SYMPOSIUM REPORT

Background synaptic activity in rat entorhinal cortical neurones: differential control of transmitter release by presynaptic receptors

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The entorhinal cortex (EC) is a key brain area controlling both hippocampal input and output via neurones in layer II and layer V, respectively. It is also a pivotal area in the generation and propagation of epilepsies involving the temporal lobe. We have previously shown that within the network of the EC, neurones in layer V are subject to powerful synaptic excitation but weak inhibition, whereas the reverse is true in layer II. The deep layers are also highly susceptible to acutely provoked epileptogenesis. Considerable evidence now points to a role of spontaneous background synaptic activity in control of neuronal, and hence network, excitability. In the present article we describe results of studies where we have compared background release of the excitatory transmitter, glutamate, and the inhibitory transmitter, GABA, in the two layers, the role of this background release in the balance of excitability, and its control by presynaptic auto- and heteroreceptors on presynaptic terminals.

(Received 28 September 2004; accepted after revision 20 October 2004; first published online 21 October 2004)
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Neurones in most areas of the brain, particularly in cortical regions, undergo constant bombardment in the form of synaptic potentials mediated by the spontaneous release of transmitter from inputs impinging on their somata and dendrites. This background activity or synaptic 'noise' has at least two components, that driven by action potentials arriving at the terminals (activity dependent) and a second component that is composed of mono-quantal events (miniature release, which is activity independent). In recent years there has been considerable discussion over whether background synaptic noise is deterministic in controlling cortical neurone function. Evidence is beginning to suggest that such activity contributes to a form of stochastic resonance, whereby noise enhances signal detection, can modulate input–output characteristics by gain modulation, and generally determines overall excitability. (e.g. Hausser & Clark, 1997; Pare *et al.* 1998*a*,*b*; Stevens & Zador, 1998; Destexhe & Pare, 1999; Harsch & Robinson, 2000; Ho & Destexhe, 2000; Stacey & Durand, 2000, 2001; Chance *et al.* 2002; Fellous *et al.* 2003; Shu *et al.* 2003; Rudolph *et al.* 2004). There is no doubt that the synaptic background activity is extremely intense in cortical neurones *in vivo* and this generates what has been termed a 'high-conductance' state (Destexhe *et al.* 2003). In reduced preparations such as brain slices maintained *in vitro*, the overall level of spontaneous transmitter release is much lower as a result of deletion of many active synapses by the slicing procedure. Nevertheless, slice preparations can provide mechanistic insights into the role of background synaptic activity, its nature and control. In this article we will discuss differences in synaptic background activity in two subpopulations of neurones in the EC studied *in vitro*, describe possible mechanisms underlying these differences, and speculate on their implications for physiological function and pathological dysfunction.

This article is dedicated to the memory of Eberhard Buhl, a dear friend, a great man and a brilliant scientist. He is sadly missed. It was presented at The Journal of Physiology Symposium in honour of the late Eberhard H. Buhl on Structure/Function Correlates in Neurons and Networks, Leeds, UK, 10 September 2004. It was commissioned by the Editorial Board and reflects the views of the authors.

Network function and dysfunction in the entorhinal cortex

The EC has long been considered to be the gateway to the hippocampus, acting as a dynamic processor

 DOI: 10.1113/jphysiol.2004.076133

of information both entering and leaving the latter structure. The intimate association of hippocampus and EC has been taken to indicate a complementary role of these areas in memory processes (e.g. see Eichenbaum, 2001; Squire *et al.* 2004). The perforant path provides the major source of input to the hippocampus, and it arises primarily from the layer II and layer III neurones of the EC, which receive convergent input from higher order cortices, both directly and via adjacent cortices (perirhinal, parahippocampal). Processed output from the hippocampus is principally directed back to the neocortex from CA1 and subicular projections to neurones in layer V–VI. In addition, these deeper neurones have associative connections with the superficial neurones, and provide an anatomical basis for reverberant activity, which may be involved in reinforcement of stored information. Given this connectivity, both the dynamic balance of inhibitory and excitatory synaptic interactions, and the level of excitability of neurones within the different layers of the EC are likely to be important in determining the processing of (and destination of) information entering and exiting the hippocampus (Jones, 1993; Witter *et al.* 2000).

This rather neat compartmentalization of hippocampal input and outputs in the EC is an oversimplification and has been called into question recently (Seward & Seward, 2003). Nevertheless it is still widely accepted, and knowledge of the properties of the neurones of the deep and superficial layers and their connectivity is fundamental to our understanding of hippocampal–entorhinal interactions.

Dysfunction of the EC has long been implicated in a variety of neurological disorders including schizophrenia, Alzheimer's disease, Parkinson's disease (e.g. see Kovari *et al.* 2003; Prasad *et al.* 2004; Pennanen *et al.* 2004) and, in particular, epilepsy. Epilepsies involving the limbic system and temporal lobe (TLE) are the most prevalent form in man, and there is increasing evidence that the EC may be a major site of seizure initiation. A recent study concluded that focal onset hippocampal seizures remain confined to this structure and are not associated with clinical signs, whereas seizures arising in parahippocampal cortex or amygdala are more likely to propagate and give rise to clinical manifestations (Wennberg *et al.* 2002). Temporal lobe resection to control refractory epilepsy invariably removes entorhinal tissue (Sperling *et al.* 1996) and a successful outcome is positively related to the amount of parahippocampal tissue (including EC) that is resected (Siegel *et al.* 1990). Goldring *et al.* (1992, 1993) suggested that the successful outcome of surgery was dependent on removal of the EC. Seizures may arise independently or preferentially in the EC (Rutecki *et al.* 1989; Lothman *et al.* 1990; Spencer & Spencer, 1994), and electrographic seizure activity induced by kainic acid in rats arises initially in the EC before propagation to the hippocampus (Ben-Ari *et al.* 1981). In both human TLE and animal models there is a characteristic loss of cells in the EC (Du & Schwarz 1992: Du *et al.* 1993, 1995; Kim *et al.* 1990) and its volume is significantly reduced (Bernasconi *et al.* 1999) in human TLE patients.

In vitro experiments in rat brain slices have demonstrated a pronounced susceptibility of the EC to acutely provoked epileptogenesis (Walther *et al.* 1986; Wilson *et al.* 1988; Jones, 1988, 1994; Jones & Lambert, 1990*a*,*b*; Bear & Lothman, 1993; Rafiq *et al.* 1993; Avoli *et al.* 1995). Pharmacologically induced seizures arise predominantly in the EC and propagate to adjacent cortical and hippocampal areas (Jones & Lambert, 1990*a*; Iijima *et al.* 1996; Avoli *et al.* 1996; Weissinger *et al.* 2000; D'Arcangelo *et al.* 2001; Buchheim *et al.* 2002). More specifically, epileptiform activity appears to be initiated in deep layers of the EC and to propagate to more superficial layers and to the hippocampus (Jones & Lambert, 1990*a*,*b*; Avoli *et al.* 1996; Lopantsev & Avoli, 1998; D'Arcangelo *et al.* 2001) suggesting that deep layers may be 'seizure sensitive' and more superficial layer 'seizure resistant' (Jones & Lambert, 1990*a*,*b*; Jones, 1993). Similar conclusions have been reached regarding laminar differences in seizure initiation in neocortex (Hoffman & Prince, 1995; Barkai *et al.* 1995; Badea *et al.* 2001; Yang & Benardo, 2002).

Laminar differences in inhibition and excitation

In this laboratory we have been engaged in determining laminar differences in the balance between inhibitory and excitatory network activity that may determine how deep and superficial neurones subserve function, and how this may contribute to the propensity to participate in pathological synchronization. We provided the first description of the synaptic and intrinsic properties of layer V pyramidal neurones in the EC (Jones, 1987; Jones & Heinemann, 1988). These studies showed that synaptic responses evoked by low frequency electrical stimulation of afferent pathways was dominated by excitation, with NMDA receptor (NMDAr)-mediated excitatory postsynaptic potentials (EPSPs) being particularly prominent. Recurrent and feed-forward synaptic inhibition was generally weak and inconsistent (Jones & Heinemann, 1988). In contrast, examination of responses in layer II neurones revealed that the dominant response to synaptic stimulation was GABA mediated inhibitory postsynaptic potentials (IPSPs; Jones, 1994; Glovelli *et al.* 1997; Heinemann *et al.* 2000). This laminar bias towards excitation in the deep neurones and inhibition in superficial has been confirmed by others for EC and for other parahippocampal areas (pre- and parasubiculum; Funahashi & Stewart, 1998). Also, synaptic inhibition has been shown to be much

more robust in layer II than in layer V in cat (van Brederode & Spain, 1995) and mouse neocortex (Silva *et al.* 1991).

However, it should be noted that synaptic inhibition in layer II of the EC is not static and invariant. We have shown that IPSPs in layer II, as in other cortical neurones (e.g. McCarren & Alger 1985; Deisz & Prince, 1989), are markedly labile with increasing frequency and can be replaced by excitation at frequencies much above 1 Hz (Jones, 1993, 1994, 1995; Glovelli *et al.* 1997). Under similar conditions, the powerful excitation of layer V neurones facilitates markedly and can give rise to repetitive firing and even the generation of synchronized population discharges (Jones, 1993, 1994; Cunningham *et al.* 2000). In order to quantify the degree of frequency-dependent facilitation of excitation, and the decrement in inhibition, individual components of the evoked synaptic responses were isolated pharmacologically. Figure 1*A* shows that AMPA receptor (AMPAr)-mediated EPSPs, recorded intracellularly in layer V, show a slightly greater degree of facilitation than those in layer II neurones with increasing frequency, but this is not significant. However, NMDAr-mediated EPSPs clearly facilitate to a greater degree in the deep layer. In addition, the frequency-dependent depression of both $GABA_A$ and $GABA_B$ receptor-mediated IPSPs is significantly greater in layer V than in layer II (Fig. 1*B*).

Thus, our results and those of others clearly indicate a dominance of inhibition over excitation in layer II and *vice versa* in layer V, and this is maintained, to some extent, under conditions where impulse traffic increases. To look at the laminar differences in more detail we have used whole cell patch clamp recordings to monitor glutamate and GABA release in the two layers.

Spontaneous background excitation

All cortical neurones suffer a continuous level of excitatory activity as a result of glutamate released spontaneously from presynaptic terminals, and the EC is no exception (Berretta & Jones, 1996*a*). This continuous excitation can be monitored as spontaneous excitatory postsynaptic currents (sEPSCs) in whole cell patch clamp recordings. In both layer V and layer II neurones sEPSCs occur at a frequency of 1–5 Hz, and they often occur in high frequency bursts in layer V (though less often in layer II). Examination of a large number of neurones in the two layers has shown that the frequency of sEPSCs, on average, is slightly, but significantly higher in layer V than in layer II. In addition, the amplitude of events in layer V is greater than in layer II (Fig. 2*A*) and they have faster rise and decay times. This may suggest that excitatory terminals are more proximally located in layer V, although differences in electrotonic length of the two populations indicate that EPSCs are likely to undergo greater dendritic filtering in layer II. In both layers, the bulk of the background synaptic activity is action potential independent, since application of TTX reduced the frequency of sEPSCs by only 15–20% (Berretta & Jones, 1996*a*). The larger and more frequent sEPSCs in layer V could be due to fundamental differences in release mechanisms in excitatory terminals in the two layers. However, it could also simply reflect a greater number of terminals and/or connectivity between neurones. Using paired intracellular recording, we have shown that recurrent excitatory connections between

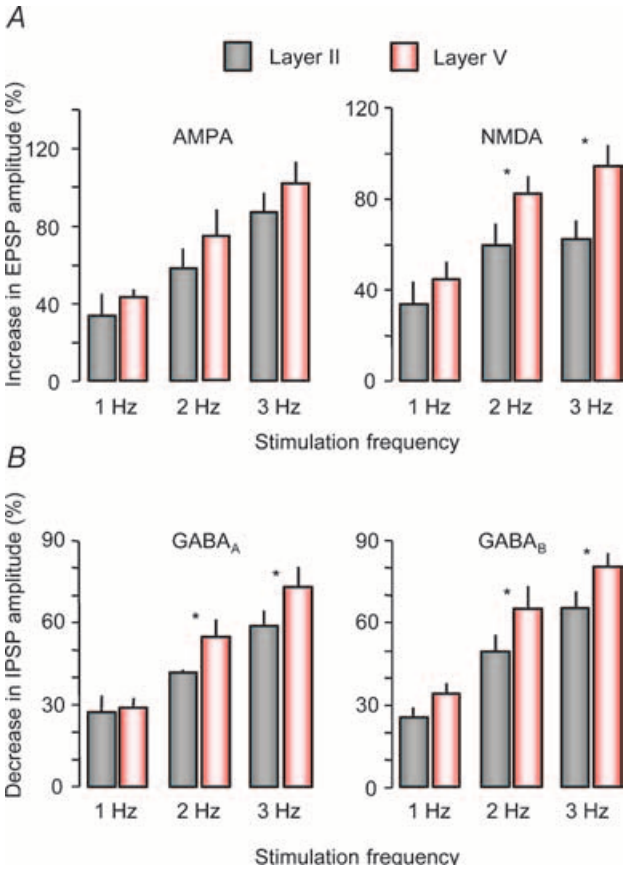
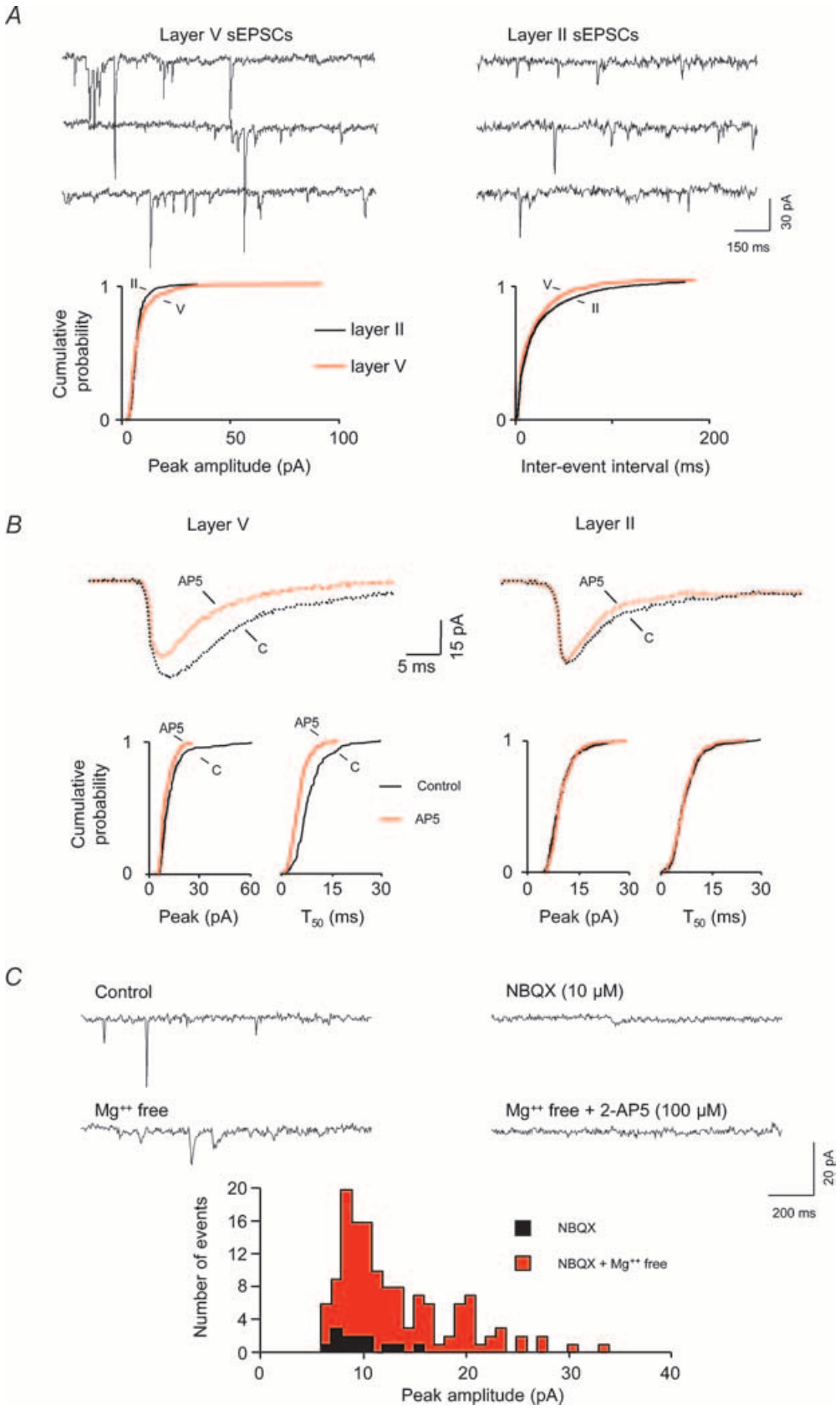


Figure 1. Frequency-dependent changes in synaptic potentials in the EC
Intracellular recordings were made from neurones in either layer II or layer V in interface slices of rat EC. Synaptic responses were evoked by stimulating afferent pathways, and using appropriate receptor antagonists to isolate different components of the synaptic EPSPS and IPSPs (e.g. 2-AP5, bicuculline and CGP55845A for AMPAr-mediated EPSPs). Amplitudes of each component were determined as a percentage of the control response evoked at low frequency (0.1 Hz). At least 7 neurones were tested in each case. *A*, the facilitation of AMPA receptor-mediated EPSPs was slightly but not significantly greater in layer V at all three higher frequencies. NMDA-dependent EPSPs, however, showed a greater facilitation in layer V, which was significant ($P > 0.05$, paired *t* test) at 2 and 3 Hz. *B*, frequency-dependent depression of both $GABA_A$- and $GABA_B$-mediated IPSPs was significantly ($P > 0.05$, paired *t* test) greater in layer V at both 2 and 3 Hz. (Data adapted from Dhillon, 1996 and A. Dhillon & R.S.G. Jones, unpublished observations).



layer V neurones are highly prevalent, whereas we were unable to record any such connections in layer II (Dhillon & Jones, 2000). Although the properties of sEPSCs in other cortical neurones have often been described, to our knowledge there are no other studies where a direct and detailed laminar comparison has been made. However, a study in rat frontal cortex suggests that sEPSCs in layer V neurones may be slightly larger and more frequent than those in layer II/III pyramids (Lambe *et al.* 2000).

Examination of the pharmacological basis of the sEPSCs revealed some very interesting differences between sEPSCs in the two layers (Berretta & Jones, 1996*a*). Thus, although virtually all events in both layers are mediated by AMPAr, both the peak and decay phase of those in layer V showed a significant contribution from spontaneously activated NMDAr, whereas an NMDAr component was almost undetectable in layer II (Fig. 2*B*). However, when AMPAr were blocked, most sEPSCs were abolished, but in both layers we could record sEPSCs that were enhanced in Mg^{2+}-free medium and blocked by an NMDAr antagonist (Fig. 2*C*), allowing us to conclude that they were pure NMDAr-mediated sEPSCs. These were considerably more frequent in layer V than in layer II. These data could suggest that there is a greater expression of NMDAr at synapses on layer V neurones. Alternatively, such receptors may be less accessible to spontaneously released glutamate in layer II, perhaps reflecting a greater expression at extrasynaptic, as opposed to subsynaptic, locations (see Nusser, 2000). Whatever the mechanisms involved, these studies point to a greater role of post-synaptic NMDAr in background excitation in layer V neurones compared to layer II. This coupled with the larger and more frequent sEPSCs suggests that background excitation may be more effective in layer V, making these neurones more excitable than their layer II counterparts, and perhaps more predisposed to synchronization.

Spontaneous background inhibition

Spontaneous transmitter release is not limited to excitatory terminals. EC neurones, like other cortical neurones (e.g. Otis *et al.* 1991; Soltesz & Mody, 1994; Salin & Prince, 1996), are constantly bombarded with GABA. We have recently completed a detailed comparison of background inhibition in layer II and layer V neurones (Bailey *et al.* 2004; Woodhall *et al.* 2004). As in other neurones, background inhibition occurs in the form of spontaneous inhibitory postsynaptic currents (sIPSCs) mediated via $GABA_A$ receptors. A second form of background inhibition has been demonstrated in some neurones, which is manifested as a tonic, standing $GABA_A$-mediated conductance (e.g. Brickley *et al.* 1996; Salin & Prince, 1996). We have not been able to demonstrate such a conductance in either deep or superficial EC neurones (Woodhall *et al.* 2004).

Whilst the pharmacological nature of sIPSCs was the same whether recorded in layer V or layer II, there were clear-cut differences in spontaneous GABA release at terminals onto neurones in the deep and superficial layers (Woodhall *et al.* 2004). The most pronounced difference was in the frequency of spontaneous inhibitory events (Fig. 3). In layer II neurones, sIPSCs occurred on average at over 4–5 times the rate recorded in layer V (around 12 Hz compared to 2.5 Hz). In both layers, sIPSCs could occur in high frequency bursts, but such bursts were much more frequent, and contained more sIPSCs per burst in layer II compared to layer V. In addition to the much greater frequency of sIPSCs in layer II, the amplitudes in the superficial layer were slightly more skewed towards larger events, although this was not a dramatic difference (Fig. 3; Woodhall *et al.* 2004). The only other study that we are aware of that has made a similar laminar comparison is that of Salin & Prince (1996), who looked at sIPSCs in pyramidal neurones

Figure 2. Spontaneous synaptic excitation in the EC
All recordings in this and subsequent figures (3–6) were made in submerged slices of rat EC using whole cell patch clamp recordings at a holding potential of −60 mV, to monitor transmitter release. *A*, records at the top show sEPSCs in individual layer V and layer II neurones. sEPSCs were on average larger and more frequent in layer V neurones compared to layer II. Note also the burst of sEPSCs at the beginning of the upper trace in the layer V neurone, which were prevalent in deep but not superficial neurones. The graphs show cumulative probability curves for pooled data (200 events per neurone, $n = 17$ neurones in each layer) for interevent interval and peak amplitudes. *B,* the traces are averaged sEPSCs ($n = 20$ in each case). In the layer V neurone, addition of the NMDAr antagonist, 2-AP5, reduced both the peak and decay phase of the sEPSCs, whereas neither was greatly altered in the layer II neurone. These effects are confirmed by the cumulative probability analyses of peak amplitude and time to 50% decay of sEPSCs in pooled data (at least 150 events per neurone both before and after drug application, $n = 7$ neurones in each case) shown in the graphs below the traces. *C*, pure NMDA-mediated sEPSCs could be recorded. The records are from a layer V neurone. Addition of the AMPA receptor antagonist, NBQX, removed most of the sEPSCs although small occasional events with slow kinetics remained. These were increased in number and amplitude by removal of the voltage-dependent block of the NMDAr in Mg^{2+}-free medium. Subsequent addition of the NMDAr antagonist abolished the slow sEPSCs. The frequency histogram shows the distribution of event amplitudes in the presence of the AMPAr antagonist alone and following removal of Mg^{2+}. (Data adapted from Berretta & Jones, 1996*a*.)

in layers II–III, layer IV and layer V in somato-sensory neocortical slices. The general characteristics of sIPSCs in their study were similar to those in the EC. However, although the frequency of events in superficial neurones was similar to that in layer II neurones of the EC, in contrast, the frequency in layer V was considerably higher, averaging around 23 Hz, about 10-fold higher than we have found in layer V of the EC. Thus, there appear to be substantial differences in inhibitory input to principal cells of somato-sensory cortex and EC.

Another difference between layers in the EC was in the ratio of action-potential-dependent release to activity-independent quantal release. TTX reduced the baseline frequency of IPSCs in both layers to a similar extent in terms of absolute frequency (by around 1–2 Hz). However, in layer II, this reduction was superimposed on an approximately 10-fold greater frequency of sIPSCs. Thus, in layer II the dominant mode of release is clearly action potential independent, with mIPSCs accounting for some 90% of recorded postsynaptic currents. In layer V, the contribution of action-potential-independent release was approximately equal to that of activity driven release.

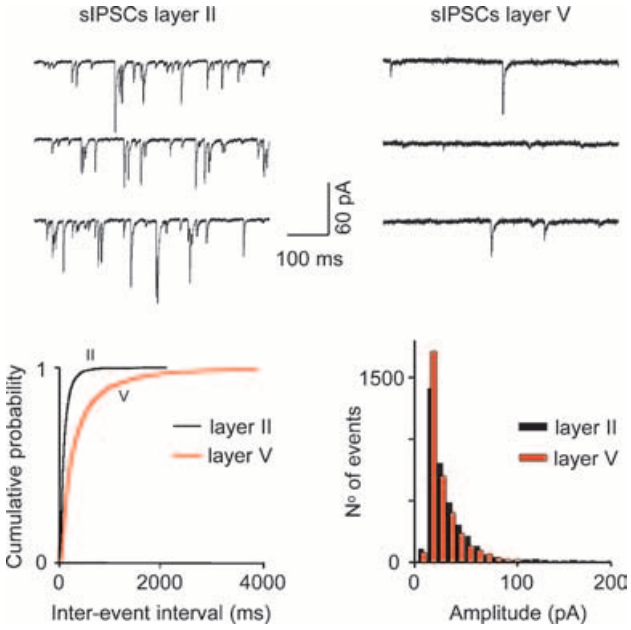


Figure 3. Spontaneous synaptic inhibition in the EC
The voltage clamp traces were recorded at a holding potential of −60 mV with symmetrical intra- and extracellular Cl^- concentrations and show sIPSCs mediated by activation of $GABA_A$ receptors (see Bailey *et al.* 2004). The frequency of sIPSCs was dramatically higher in layer II compared to layer V. The pooled analyses of data from 15 neurones shown in the graphs confirm the much greater frequency in layer II and show that there was a slightly greater preponderance of larger amplitude events in the superficial neurones, and this was reflected by slightly larger mean and median amplitude values in layer V. (Data from Woodhall *et al.* 2004.)

What underlies the much higher level of background inhibition in layer II? It seems likely that there may be more inhibitory terminals on layer II cells, either as a result of a greater number of interneurones or more synaptic contacts per interneurone. There is evidence for both possibilities. Kohler *et al.* (1985) showed that layer II contained the highest number of GABA and GAD immunoreactive neurones in the EC, and also a much greater density of immunoreactive terminals than other layers. Layer V was the least densely innervated layer. In layer II, GABAergic terminals and axons form extensive pericellular plexuses around principal neurones (Kohler *et al.* 1985; Jones & Buhl, 1993) but these are rarely seen in layer V (Kohler *et al.* 1985; R. S. G. Jones & E. H. Buhl, unpublished observations). Other studies which have looked at colocalization of GABA with neuropeptides provide the same general picture, with inhibitory neurones and terminals heavily concentrated in superficial layers, and a relative paucity in layer V (Kohler & Chan-Palay, 1982, 1983; Lotstra & Anderhaeghen, 1987; Wouterlood *et al.* 1995, 2000; Fujimaru & Kosaka, 1996; Miettinen *et al.* 1996, 1997; Mikkonen *et al.* 1997; Wouterlood & Pothuizen, 2000; Arellano *et al.* 2002). In particular, basket cells and chandelier cells, which are amongst the most powerful of inhibitory interneurones, show a preferential location to superficial layers (Tunon *et al.* 1992; Wouterlood *et al.* 1995; Miettinen *et al.* 1996, 1997; Arellano *et al.* 2002). Chandelier cells, by virtue of their dense innervation of the axon initial segment, are able to provide powerful inhibitory control, and these appear to be restricted to layers II and III (Soriano *et al.* 1993; Martinez *et al.* 1996; Arellano *et al.* 2002). Thus, there is a strong possibility that layer II neurones are recipients of many more GABAergic terminals than their counterparts in layer V, and this could account for the much higher frequency of spontaneous events.

A greater density of GABAergic innervation would help to explain the comparative weakness of evoked inhibition in layer V and its dominance in layer II. It was also noted above that frequency-dependent decrement of IPSPs is more pronounced in layer V. The high frequency of sIPSCs in layer II could indicate that superficial terminals have a high safety factor for GABA release compared to those in layer V, and this could help sustain inhibitory effects under conditions of increased afferent activity.

Kinetic properties of sIPSCs in layer II and layer V suggested that there were subpopulations of inhibitory events. At least three classes could be distinguished in layer V neurones, and two in layer II (Woodhall *et al.* 2004). There was a correspondence between kinetically distinct classes of IPSCs within individual recordings and within mean data from groups of recordings, which indicates that whilst all neurones show all classes of IPSC, one kinetic

subtype may predominate. It is quite possible that the different sIPSCs may reflect inputs from separate classes of interneurones. Paired intracellular recordings from principal cells and interneurones in other areas have shown that subtypes of GABA neurones provide anatomically and physiologically compartmentalized inputs to principal cells (Miles *et al.* 1996; Maccaferri *et al.* 2000; Tamas *et al.* 2000), giving rise to IPSCs with quite different kinetics. However, a more detailed analysis will be needed to determine if input from one type of neurone may predominate and possibly underlie the high frequency of sIPSCs in layer II.

Thus, layer II neurones are certainly subject to more profound background inhibition than their counterparts in layer V, and this may greatly reduce sensitivity to excitatory inputs to superficial neurones (cf. Soltesz *et al.* 1995; Hausser & Clark, 1997; Pare *et al.* 1998*b*; Stevens & Zador, 1998; Harsch & Robinson, 2000). As background excitation may be more effective in layer V compared to layer II (Berretta & Jones, 1996*a*), the overall balance is clearly in favour of inhibition in the superficial layer and this may result in a reduced propensity for synchronization to occur.

Factors underlying laminar differences in background synaptic activity

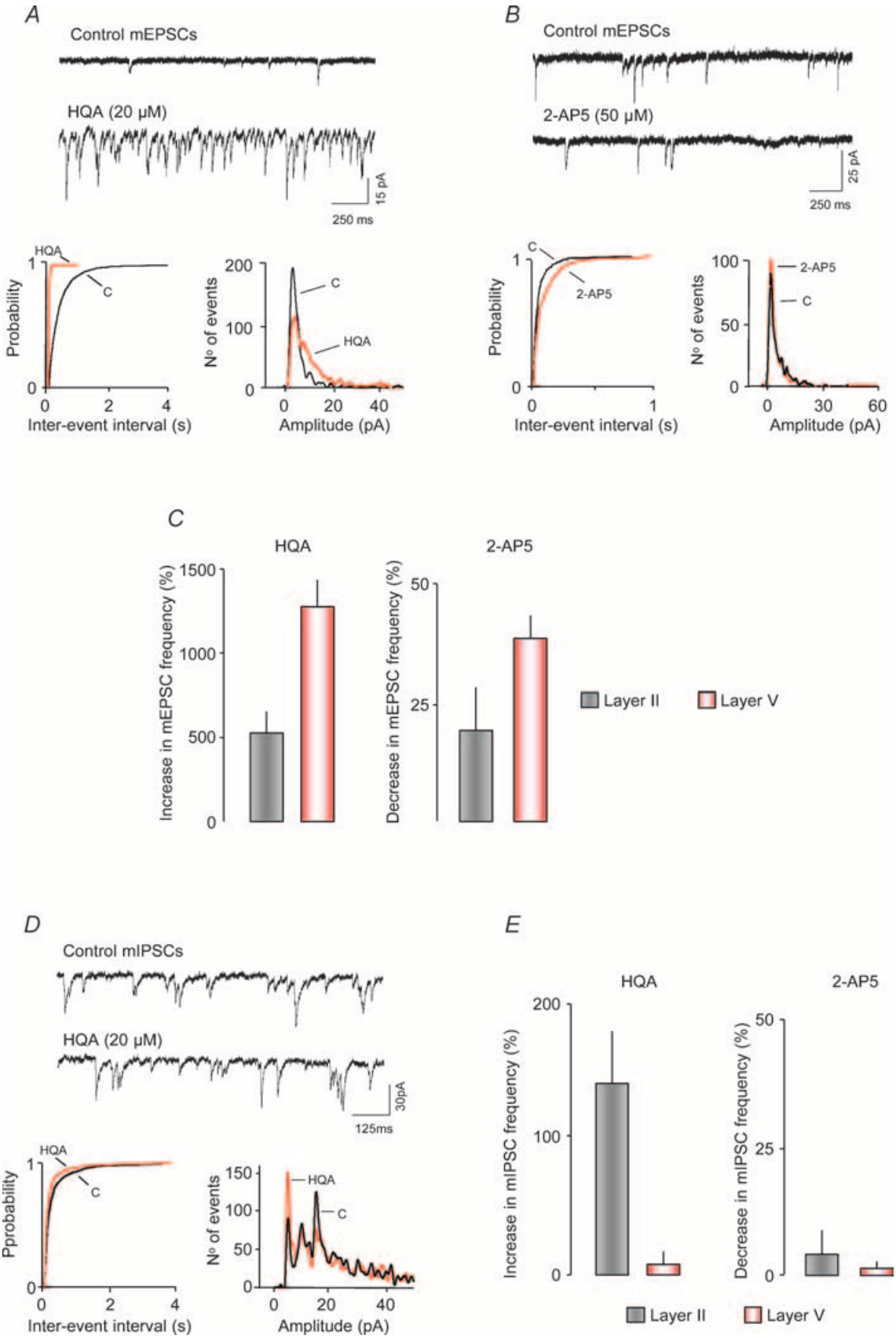
Among the possible underlying mechanisms for laminar differences in background synaptic activity we have considered a differential control of both glutamate and GABA release by presynaptic receptors.

Presynaptic NMDA receptors. During our investigation of the pharmacological properties of sEPSCs in EC (Berretta & Jones, 1996*a*), we found that application of the NMDAr antagonist, 2-AP5, reduced the frequency of sEPSCs in layer II neurones without affecting their amplitude (see Fig. 4*B*) suggesting that the antagonist may be having a presynaptic effect at NMDAr. To investigate this further we developed a novel means of blocking the postsynaptic receptors without affecting the presynaptic terminal. This involved inclusion of the NMDAr open channel blocker, MK801, in the patch pipette solution. Repeated synaptic activation, or membrane depolarization enabled the drug to enter the channel from the inside and block postsynaptic NMDAr allowing investigation of the effect of activating or blocking receptors on the terminals. Subsequent investigations showed that the effect of 2-AP5 on sEPSC (and mEPSC) frequency was due to blockade of tonic facilitation of glutamate release by presynaptic NMDA autoreceptors on the excitatory terminals (Berretta & Jones, 1996*b*). We have also shown that these receptors tonically facilitate release in layer V, and that they probably do so by increasing Ca^{2+} entry into the terminals via the NMDAr ionophore (Woodhall *et al.* 2001*a*). In addition, we have shown that the frequency-dependent facilitation of excitatory transmission in both layer II and V, described above, is likely to depend, in part, on activation of the NMDA autoreceptor (Berretta & Jones, 1996*a*; Woodhall *et al.* 2001*a*)

So can a differential activation of this receptor explain the higher frequency of sEPSCs in layer V? Activation of the autoreceptor with the agonist homoquinolinic acid (HQA) dramatically increases sEPSC frequency in both layers (Fig. 4*A*; Woodhall *et al.* 2001*a*). However, the percentage increase in frequency in layer V is greater than in layer II (Fig. 4*C*). More significantly, the decrease in frequency induced by 2-AP5 is more marked in layer V neurones. Taken together, these data indicate that the small, but consistently higher frequency of sEPSCs in layer V is partly maintained by a greater tonic activation of the autoreceptor in the deep layer. Indeed, this difference in frequency between the two layers disappears in the presence of NMDAr antagonists (not shown). Whether the terminals in layer V express a higher density of autoreceptors, or whether they are more accessible to the synaptically released transmitter is a matter for conjecture. However, the greater effect of the autoreceptor in layer V could contribute to the greater degree of frequency dependent facilitation of evoked responses in this layer that was discussed above.

We have also shown that NMDAr exist as facilitatory heteroreceptors on GABA terminals in layer II, but they are not present in layer V (Fig. 4*D* and *E*; Woodhall *et al.* 2001*a*). However, 2-AP5 does not alter the frequency of sIPSCs in either layer (Fig. 4*E*). Thus, it is unlikely that the much higher level of background inhibition in layer II is dependent on tonic facilitation of GABA release by glutamate spillover onto presynaptic NMDA heteroreceptors.

Presynaptic group III metabotropic receptors. Group III metabotropic glutamate receptors (mGlur) have been shown to act as autoreceptors at excitatory synapses, where they are widely held to depress glutamate release (see Conn & Pin, 1997). However, we have demonstrated a novel and unusual effect of a group III mGlur, in layer V of the EC, where a specific agonist (ACPT-1) at the group III receptor, mGlur4, produced a robust increase in sEPSC frequency (Fig. 5*A*; Evans *et al.* 2000, 2001). The effect persisted in TTX, indicating that it was likely to be a direct action on the glutamate terminals, rather than a network effect resulting from activation of receptors on the somata or dendrites of presynaptic neurones. The novelty of the effect was reinforced by the observation that the same agonist induced the expected decrease in sEPSC frequency in layer II neurones (Fig. 5*A*; Evans *et al.* 2000). Thus, terminals in layer V possess two mechanisms for mediating what we have termed glutamate-induced glutamate release, namely facilitatory NMDA and mGlur receptors. However, unlike the former,



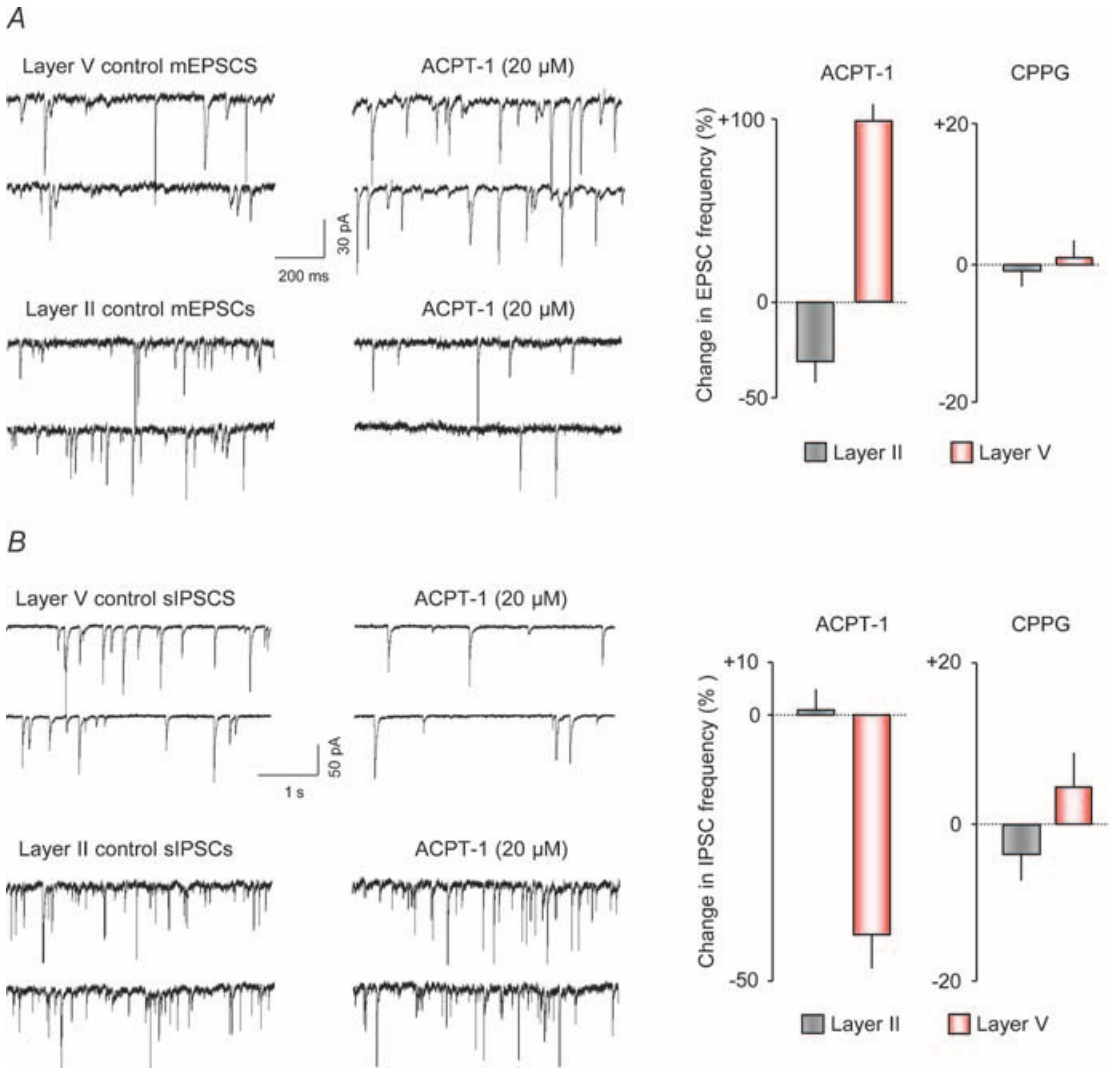


Figure 5. Presynaptic mGlurs in the EC
A, addition of ACPT-1 (20 μM), a specific agonist at the group III receptors, greatly increased the frequency of mEPSCs recorded in the presence of TTX (1 μM) in a layer V neurone. In contrast, the same agonist significantly decreased the frequency of mEPSCs in layer II. The summary data (14 layer V neurones and 5 layer II) show a doubling of mEPSC frequency in the deep layer and around a 30% decrease in layer II. Neither effect is tonically operative since the group III antagonist, CPPG had no effect on frequency in either layer. *B,* mGlur activation reduces the frequency of sIPSCs in layer V neurones, but not in layer II. The summary data ($n = 10$ in each layer) show this differential effect and also that again the group III antagonist does not affect frequency in either layer. (Data adapted from Evans *et al.* 2000 and Woodhall *et al.* 2001*b*.)

Figure 4. Presynaptic NMDA receptors in the EC
All recordings in this figure were conducted with the open channel NMDAr blocker, MK801 in the patch pipette. This approach allows blockade of postsynaptic NMDAr on the recorded neurone (see Berretta & Jones, 1996*b*). *A,* the records show AMPAr-mediated miniature EPSCs in a layer V neurone recorded in the presence of 1 μM TTX. Addition of an NMDA receptor agonist, homoquinolinic acid (HQA), dramatically increased the frequency of sEPSCs and resulted in a shift towards larger amplitude events. The graphs show pooled data from 5 layer V neurones. *B,* the facilitatory effect of the presynaptic NMDAr on glutamate release was tonically operative, since the NMDAr antagonist, 2-AP5, reduced the frequency of mEPSCs with little effect on amplitude. *C*, presynaptic NMDAr are present in both layer V and layer II. However, the summary data ($n = 5$, layer V and $n = 4$ layer II) show a greater effect of the agonist in increasing EPSC frequency in layer V and a greater reduction in frequency with the antagonist, compared to layer II neurones. *D*, presynaptic NMDAr also act to facilitate GABA release in the EC. The records are $GABA_A$r-mediated mIPSCs recorded in TTX in a layer II neurone. Addition of HQA increased the frequency with little effect on amplitude. This effect was weak compared to that seen with mEPSCs, and the pooled summary data in *E* show that this effect is restricted to layer II, and that the NMDAr antagonist did not alter mIPSC frequency in either layer. (Data adapted from Woodhall *et al.* 2001*a*.)

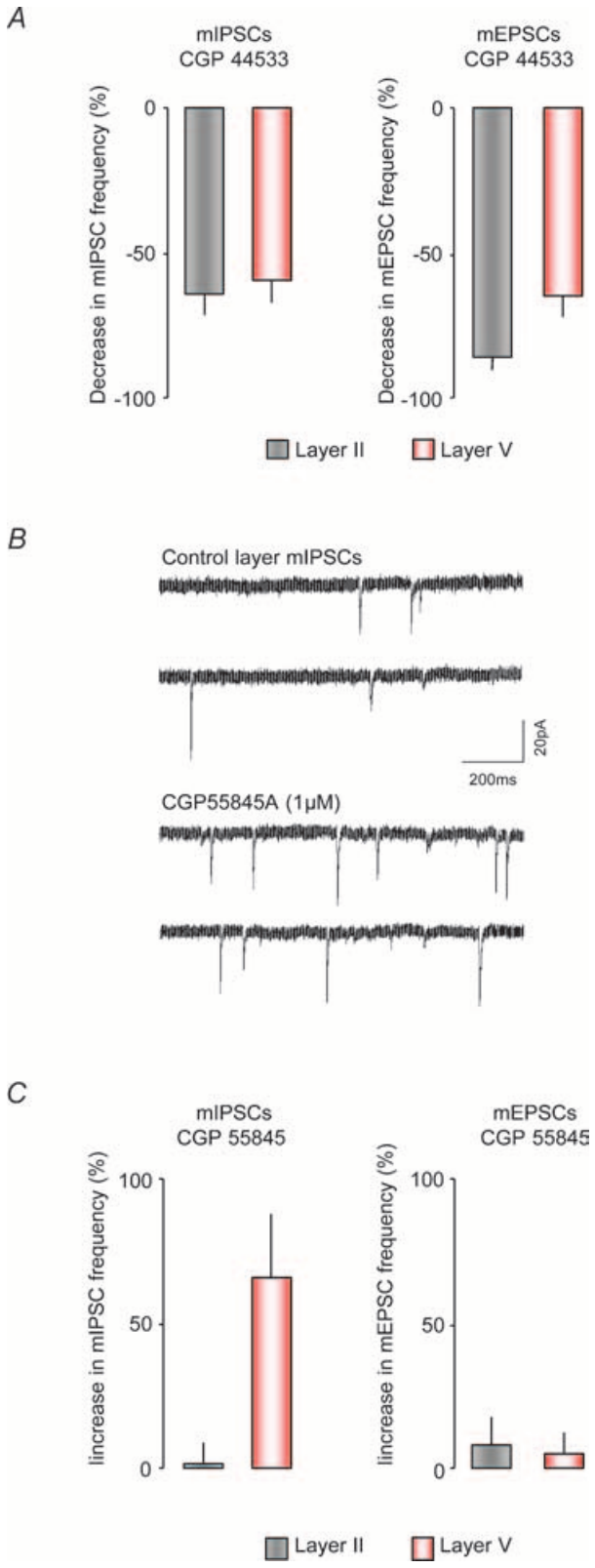


Figure 6. Presynaptic $GABA_B$ receptors in the EC
A, summary data show that activation of $GABA_B$r with the agonist CGP 44533 (10 μM) reduced the frequency of mIPSCs recorded in both layer II and layer V to a similar extent ($n = 17$ in each case). A similar reduction in mEPSC frequency was seen in layer V, but in layer II, this heteroreceptor effect was significantly more pronounced. *B,* the $GABA_B$r was tonically operative at autoreceptors in layer V neurones, as the antagonist CGP 55845 increased the frequency of sIPSCs. *C,* the summary data show that the tonic autoreceptor effect is restricted to layer V, and is not present in layer II. In addition, the same antagonist, CGP 55845 (10 μM), failed to affect the frequency of mEPSCs in either layer. (Data adapted from Bailey *et al.* 2004 and Thompson, 2004 including unpublished observations from S.E. Thompson & R.S.G. Jones).

the latter does not operate tonically, as a group III mGlur antagonist (CPPG) does not alter sEPSC frequency (Fig. 5*A*). A tonic inhibitory effect of the receptor on glutamate release in layer II does not occur either (Fig. 5*A*). It is unlikely therefore that mGlur activation contributes to the higher level of background excitation seen in layer V compared to layer II, at least in the EC slice preparation.

We have also examined the role of mGlurs as heteroreceptors on GABA terminals (Woodhall *et al.* 2001*b*). ACPT-1 substantially decreased sIPSC frequency in layer V but not in layer II (Fig. 5*B*). Again, the effect was not tonically operative as the antagonist failed to alter sIPSC frequency (Fig. 5*B*). Nevertheless, the overall profile of the effect of mGlur4 (increase in sEPSC and decrease in sIPSC frequency in layer V, decrease in sEPSC frequency in layer II) would support the dominance of excitation in the deep layer compared to the superficial, and it is possible that such mechanisms could contribute during the more intense network activity that is likely to occur *in vivo.*

Presynaptic $GABA_B$ receptors. Release of both glutamate and GABA are subject to inhibitory control by presynaptic $GABA_B$ receptors ($GABA_B$r) at cortical synapses (see Bowery *et al.* 2002), and again the EC is no exception. We have recently reported that activation of $GABA_B$ autoreceptors by $GABA_B$r agonists depresses sIPSC frequency to a similar extent in layer II and layer V neurones (see Fig. 6*A*; Thompson *et al.* 2003; Bailey *et al.* 2004). However, this depression is tonically operative in layer V as the $GABA_B$-receptor antagonist, CGP 55845, increased IPSC frequency. This was not seen in layer II (Fig. 6*B* and *C*; Bailey *et al.* 2004). Thus, this tonic feedback may contribute to, but does not totally account for, the higher level of background inhibition seen in layer II neurones.

$GABA_B$r agonists also depress the frequency of EPSCs in both layers (Fig. 6*A*; Thompson *et al.* 2003), but a significantly greater effect was seen in layer II, suggesting that $GABA_B$ heteroreceptor control of glutamate release may play a greater role in dampening background excitation in layer II. However, $GABA_B$r antagonists failed to alter the frequency of IPSCs in either layer (Fig. 6*C*), so there would not seem to be any spillover of GABA to glutamate terminals. Again it should be remembered

that network activity will be markedly higher *in vivo*, so a lack of tonic activation in the EC slice does not necessarily rule out a physiological contribution of tonic activation of presynaptic $GABA_B$r on background excitation.

Implications of background synaptic activity

Our observations show that the dominance of excitation over inhibition in layer V of the EC seen with evoked responses (and vice versa in layer II) is mirrored by background synaptic activity mediated by glutamate and GABA release in the two populations of neurones. Again, it should be stressed that these are observations made in a reduced *in vitro* preparation. The situation may be different under *in vivo* conditions, but there is no reason *a priori* to assume that this is so. If we accept that background synaptic activity is not only reflective of the state of the network, but also a determinant of its excitability, then current evidence would support the conclusion that layer V neurones may be more readily responsive to alterations in network activity, and more likely to participate in network synchrony, both functional and dysfunctional. Thus, the suggestion that layer V may be a site of seizure initiation in TLE whilst layer II may resist such pathological synchronization (Jones & Lambert, 1990*a*,*b*; Avoli *et al.* 1996; Lopantsev & Avoli, 1996; D'Arcangelo *et al.* 2001) could be supported. However, recent studies of human inferior temporal cortical tissue re-sected during surgical intervention for epilepsy demonstrated spontaneous synchronous discharges that were primarily initiated in layer II and upper layer III (Kohling *et al.* 1998, 1999). The authors suggested that this represented a change in the functional organization of cortical excitability. Other studies in human tissue (Louvel *et al.* 1992) and animal models (Silva-Barrat *et al.* 1988) have also suggested that epileptiform activity may be initiated in the upper layers. In addition, in a model of epilepsy involving focal injection of amino-oxyacetic acid into the EC of rats, there was a persistent increase in excitability in layer II (e.g. repetitive bursting to single stimuli) with much less evident changes occurring in layer V (Scharfman *et al.* 1998). These experiments could suggest a laminar reorganization in the EC in chronic epilepsy, with a shift of seizure susceptibility to the superficial layers. In recent experiments we have recorded spontaneous synchronized discharges in EC slices from chronically epileptic animals, which appear to be restricted to layer II (R. S. G. Jones, unpublished observations). In addition, we have reported that both spontaneous background inhibition and excitation are increased in both layers of the EC in the same model, albeit to different degrees (Woodhall *et al.* 2001*c*). The extent to which this alters network excitability and could result in a shift in seizure focus is currently a focus of investigation.

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Acknowledgements

We acknowledge the contributions of Drs Nicola Berretta, Arvinder Dhillon, Sarah Bailey, Mark Cunningham, Ieuan Evans, Anne Stacey, Sarah Thompson and Goher Ayman to the work discussed in this article. Financial support from the Welcome Trust, the MRC, the BBSRC, the Epilepsy Research Foundation, The Royal Society and the Taberner Trust has been gratefully received.