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The role of the anterior intralaminar nuclei and N-Methyl D-Aspartate receptors in the generation of spontaneous bursts in rat neocortical neurones

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Summary. The nature of spontaneous unitary activity of rat neocortex was investigated during slow wave sleep and urethane anaesthesia. Neurones in layer IV and V locations fired in a burst-pause pattern at a low burst repetition rate (0.5–4 per second) during both stage 3/4 sleep and urethane anaesthesia. Occasionally an alternative mode of firing (spindle clusters), associated with focal spindle wave activity, was also found to occur in both states. Using dual microelectrode implants it was found that the onset times of bursts (but not spindle clusters), coincided in the same and opposing cortices, whether in functionally similar or disparate areas. The highest probability was that burst onsets occurred simultaneously (resolution = 2.56 ms, interquartile range = 40 ms). Spontaneous unitary activity was investigated in the thalamus for temporal correlation with spontaneous unitary activity in neocortex under urethane anaesthesia. Neurones of the anterior intralaminar group (aIL) consistently fired in a burst-pause pattern such that each aIL burst showed a strong tendency to precede a cortical burst. Unilateral electrical stimulation of the aIL nuclei evoked widespread bilateral entrainment of cortical bursts. In contrast stimulation of VPl, or cutaneous sites, evoked only short duration spike responses together with burst abolition in the appropriate restricted Sml area. Ionophoresis of NMDA (N-Methyl D-Aspartate) onto Sml neurones increased the probability of cortical burst responses to aIL stimulation in addition to decreasing the latency by 20–40 ms (n = 11). Ionophoresis of 2APV (2-amino 5-phosphono valeric acid) caused simultaneous abolition of spontaneous cortical bursts and bursts evoked by aIL stimulation. Short latency responses to cutaneous and VPl stimulation were unaffected by ionophoresis of 2APV sufficient to cause burst elimination, suggesting that this pathway does not operate via a 2APV sensitive receptor mechanism. Anatomical features of the aIL nuclei and their overall cortical projection pattern are discussed in relationship to these findings. The activation of cortical NMDA/APV sensitive receptors by aIL afferents in the "spontaneous" generation of bursts in cortical cells is discussed.

Key words: Intralaminar nuclei – Neocortex – Somatosensory – NMDA – 2APV – Thalamo-cortical

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Introduction

In rats and rabbits anaesthetized with urethane, layer V neocortical neurones are known to fire in regular low frequency bursts of spikes (Creutzfeldt and Houchin 1974; Armstrong-James and Fox 1983). Similar bursts occur during slow wave sleep (SWS) in a variety of mammals (Hubel 1959; Evarts 1962, Armstrong-James and Fox 1984). Such bursts have recently been stated to occur simultaneously at widely displaced cortical sites bilaterally and homolaterally (Armstrong-James and Fox 1984). During arousal bursting does not occur. In view of these observations we have investigated the possibility that such bursts may be initiated by a subcortical site which has "nonspecific" projections to both hemispheres. The number of subcortical candidates with the required global cortical innervation is rather few, the major contenders being the dorsal raphé nuclei (DRN) (Moore et al. 1978), the locus coeruleus (LC) (Moore and Bloom 1979), the basal forebrain nuclei, (BFb) (Divac 1975), and the intralaminar nuclei of the thalamus (IL) (Walker 1938).

Although the serotoninergic cells of the DRN are strongly implicated in sleep mechanisms (Jouvet

1972), their spontaneous activity decreases progressively during SWS and fails to match the pattern of the cortical bursting rhythm or EEG (Trulson and Jacobs 1979). The noradrenergic innervation which arises from the LC has been implicated in cortical arousal mechanisms (Jouvet 1972) although on the basis of lesion experiments denied any role in sleep or waking mechanisms by others (Jones et al. 1977; Robinson et al. 1977). Ionophoresis of noradrenaline onto cortical cells causes either inhibition, or a lasting change from bursting to a continuous or sporadic pattern of firing (Armstrong-James and Fox 1983). Less is known about the BFb area than other regions; stimulation of the area can induce sleep after a few minutes, although this appears to evoke EEG spindles unrelated to stimulation frequency (Sterman and Clemente 1962). Widespread destruction in the area causes a decreased incidence of REM sleep, after a delay of a few days (Sterman and Clemente 1962; McGinty and Sterman 1968). The BFb direct projection to cortex appears to be rather poor in the rat, and overwhelmingly cholinergic (Saper 1984). The effect of ionophoresed acetyl choline on cortical cells is either total inhibition or to cause tonic firing in cats anaesthetised with Dial-urethane (Krnjevic and Phillis 1963; Spehlman and Smathers 1974).

These observations would seem to mitigate against any of the above systems acting as direct routes for initiating bursts characteristic of SWS. However, stimulation of the IL nuclei at low rates produces slow wave sleep in cats, (Akert et al. 1952) and at somewhat higher rates the well known recruiting waves (Morison and Dempsey 1942). The IL nuclei are known to possess direct diffuse cortical projections (Jones and Leavitt 1974) and intimate midline interconnections (Scheibel and Scheibel 1967). In addition there is strong anatomical evidence that the IL-cortical pathway is either aspartergic or glutaminergic (Ottersen et al. 1983; Streit 1980) and it has recently been shown that activation of a cortical amino acid receptor of the N-Methyl D-Aspartate (NMDA) variety is necessary for generation of neuronal cortical bursts (Armstrong-James, Caan and Fox 1985).

The present study was designed to investigate whether there was a role for the IL nuclei in the production of 'spontaneous' neuronal firing patterns found in urethane anaesthetised rat cortex, and by implication in SWS. The results suggest that the anterior members of the IL group act as a site for generation of cortical bursting activity, and that they do so either directly or indirectly by phasic activation of N-Methyl D-Aspartate (NMDA) sensitive receptors on cortical neurones.

Methods

Surgical preparation and recording techniques

Acute animals. Adult male Sprague-Dawley rats were used for most of the experiments, together with a few male Wistars. Animals were anaesthetised with fresh urethane (20% w/v in distilled water) at a dose of 1.5 g/kg body weight. Anaesthesia was maintained by supplementary intraperitoneal injections of 0.2–0.5 ml when necessary, as judged by monitoring the burst rate of cortical cells (see Results).

Rectal temperature was maintained at 36.5°C by thermistor control. Single or double craniotomies were performed, usually exposing an area approximately 2 mm square just caudal to bregma and 2–5 mm lateral to the midline in order to record from the principal hindpaw and forepaw representations of Sml cortex. Other cortical areas were additionally exposed where necessary. The exposed area was covered with agar gel (2% w/v in 0.9% saline). Evaporation from the gel surface was prevented by a covering layer of silicone oil.

Single-barrelled and multi-barrelled carbon fibre microelectrodes (Armstrong-James et al. 1980) were used to record extracellularly. In order to facilitate the positioning of two microelectrodes unilaterally in a small area, one microelectrode would be bent approximately 20° from the normal, 5 mm or so behind the electrode tip, by heating cautiously in a needle bunsen flame. The electrodes were adjusted in the micromanipulators for a penetration normal to the pial surface. The bent electrodes were unneccessary in bilateral penetrations.

Stereotaxic co-ordinates for intralaminar penetrations were calculated from the atlas of Paxinos and Watson (1982). Penetrations were made at an angle of 15° to the normal of the cortical surface such that the electrode tip pointed in toward the midline. This method enabled the greatest extent of the intralaminar nuclei to be encountered in a single penetration and also obviated the need to expose the cortex too near to the saggital sinus.

Chronic animals. Adult male Sprague-Dawleys rats were anaesthetised intraperitoneally with Saffan (Glaxo Labs.) at a dose of 1.1 ml/100 g, which usually induced anaesthesia for four to five hours. Wound margins were treated with lignocaine.

Prodecures were identical to those described above for acute animals with the exception of the electrode implant. The earth connection was made via a soldered connection to a small screw located in the frontal bone. Microelectrodes were manufactured in the usual way but the shank was cut to give an overall length of approximately 10 mm. The shafts were then bent into an L shape 2–3 mm behind the tip. Pairs of electrodes were then mounted on a small plastic platform, finely adjusted until they were parallel with one another and arranged with the tips overhanging the platform by the same length. The platform was held with a pair of modified forceps on a micromanipulator enabling the electrodes to be positioned in the cortex.

The cortex was protected by agar gel from dental cement (Duralon) used to fix the implant in place. Antibiotics were applied topically before final cementing and the whole structure covered with plastic skin (Smith and Nephew Ltd.).

Animals usually recovered within 8 h of primary induction of anaesthesia. Advance warning of recovery from Saffan was signalled by greatly increased spontaneous activity of cortical cells. A period of ataxia sometimes persisted for an hour or two after regaining consciousness. Thus, recordings were only made after it became obvious that the animal was behaving normally, i.e. grooming, feeding, and briskly investigating its box environment. Sleep states were defined according to the protocol set out in a recent comprehensive review of sleep states as defined by EEG patterns (Rechtschaffen and Kales 1976). Most recordings were

carried out during focal EEG activity resembling stage 3 or 4 sleep, during which delta waves predominate and spindle waves are rare or absent. Sleep episodes could last for 20 to 30 min.

This group of animals was later anaesthetised with urethane after observations during sleep had been made in order to compare spontaneous activity of the same neurones in the two states.

Histology

All thalamic penetrations were reconstructed histologically. The bottom of the thalamic tracks were marked by iontophoresis of procion blue dye (1000 nA, 10 min), having first made a small focal lesion (2.5 μA tip negative, 10 s) and/or by cutting off the carbon fibre microelectrode in situ. The brain was then fixed by perfusion with 10% paraformaldehyde in saline. Fifty to 75 μm sections were then cut on a freezing microtome and stained with nuclear fast red.

Iontophoresis

Three-barreled carbon fibre microelectrodes were used for iontophoresis (Armstrong-James et al. 1980). 2APV and NMDA (Cambridge Biological Research Chemicals) were both dissolved in distilled water to a concentration of 50 mM. The pH was then adjusted to between 7.2 and 7.5 for both solutions. A Neurophore BH2 (Medical Systems Corp.) was used to provide ejecting and retaining currents for the iontophoretic barrels.

In these experiments, the ejecting current was varied continuously downwards during the period of the iontophoretic trial in a manner found previously to maintain a steady concentration of drug at the electrode tip (Armstrong-James and Fox 1983) hence maintaining the firing rate of the cell under observation constant.

Electrical microstimulation

Stimulus pulses were produced by a Digitimer D100 and fed into a Neurolog NL510 pulse buffer feeding into a Neurolog NL800 stimulus isolation unit. Stimuli were delivered through multibarrelled or single-barrelled carbon fibre microelectrodes. Stimulus parameters and intensities employed are described in Results.

Data analysis

Two or three recording electrodes were used in these experiments. For one of the two recording channels wideband filters were employed (0.1 Hz–10 kHz). The focal EEG on this channel was derived by bandpassing from 0.1 to 30 Hz. Spike recordings on the same and remaining channels were derived by bandpassing at 800 Hz to 8 kHz. Surface EEG recordings were made using a 0.2 mm chlorided silver ball electrode in contact with the pia. Band-passing at 0.1–35 Hz (2nd order Butterworth filter) was used.

Most analysis was made off-line from raw data recorded on a Racal store 4 tape-recorder and analysed using a Biomac 1000 (Data Laboratories Ltd.) with suitable conditioning of input data.

When required, single action potentials were discriminated by their amplitude and timecourse and displayed on a digital oscilloscope to ensure that only a single cell was studied at one time. Interval histogram analysis was carried out for confirmation.

The nature of spontaneous multi-unit activity during urethane anaesthesia and some periods of slow wave sleep consists of coherent bursts of action potentials, synchronised for all active cells at the recording site (Armstrong-James and Fox 1983). For temporal analysis these compound bursts were considered as single events. Briefly, where such bursts were recorded, the largest three cells at a locus were discriminated and used to activate a retriggerable monostable with a 150 ms retrigger interval. If a period of 150 ms or more occurred between the main spikes at a locus, then such periods constituted interburst intervals. Burst duration and interburst duration and interval histograms for single cells were constructed as described previously (Armstrong-James and Fox 1983). The difference between bursts of unitary activity and spindle cluster unitary activity is given in the Results section.

Temporal cross-correlation between bursts was performed by executing latency histogram analyses between the relative onset times of the bursts at the two recording sites. Bursts which tended to start synchronously produced a peak in the burst onset latency histogram (BOLH) distribution at zero milliseconds. An additional refinement to the BOLH technique was to subtract the BOLH for synthesised asynchronous trains of bursts from the experimentally obtained BOLH. One burst train was supplied by the cortical bursts under investigation, whilst burst onset pulses representing a synthesised burst train were produced by a pulse generator. The pulse generator was set to the same interpulse interval as the intralaminar sites' mean burst repetition interval. Since the two inputs were asynchronous, the BOLH represented that expected from two unrelated sites producing bursts at similar rates. The same number of bursts was used to construct the 'random' control BOLH as the histogram from the real experimental data; hence by subtracting the control from the experimental BOLH, it was possible to see which latencies occurred at a greater than random probability and which at less.

This method increased the variance in the individual bin heights but was nevertheless found to be useful. Positive values represent burst events occurring at greater than chance probabilities, negative values those at less than chance probabilities.

Results

Activity of cortical neurones

Single and multiunit neuronal activity was recorded from 608 cortical sites in 101 rats. Sites were located mainly in somatosensory cortex (Sm1), but when necessary other cortical areas were sampled as indicated in the text. Although the cells were sampled from all cortical layers, principle findings relate to layer V cell locations which usually exhibit profound bursting activity under urethane anaesthesia (Armstrong-James and Fox 1983).

Spontaneous neuronal activity recorded within the cortex during natural slow wave sleep and under urethane anaesthesia could be classified broadly into two main types; spindle cluster activity, as described by Andersen et al. (1967) in the thalamus for barbiturate anaesthetised cats, and a quite different burst-pause pattern of activity. Examples of these are shown in Fig. 1. The lower frequency burst-pause pattern was the dominant type of activity in the urethane anaesthetised animals. Urethane differs in this respect from some other anaesthetics, such as sodium pentobarbitol, which promotes spindle wave

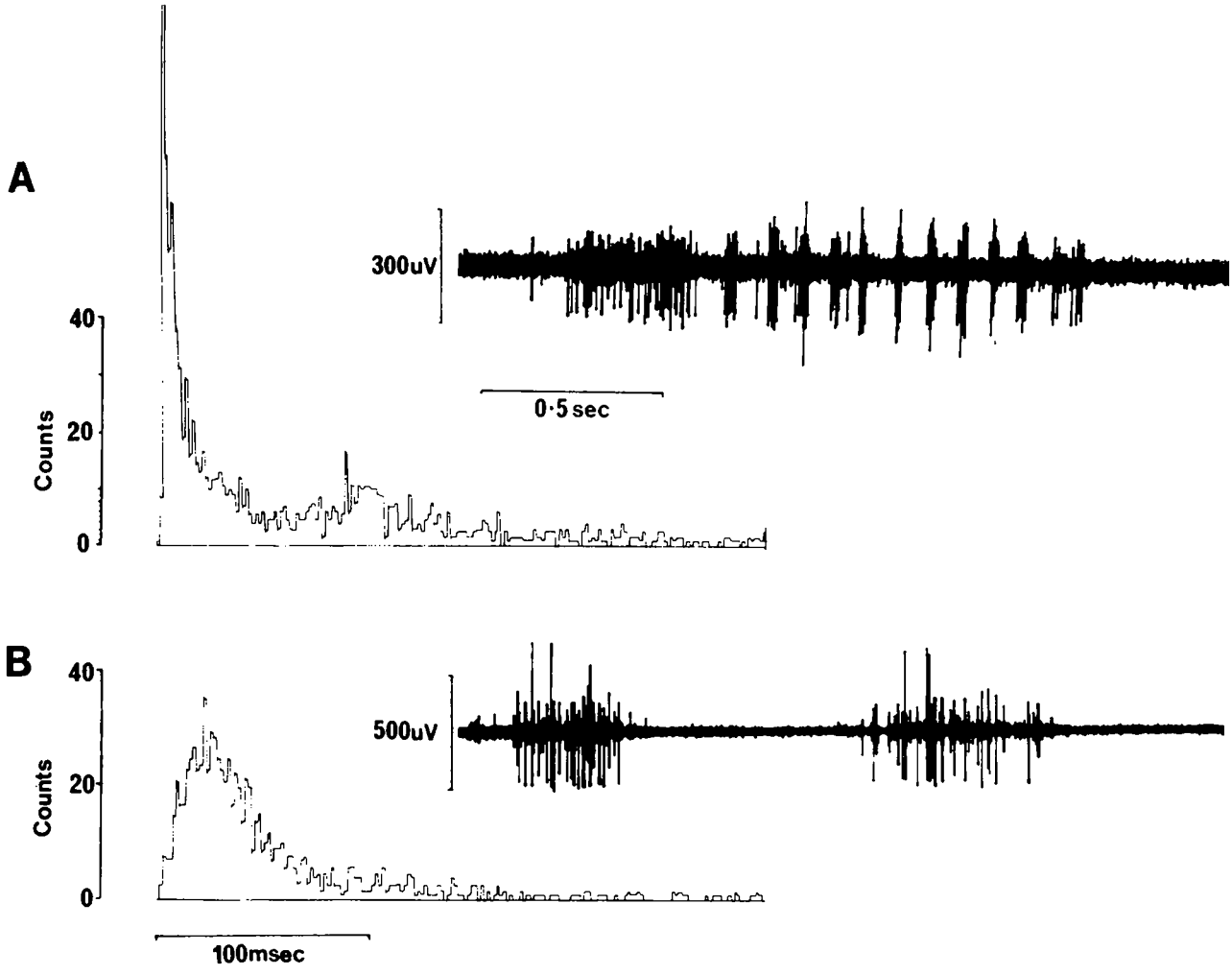

Fig. 1. A Interval histogram (IH) (left), and unitary activity (right) during mixed bursting and spindle cluster activity recorded at 800 μm depth in Sml neocortex; smaller unit discriminated to produce IH (see Methods section). The cortical burst is followed by twelve distinct minibursts which compose the spindle, the latter dominating the IH distribution. Modal intervals occur at approximately 4 ms (representing a 250 Hz firing rate within clusters) and 90–115 ms (representing a 11–8.7 Hz cluster repetition rate). **B** IH for same unit during pure cortical burst activity (spindling is absent). The IH is dominated by intraburst intervals: the mode of 18 ms represents a firing frequency of 55 Hz. Interburst intervals (circa 600 ms) are beyond the histogram interval scale

activity in the cortex and spindle cluster unit activity in the thalamus (Andersen et al. 1967).

Spindle clusters

From Fig. 1 it can be seen that single unit spindle cluster activity characteristically shows a bimodal interval distribution. The longer modal period represents the interval between the clusters of spikes whilst the shorter modal interval represents the interspike intervals within the clusters. Cells fired at a lower maximal frequency during bursts than during the spindle clusters, as shown by the lower histogram of Fig. 1. The spike clusters ranged from singleton spikes to groups of spikes up to 30 ms in duration, each cluster being rhythmically repeated at spindle wave frequencies (8–14 Hz). In contrast bursts were repeated at irregular intervals (about 0.5 to 4 per second), being associated with slow waves at the delta wave frequency (see Fig. 2). The spikes composing the spindle clusters occurred at very short (5 ms) intervals and occurred with high amplitude waves which appeared entirely similar to classical EEG spindle waves (see Fig. 2)

Cross-correlation of the times of onset of spindle clusters was tested between pairs of homolateral or bilateral cortical sites exhibiting spontaneous spindle cluster activity. However, spindle clusters onset times were found to be unrelated within a 200 ms time aperture (2.56 ms/bin).

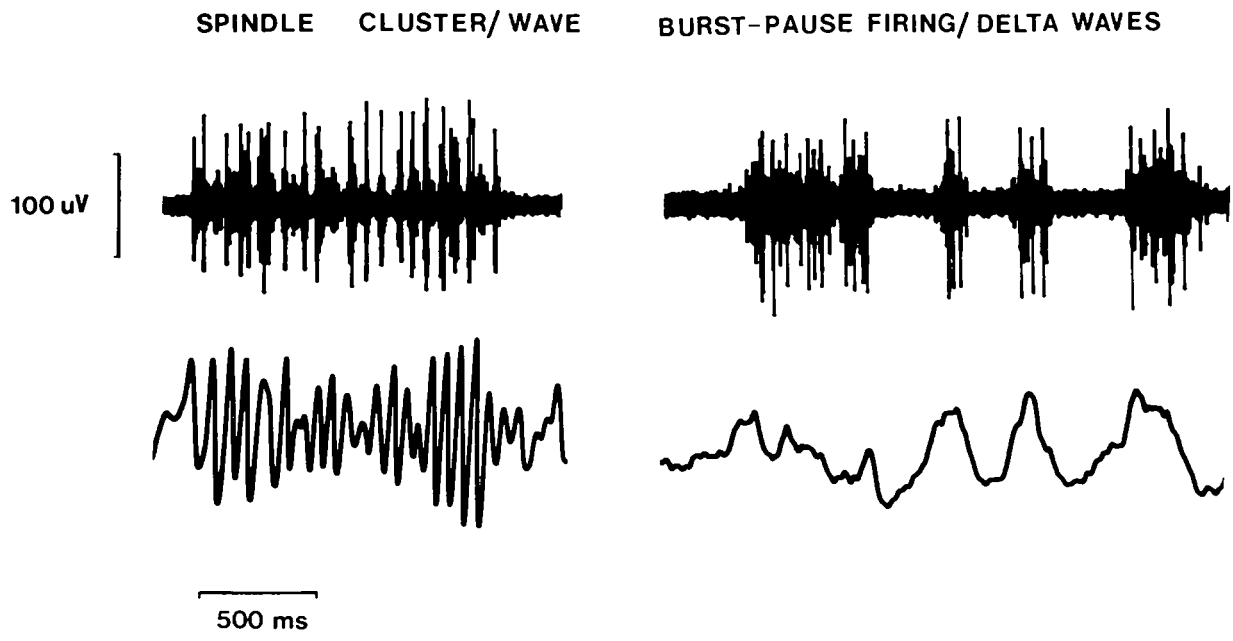

Fig. 2. Left: Spontaneous spindle cluster unitary activity (upper trace), recorded at 720 μm depth in forepaw zone of Sml neocortex in association with spindle waves (lower trace) recorded focally at the electrode site. Right: Unitary burst-pause activity (upper trace), recorded at 1050 μm depth in hindlimb zone of Sml neocortex in association with slow waves at delta wave frequency (lower trace) recorded from the pial surface close to the electrode penetration site

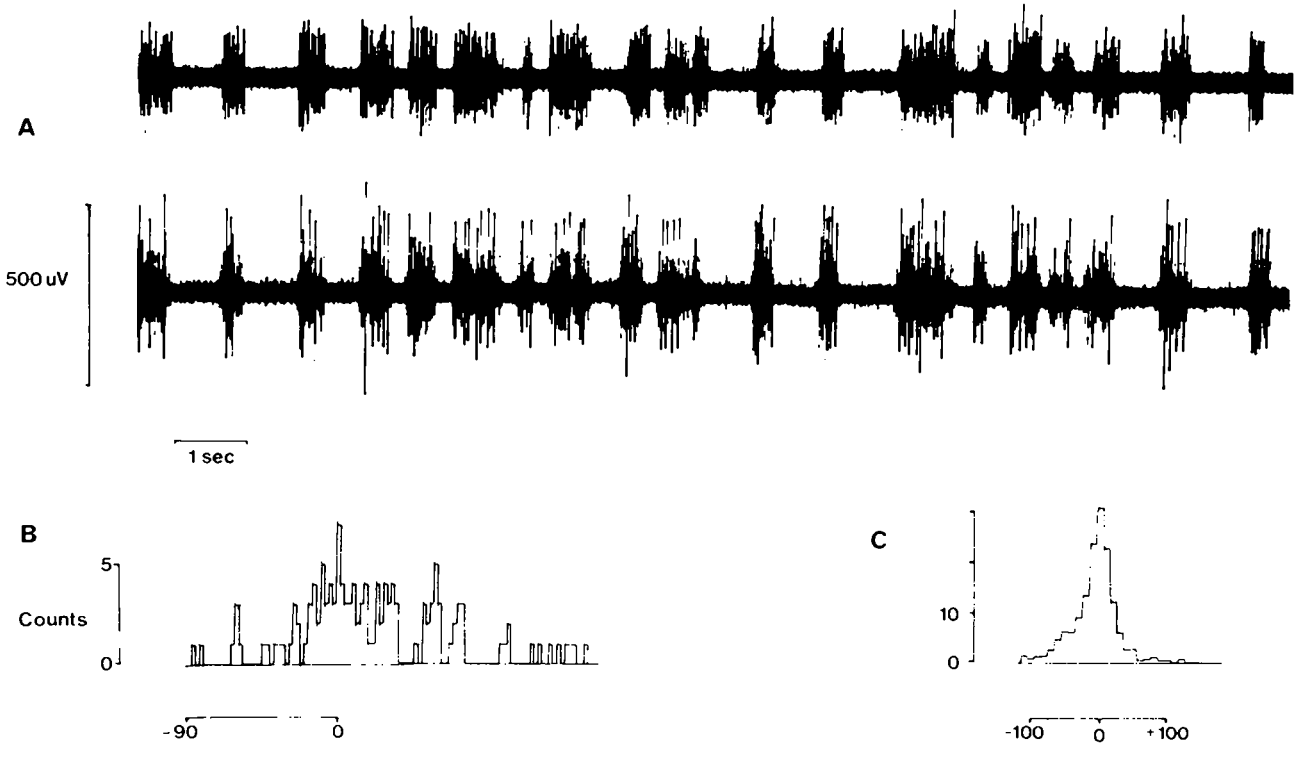

Fig. 3A–C. Urethane anaesthetised rat. **A** Unitary activity simultaneously recorded at paired homolateral recording sites, each at 1000 μm depth (layer V). Lateral separation of microelectrodes was about 2 mm. Modal burst repetition rate was 1.2 per second. **B** Histograms constructed for cross-correlation of burst onset latencies (BOLH) at the two sites. Time zero (ordinate) represents coincident onset of bursts. Ordinate scale is in milliseconds, bin width 2.56 ms. **C** BOLH as for **B** constructed using a bin width of 10.24 ms

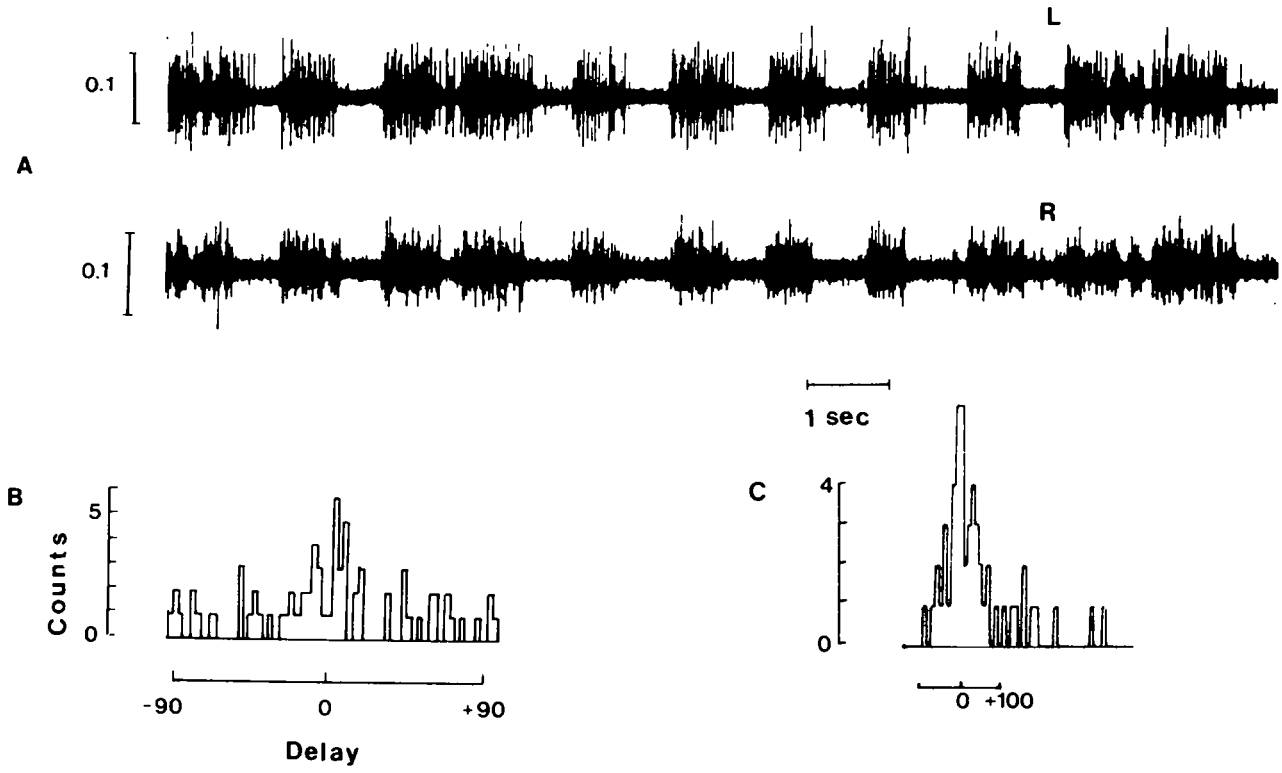

Fig. 4A–C. Unanaesthetised rat. **A** Simultaneously recorded multi-unit bursts at a depth of 950 μm in left (L) Sml neocortex, and 980 μm in right (R) Sml neocortex during natural stage 3/4 slow wave sleep. Vertical bar 100 μV. Modal burst rate was 0.92 per second. **B, C** BOLHs constructed for cross-correlation of burst onset times (see Fig. 3 for details). Bin width in **B** 2.56 ms, in **C** 10.24 ms

Bursts

During natural sleep a distinctive burst-pause pattern of firing prevailed in deep cortical locations when the focal EEG pattern was dominated by low frequency slow waves in the delta wave range. Under these conditions spindle waves occurred infrequently indicating that the naturally sleeping animal was exhibiting stage 3/4 SWS (Rechtschaffen and Kales 1976). Entirely similar focal EEGs occurred in the urethane anaesthetized animals when a burst-pause pattern of firing occurred suggesting that urethane anaesthesia produces an analogous cortical condition to stages 3/4 sleep under these circumstances (Fig. 2).

Correlation of onset times of bursts at paired microelectrode sites was evaluated by constructing burst onset latency histograms (BOLHs; see Methods). During delta-wave SWS and urethane anaesthesia bursts were found to start at the same time bilaterally and homolaterally. Figures 3 and 4 show typical results from such experiments.

In these experiments, the widest separation of recording sites in the same hemisphere was 7 mm (frontal and Sml cortical locations) and high correlations of simultaneous onset of bursts at the two sites were observed. A similar degree of onset correlation was also always present between paired sites in opposite hemispheres (see Fig. 4).

It is important to note that only the *onset* times of bursts correlated well. There was no time invariant relationship between the firing of *individual* cells within a burst. Neither were the ends of the bursts highly correlated between two displaced sites, unless such sites were exceptionally close to one another and bursts were of very similar duration. BOLHs which were computed for bursts at the same depth, but at horizontally displaced sites, showed latencies evenly distributed around the mode of 0 ms (Figs. 3 and 4). Hence cells at either site were equally likely to fire a burst first. The interquartile ranges of about 40 ms therefore imply that 50% of the bursts started within 20 ms of one another.

Thalamic neurone sampling

Thalamic spike recordings were largely confined to the ventro posteriolateral nucleus (VPl) and the anterior intralaminar nuclei (aIL), comprising centralis lateralis, paracentralis, and centralis medialis.

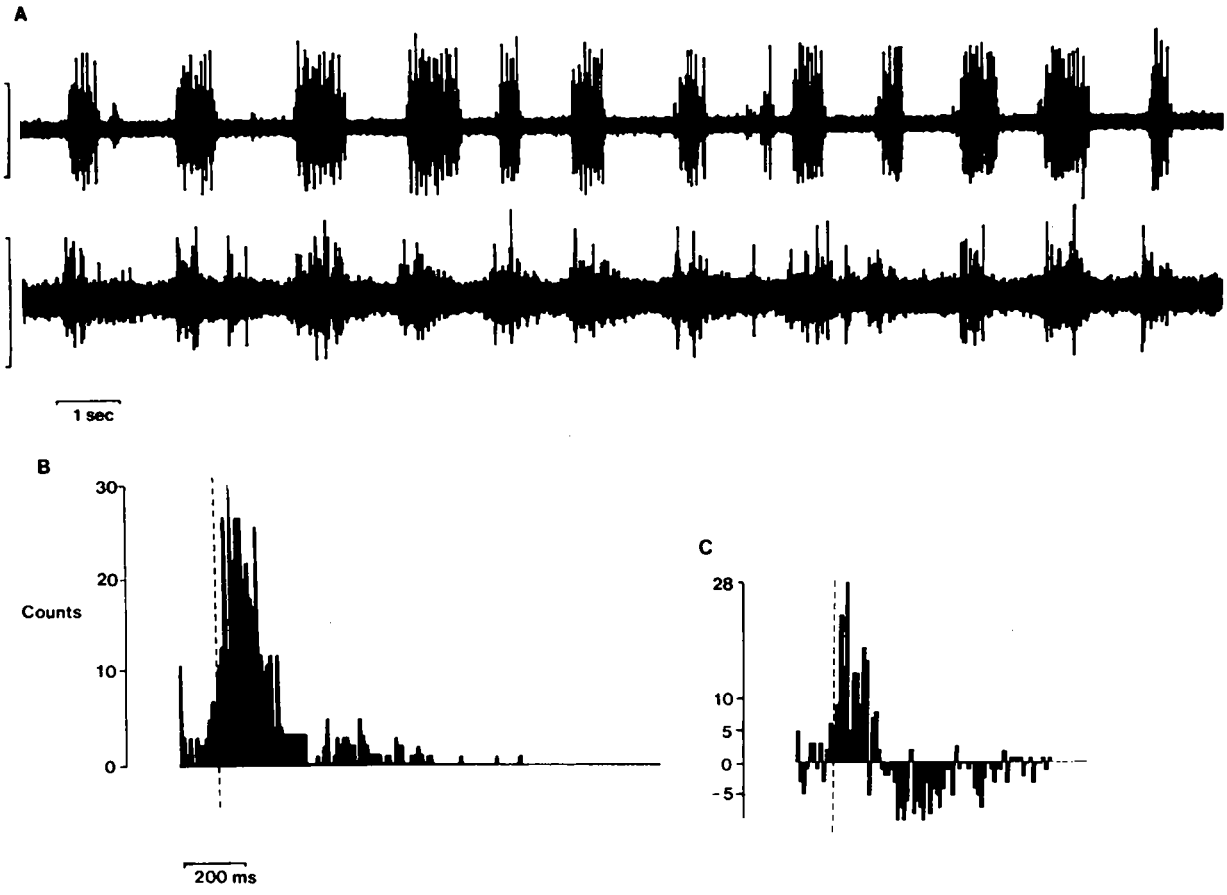

Fig. 5A–C. Urethane anaesthetised rat. **A** Simultaneous recordings of spontaneous activity from a pair of microelectrodes, one situated in Sm1 neocortex (upper trace) at 1150 μm depth, the other within the ipsilateral n. paracentralis (Pc), (lower-trace), vertical bars 100 μV. Note the one-to-one burst relationship between bursts at the two sites. **B** Cross-correlation of onset time of cortical burst with respect to burst onsets in Pc. Dotted line indicates burst onset time at Pc locus. Note cortical bursts start after Pc bursts. **C** As for **B** after subtracting identical numbers of randomly cross-correlated bursts. See Methods for analytical techniques

All sites were histologically identified after the experiment (see Methods). The electrode was advanced 200 to 300 μm between sample sites without regard to the spontaneous activity encountered. This often led to multi-unit recording locations, which in this particular study was desirable. This protocol reduced the possibility that any one cell was included twice by being within recording range at two locations. With online monitoring of action potential shapes it was our experience that very few cells could be tracked for distances greater than 100 μm. Furthermore, sampling was evenly distributed throughout the nucleus without bias for any particular subsection of it. A number of cells was not clearly located in a nucleus, and where this was the case the cell was classified as borderline. Although these cells represent valid data, they were excluded from the statistical tests used to compare spontaneous activity in different nuclei.

Spontaneous activity of thalamic neurones

Neurones located within the aIL nuclei tended to fire spontaneously in a burst pause pattern when the EEG pattern exhibited high voltage low frequency (HVLF) waves in the delta wave range. Their burst repetition rate of 1–1.2 bursts per second was entirely similar to the cortical units burst repetition rate. The aIL bursts were, in general, less coherent than those recorded in the cortex (see Fig. 5). When recordings were made from two aIL sites simultaneously however, the individual bursts appeared to sum to a single more coherent burst.

On eight occasions recordings were made from pairs of aIL neurons located on opposite sides of the midline. Median values for spontaneous burst onset latencies at paired aIL sites ranged from 0–40 ms. Interquartile ranges were within 100 ms on six occasions and 150 ms on two occasions indicating high

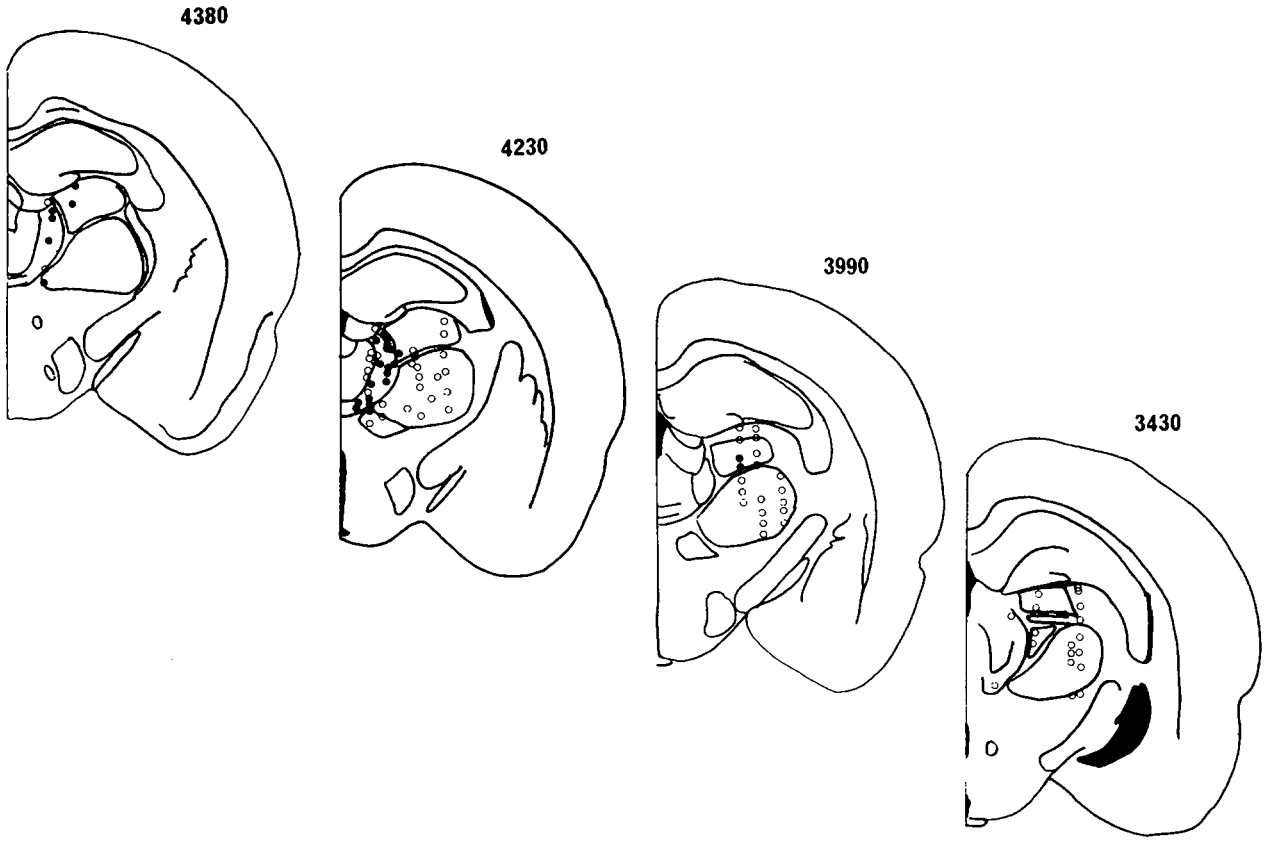

Fig. 6. Filled circles show distribution of histologically identified cells in the thalamus which consistently produced bursts whose onset time just preceded onset times of cortical bursts (results pooled from 11 rats). Such cells are clustered within the anterior intralaminar nuclei. Unfilled circles represent all other cells showing no such relationship during cortical bursting activity. Eleven cells firing bursts preceding the cortical bursts which were located in aIL have been omitted for clarity. The atlas of Konig and Klippel was used for this illustration

temporal cross correlation. These quantitative results lend support to the observation that bursts at different aIL sites appear to be components of a coordinated discharge of the whole aIL nucleus.

Figure 5B shows the BOLH for a cell located in aIL and an ipsilateral Sm1 site at a depth of 1150 μm. There is clearly a far greater probability of the aIL cell spontaneously starting a burst prior to the cortical burst onset than after it. In this case the cortical burst onset modal latency was 102 ms. The relationship between the two sites can be seen more clearly when the probability of one site firing randomly is subtracted, as shown in Fig. 5C, (see Methods). There is a reduced probability (indicated by negative values) of IL firing 400–600 ms before the cortex corresponding to the silent interburst period before the aIL neuron fires a burst.

To give some measure of the tendency for aIL cells to fire before the cortex the lower inter quartile was calculated for each burst onset latency histogram. In 21 of the 23 cases tested the lower interquartile was situated beyond the aIL burst onset indicating more than 75% of cortical bursts occurred after the start of the recorded aIL burst. Of the remaining two cases, which were located in a single electrode penetration, the cortex fired after one aIL site 63% and the other 60% of the time.

The interquartile ranges for the aIL to cortex burst onset latency histograms lay between 100 and 250 ms for the population of cells studied. This is a far greater spread of values than for latencies between burst onsets at paired cortical sites where interquartile ranges were about 40 ms. This suggests that if the aIL group is involved in triggering the cortical bursts then there must be an element of cooperation between many aIL neurons such that their summated output determines the cortical burst onset. This notion is supported by the results of experiments described below.

There was a greater probability of finding a cell exhibiting a burst pause firing pattern in the aIL nuclei than in any other thalamic nucleus in our sample, ($\chi^2 = 28.77$, df = 1, N = 117, $p < 10^{-4}$). This value is highly significant, but since most of the

Table 1. Summary of cross correlations between cortex and various thalamic nuclei. POST; thalamic cell fires a burst of spikes starting after the cortical burst. PRE; thalamic cell fires a burst before cortical burst. UNCORRELATED; thalamic cell shows no temporal relationship with cortical bursts. MD; Medio-dorsal n., LD; Lateralis Dorsalis n., VPl; Ventro-posteriolateral n., aIL; anterior Intralaminar n.

Nucleus	No. in sample	Uncorrelated	Correlated	
			Post	Pre
VPl	48	39	6	3
aIL	44	1	2	41
LD	13	2	6	5
MD	3	0	3	0
Others	9	9	0	0
Borderline	14	5	5	4
Total	131	56	22	53

samples outside aIL were located in VPl it is partly a reflection of the tendency for VPl not to fire in bursts. Recordings from VPl largely confirmed the findings of other workers (Andersen et al. 1967; Baker 1971) that cells were either silent, tonically active, or firing neuronal spindle clusters. A specific analysis was made of the distribution of spontaneously bursting cells which were found to commence firing before cortical burst onsets. Such cells were found to be almost exclusively located in the aIL nuclei as can be seen from Fig. 6. The numerical description accompanying this data is to be found in Table 1.

Evoked activity

The sites chosen for stimulation were those which showed a high burst correlation with the cortex and fired before the cortex. All stimulation sites chosen in this way proved to be located in aIL. The method did not seem to bias the sites in favour of any particular part of the aIL group.

It was found empirically that a train of four 1 ms current pulses with an interpulse interval of 10 ms was sufficient to evoke responses bilaterally in the cortex, shorter pulses of 100 μs being totally ineffective. During stimulation, recordings were usually made from two electrodes located bilaterally in the deeper layers of Sml or Ms1 cortex. The thresholds for these responses were between 40 and 50 μA, though contralateral sites occasionally required pulses of up to 70 μA. The threshold was defined as that pulse current which evoked a response to 50% of the stimuli. The stimulus strength then used in the subsequent trials was 1.5 times greater than threshold. This resulted in current pulses falling within the range 60 to 75 μA for all ipsilateral sites.

Figure 7 shows the responses of two cortical sites located bilaterally in Sml to stimulation of the aIL group. The same stimulation site was found to evoke responses at several locations within aIL when the contralateral of the two electrodes was later advanced into aIL. Threshold activation of aIL cells by stimulation of the opposite aIL only required current stimuli of two pulses rather than the four pulses needed for cortical activation. Thus the threshold for activation of one aIL nucleus by stimulation of the other was effectively less than for activation of cortical bursts by stimulation of the same aIL site. Response latencies for aIL activation of units in the opposite aIL site fell within the range 10 to 40 ms (n = 8).

Contralateral and ipsilateral cortical sites responded to aIL stimulation by producing bursts of action potentials similar in form to the bursts that occurred spontaneously at these sites. The burst durations were almost identical during stimulation and spontaneous activity. Optimal driving of cortical

Fig. 7. Urethane anaesthetised rat. Multi-unit burst responses recorded bilaterally in neocortex in response to stimuli (triangles) delivered through a carbon fibre microelectrode; (4 pulses, 70 μA (1.5 T), 30 ms train). The stimulation rate was 2/s, and modal burst rate in the absence of stimulation was 1.25 per second. Note consistently longer latency at the contralateral site (CON)

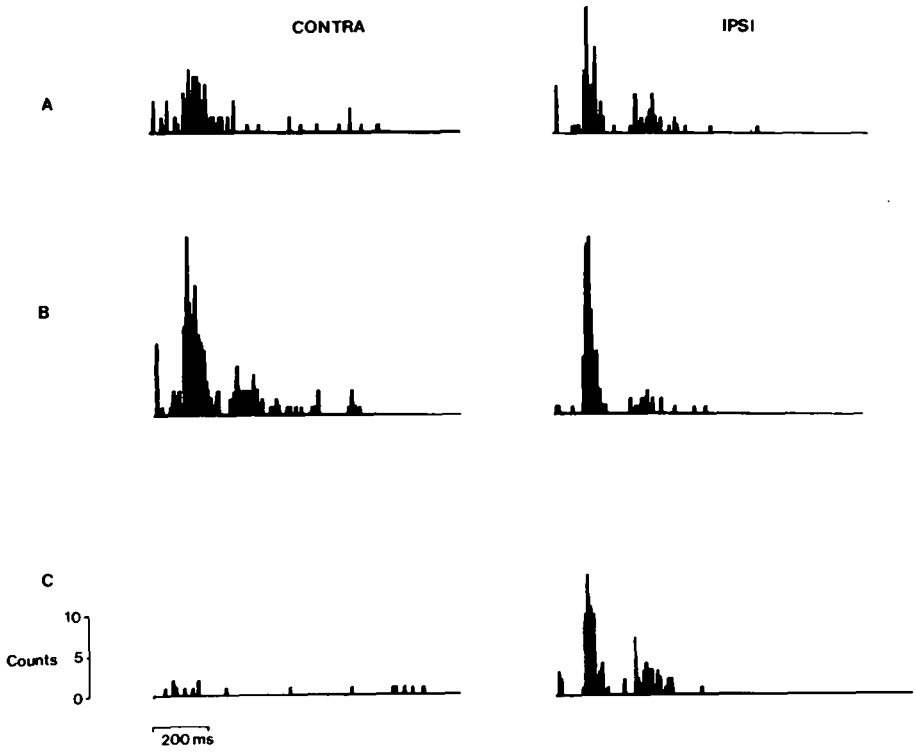

Fig. 8A–C. Burst onset latency histograms for a pair of single units, simultaneously recorded from opposite Sm1 neocortical sites, in response to 100 stimuli to aIL nucleus. Left column; contralateral to aIL site, unit at 950 μm depth, Right column; ipsilateral to aIL site, unit at 1025 μm. **A** Control recordings. **B** During ionophoresis of 4 nA of NMDA onto unit at contralateral site. **C** With ionophoresis of 10 nA of 2APV onto unit at contralateral site. No drugs were ejected onto ipsilateral unit. Note potentiation by NMDA and elimination by 2APV of evoked bursts at the contralateral site. NMDA ionophoresis also reduced modal onset latency at the contralateral site

bursts was achieved by aIL stimulation at rates of between 0.8 to 2 per second. Pilot experiments suggested that the exact following frequency was dependent on the spontaneous burst rate, and thereby the anaesthetic level. While stimulation was able to reschedule bursts it was not always possible to completely overide the inherent natural rhythm of burst generation when using just suprathreshold stimulation.

Response latencies were extremely consistent in trials for any given pair of aIL and cortical sites. Ipsilateral modal latencies ranged between 36 and 72 ms for various sites. Contralateral cortical responses were consistently 10–40 ms later than ipsilateral repsonses evoked from the same aIL site. In addition the responses were poorer in that they occasionally failed to fire a burst, (although ionophoresis of NMDA greatly improved the occurrence of contralateral burst responses and reduced their latencies as described in the following section).

Electrical stimulation of VPl was studied for comparison with responses in reply to aIL stimulation. The VPl responses were of much shorter latency than those evoked from aIL and produced a short volley of spikes rather than the long bursts caused by aIL stimulation. Cortical neurones could follow much higher stimulus repetition rates to VPl which in common with cutaneous stimulation tended to completely abolish the burst-pause firing pattern at the restricted cortical innervation site. Such stimulation was ineffective on activity at control recording sites outside ipsilateral Sm1 cortex.

Ionophoresis experiments

A previous study has implicated NMDA/2APV sensitive receptors in the generation of the burst-pause pattern of cortical neurones (Armstrong-James et al. 1985). The results described in the present study suggest a role for the aIL nuclei in spontaneous burst generation and so we attempted to ascertain whether the aIL neurones mediate their effect on the cortex via NMDA/2APV sensitive receptors. This was tested by electrically stimulating aIL and testing the effect of ionophoresing 2APV or NMDA onto the responding cortical neurones.

The reliability of the response of cortical neurons to aIL stimulation was increased by ionophoresis of NMDA at both ipsilateral and contralateral sites. 2APV however completely abolished the cortical response to aIL stimulation. Both effects are shown in Fig. 8. The ipsilateral site serves as a control allowing monitoring of the stimulus efficacy throughout the experiment. As shown in Fig. 8 the stimulus efficacy remains fairly constant by comparison with the changes caused by the drugs at the contralateral site as was the case in all such experiments. Recovery to control values occurred in all ionophoretic tests,

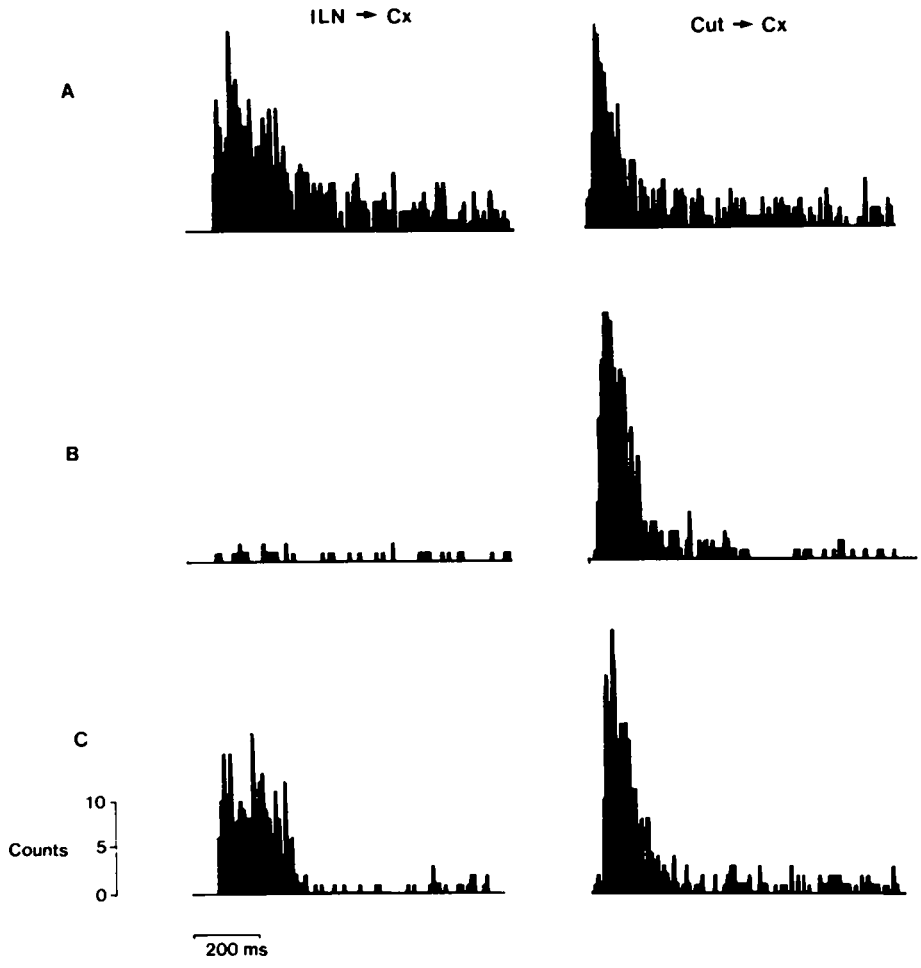

Fig. 9A–C. Left column: PSTHs constructed for a single neocortical cell recorded at 920 μm depth (layer V) in response to aIL microstimulation (1.5 × threshold 70 μA pulses). Right column: PSTHs from the same cell in response to controlled 1.5 × T tactile stimulation of centre receptive field (2 ms rise time, 10 ms duration electromechanical stimulus, 1 mm^2 200 μm depression). Stimulation to aIL and skin were alternated. **A** Control responses. **B** During 2APV ionophoresis (10 nA). **C** Recovery 15 min after end of 2APV trial. Note the lack of effect of 2APV on short latency cutaneous response during elimination of aIL evoked response

usually within 8 min for 2APV and much sooner for NMDA. The recovery to control values of aIL evoked responses always occurred simultaneously with the recovery of the spontaneous bursting activity to control values, indicating a common effect on spontaneous and driven bursts.

Apart from simply improving the reliability of cortical burst response to aIL stimulation, NMDA also reduced the modal response latency by 20–40 ms (n = 11). The responses at the control site were completely unaffected in these cases implying that a local cortical factor was involved in the latency shift. It was not usually possible to measure the latency of the response to aIL stimulation during 2APV ionophoresis because the responses were practically abolished. However in one case where 2APV only partly reduced the response a latency increase of 25 ms occurred. Since NMDA decreased the latency in this particular cell by 40 ms the total range of latency modification was 65 ms.

Inhibition produced by 2APV was not a nonspecific effect due, for instance, to hyperpolarisation of the cell membrane since short latency responses to stimulation of centre cutaneous receptive fields remained constant throughout trials. Figure 9 shows an example of such an experiment. The cutaneous stimulation sequences were alternated with the periods of aIL stimulation. Presumably the short latency cutaneous response is mediated via a different population of receptors to those mediating bursts; different either by being pharmacologically different or located at a site remote from the receptors mediating the burst activity. Electrical stimulation of VPl, classically responsible for transmission of short latency sensory information to the cerebral cortex, produced brief volleys of spikes that were also unaffected by 2APV ionophoresis.

Discussion

This study has been concerned with an investigation into the burst-pause pattern of firing of cortical neurones, which predominates in urethane anaes-

thesia (Armstrong-James and Fox 1983) and slow wave sleep (SWS), (Hubel 1959; Evarts 1964; Noda and Adey 1971; Calvet et al. 1973; Armstrong-James and Fox 1984). A clear distinction is made between this type of neuronal activity and that associated with spindle waves in the EEG (spindle clusters) as previously indicated by Creutzfeldt and Jung (1961). Bursts were found to coincide at widely displaced cortical sites unilaterally and bilaterally in SWS and urethane anaesthesia. The most likely event was that the onset time of bursts was coincident, regardless of the horizontal distance of separation of electrodes within the neocortex. Although widespread bilateral synchronisation of bursts has not previously been reported by other workers, correlated firing of units recorded from widely displaced cortical sites has been demonstrated for the urethane anaesthetised rat (Holmes and Houchin 1966), and for closely spaced single units in cat cortex during slow wave sleep without spindles (Noda and Adey 1971). In both of these studies cross-correlation analysis showed periodicities at about one per second, which is appropriate for the burst-pause periodicities found in the present and the other studies quoted above.

The findings presented here in the rat support the hypothesis that the anterior regions of the intralaminar nuclei (aIL), (centralis lateralis, paracentralis and centralis medialis), are involved in initiating cortical bursts during urethane anaesthesia and by implication the bursts during delta wave SWS. The suggestion that the IL nuclei are the ultimate relay for midbrain pathways initiating the waking state (Moruzzi 1963) is not necessarily incompatible with this view. A change from a bursting pattern of firing to tonic firing has been shown for cat intralaminar neurones on transition from sleep to wake (Glenn and Steriade 1982). In the cat, repetitive stimulation of the IL nuclei can induce delta wave SWS although with an increase in stimulus intensity arousal occurs (Akert et al. 1952).

There is strong evidence that the aIL nuclei are suitable anatomical substrates for widespread bilaterally synchronous activation of cortical cells. An early study showed that complete degeneration of the IL group occurred following decortication, (Walker 1938) although restricted lesions gave little or no degeneration. This implies a diffuse widespread overlapping innervation from the IL region. More recent retrograde axonal transport studies, using horseradish peroxidase or fluorescent dyes injected into various cortical regions, has confirmed a widespread direct cortical innervation from the aIL group in the cat (Macchi et al. 1977; Bentivoglio et al. 1983) and the rat (Jones and Leavitt 1974; Herkenham 1980). The latter study showed that aIL projects to all cortical areas and most heavily to layers V and VI where bursting neurones predominate (Armstrong-James and Fox 1983). All other thalamic nuclei project to rather restricted cortical areas (Jones 1981) with the exception of the ventromedial nucleus which projects extensively to layer I (Herkenham 1980).

All of the above projections to cortex, including those of the intralaminar nuclei have been shown to be unilateral. Additionally the classical histological study of the Scheibels (Scheibel and Scheibel 1967) on the intralaminar nuclei illustrates the complexity of the axonal involvements of these nuclei. A remarkable feature is the extensive bilateral coupling between the aIL nuclei across the midline, suggesting copious opportunity for mutual bilateral synaptic involvements. Such an arrangement would facilitate spontaneous bilateral synchronisation of bursting activity in opposing aIL nuclei, and explains the low threshold initiation of bursts in one nucleus by stimulation of its contralateral counterpart. The anatomical arrangement therefore provides a structural basis for a single coherent midline pacemaker for initiating bilateral synchronous bursting activity in the cortex. It is not necessary to postulate that callosal connections are involved in the contralateral cortical response to electrical microstimulation of the aIL nuclei, although their participation cannot at present be ruled out. However, responses to contralateral aIL stimulation recorded in the hindpaw zone where callosal innervation is absent, (Wise and Jones 1976) and the tail receptive field area of cortex, where callosal connections exist, (Manzoni et al. 1980) were not found to be quantitatively different. In addition contralateral aIL sites were activated from an ipsilateral aIL site using two-pulse stimulation without evoking a cortical response, and at a shorter latency than for ipsilateral or contralateral cortical responses to four-pulse stimulation. Therefore callosal connections cannot be involved in driving units in the contralateral aIL nuclei.

The relationship between our findings and those on the well known cortical recruiting waves to non-specific thalamic stimulation is not clear. In the original study of Morison and Dempsey (1942) the authors observed "a similarity of distribution of intensity of response to that of one of the characteristic elements of the spontaneous EEG", apparently referring to waves in the 8–12 Hz range. The latter may fall into the class of either spindles or the alpha-rhythm, but not the much lower frequency waves associated with cortical bursts (Calvet et al. 1973; Creutzfeldt and Houchin 1974). Most studies on recruiting waves have been carried out on encephalé isolé preparations or under barbiturate anaesthesia, where spindle waves are the most prevalent compo-

nents of the EEG. In no case was spindle cluster activity evoked by stimulation of the aIL nuclei in this study. The difference between our study and those referred to above would seem to lie in the fact that our results relate to anaesthetic conditions in which a delta wave EEG similar to stage 3/4 sleep was present and spindle waves were infrequent.

Recently an involvement of the excitatory amino acid receptors of the NMDA type in producing trains of action potentials from neocortical cells in cortical slice preparations and in vivo has been demonstrated (Flatman et al. 1983; Armstrong-James Caan and Fox 1985). In other studies in the C.N.S. 2-amino 5-phosphono valeric acid (2APV) has been established as a specific antagonist of NMDA receptors (Watkins and Evans 1981). The novel finding in the present study that ionophoresed 2APV simultaneously abolished, and NMDA simultaneously promoted *both* spontaneous and aIL driven cortical bursts is taken as confirmatory evidence of the fundamental involvement of the aIL nuclei in spontaneous cortical burst generation. In addition the present study showed that different receptors were activated on the same bursting cortical cells by the main somatosensory pathway. 2APV ionophoresis, at levels which abolished spontaneous and aIL driven burst activity, left the short latency activity evoked by stimulation of VPl and cutaneous stimulation unaffected. These results suggest that the specific cutaneous input to Sml does not act via 2APV sensitive receptors.

During stimulation of the aIL nuclei, ionophoresis of NMDA onto cortical cells caused a 20–40 ms reduction in the latency of aIL evoked cortical bursts. In contrast it was found that submaximal ionophoresis of 2APV caused an increase in latency to the same stimulus of about 25 ms, thereby yielding a total latency modification of some 65 ms. Such effects are presumably solely attributable to post-synaptic action on the cortical cells, since the aIL-cortex pathway could only have been modified at the cortical terminal sites. A presynaptic action is unlikely in view of the well documented post-synaptic effects of NMDA (Watkins and Evans 1981). Since effective stimulation of aIL required a train of stimuli to drive cortical bursts these findings suggest that ionophoresed NMDA or 2APV respectively amplify or attenuate a relatively slow incremental excitatory effect of the aIL projection onto cortical cells.

The summed EPSP to a 30 ms train of pulses can presumably have a rising phase extending over at least 65 ms, leading to long latency bursts of action potentials in the cell. Layer V cells in neocortical slice preparations exhibit responses to current injection which are delayed in onset by ramping the current (Stafstrom et al. 1984). A slow depolarising action of NMDA on cortical neurones in vitro is also claimed (Flatman et al. 1983). These observations and deductions may help to explain the apparent slow conduction time of the aIL-cortex pathway found in this study, since slowly rising EPSP action would contribute significantly to the latency in the aIL-cortex pathway.

The aIL group was the only stimulation site to evoke widespread 300 ms or so bursts of firing bilaterally in the cortex, suggesting somewhat specialised synaptic mechanisms. Orthograde transport studies demonstrate that the intralaminar nuclei project mainly to layers V and VI (Herkenham 1980). Retrograde transport studies (Ottersen et al. 1983) support this finding since D-(^{3}H) Aspartate was found to primarily label cells in the intralaminar nuclei if injected into deep cortical layers including layers V and VI. The latter additionally implies that the pathway is either aspartergic or glutaminergic. The present study adds physiological evidence in support of this notion since 2APV abolished and NMDA promoted activity in layer V neurones evoked by aIL stimulation.

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