non-competitive antagonist R-CPP dramatically reduces the duration of the post tetanic depolarisation (and therefore oscillation) in a stimulus dependent manner. This effect was also observed with the NMDA receptor antagonist ketamine (Anis *et al*., 1983), showing a concentration dependent decrease in oscillation duration at both stimulated sites. The oscillation that remains demonstrated very erratic phase differences between sites. This may also be caused by the reduced driving force in the presence of ketamine. In the control condition the post tetanic depolarization saturates to generate a plateau potential. This ensures that both sites are driven at equal intensities. If ketamine reduces drive below this saturated level then differences in the driving force at each site would be expected. Such differences have already been shown to detrimentally affect the phase relationship between two oscillating regions (Whittington *et al*., 1997).

Thiopental, morphine and ketamine each disrupted synchrony and thus detrimentally affected communication between sites in the temporal domain. The effects of each of these drugs on synchrony occurred at aqueous concentrations relevant to clinical anaesthesia. The ED_{50} for thiopental has been estimated at 25 μM (Franks & Lieb, 1994), a concentration around which the effects on frequency and synchrony became statistically significant (Figure 1). Morphine is more commonly used as an anaesthetic adjuvant, however anaesthesia can be achieved with concentrations much higher than used for analgesia (c. 10 μM). Effects on synchrony were apparent at concentrations above 10 μM and became significant an order of magnitude higher than this. Ketamine is an effective dissociative anaesthetic at concentrations above 10 μM. Present observations demonstrated a profound effect on synchrony at concentrations of 20 μM and above. It should also be noted that it is impossible to quantify disruption of synchrony when areas do not oscillate. It is as yet unclear how long areas have to oscillate to establish pertinent temporal patterns of communication but ketamine also showed a significant reduction in oscillation duration at concentrations of 20 μM and above.

Diazepam is, arguably, not a true anaesthetic agent. It is a good hypnotic agent and the transition from the awake state, through sleep, to anaesthesia can be thought of as a continuous scale. The present observations demonstrate that diazepam reduces oscillation frequency at clinically relevant concentrations for sedation (c. 100 nM) but the lack of effect on synchrony sets this agent apart from the anaesthetics studied here.

In conclusion, the three agents capable of producing profound cognitive impairment leading to anaesthesia (morphine, thiopental, and ketamine), all disrupted communication between spatially separate brain regions in the temporal domain. This occurred despite very different primary modes of action. Diazepam did not affect this aspect of neuronal communication. The present study used the hippocampal CA1 region as a relatively simple model neuronal network. However, failure of hippocampal function *in vivo* causes amnesic and psychotic effects and not loss of consciousness *per se*. The sensory regions of the neocortex also demonstrate gamma oscillations and these oscillations, too, are labile to general anaesthetics as measured using surface EEG recordings (see Introduction). At least some of this gamma activity is generated by the mutual inhibition mechanism seen to be active in the hippocampus (Whittington *et al*., 1995; Antkowiak & Hentsche, 1997). However, the neocortex represents a far more complex neuronal network and additional mechanisms of generating gamma oscillations are present (Gray & McCormick, 1996). If the observation that general anaesthetics disrupt gamma oscillation-induced synchrony is to be developed into a hypothesis for production of general anaesthesia in general then comparable studies need to be performed on thalamocortical systems. This aside, these results demonstrate that disruption of synchronous gamma oscillations in the hippocampus constitutes a common mechanism of anaesthetic drug action.

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