Role of the ferret perigeniculate nucleus in the generation of synchronized oscillations *in vitro*

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1. The cellular mechanisms by which neurons of the ferret perigeniculate nucleus (PGN) participate in the generation of spindle waves and slowed absence seizure-like oscillations were investigated with intracellular and extracellular recording techniques in geniculate slices maintained *in vitro*.

2. During spindle wave generation, PGN neurons generated repetitive (2–9 Hz) high frequency (up to 500 Hz) burst discharges mediated by the activation of a low threshold Ca^{2+} spike by the arrival of barrages of excitatory postsynaptic potentials (EPSPs). In most PGN cells at resting membrane potentials (−60 to −70 mV) spindle waves were associated with a progressive hyperpolarization that persisted as a prolonged after-hyperpolarization.

3. The EPSPs occurring in PGN cells were highly synchronized with burst firing in the neighbouring portion of the dorsal lateral geniculate nucleus (LGNd) and were intermixed with short duration inhibitory postsynaptic potentials (IPSPs). After block of GABAergic receptors, the EPSPs occurring during the generation of spindle waves reversed polarity at around 0 mV. In addition, these EPSPs were completely blocked with the bath application of the non-NMDA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), as was spindle wave generation in both the PGN and LGNd.

4. Slowing the intraspindle frequency to 2–4 Hz with pharmacological block of $GABA_A$ receptors resulted in a marked increase in the intensity of burst firing by PGN cells such that the number of action potentials per burst increased from a maximum of thirteen to a maximum of sixty. Block of $GABA_A$ receptors also resulted in a marked increase in the amplitude and duration of the EPSP barrages arriving from the relay laminae during generation of the slowed oscillation.

5. These findings indicate that spindle waves are generated in the ferret LGNd *in vitro* through an interaction between the GABAergic neurons of the PGN and relay neurons, such that burst firing in relay neurons activates a barrage of EPSPs and a subsequent low threshold Ca^{2+} spike in PGN cells. This activation of PGN neurons inhibits a substantial number of relay cells, a few of which rebound burst after this IPSP, thus starting the cycle again. Block of $GABA_A$ receptors results in a marked enhancement of activity in PGN cells through increased excitation from relay cells and disinhibition from neighbouring PGN cells. This increased activity in PGN neurons results in a markedly enhanced activation of $GABA_B$ receptors in relay neurons and the subsequent generation of paroxysmal activity that is similar to that associated with absence seizures.

Spindle waves are a form of synchronized oscillation that have a characteristic intraspindle frequency of 6–12 Hz and an interspindle frequency of 0·1–0·3 Hz and are associated with burst firing in thalamic relay cells induced by the activation of low threshold Ca^{2+} spikes by barrages of inhibitory postsynaptic potentials (Andersen & Andersson, 1968; Deschênes, Paradis, Roy & Steriade, 1984; Jahnsen & Llinás, 1984*a*, *b*; Roy, Clerq, Steriade & Deschênes, 1984; Steriade & Deschênes, 1984; von Krosigk, Bal & McCormick, 1993; Bal, von Krosigk & McCormick, 1995). Andersen & Andersson (1968) hypothesized that these IPSPs were generated by local 'distributor' inhibitory interneurons within the thalamus. Electrophysiological and lesion experiments subsequently demonstrated that

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these IPSPs were generated by bursts of action potentials in the GABAergic neurons of the nucleus reticularis thalami (nRt) or the perigeniculate nucleus (PGN) in the case of the dorsal lateral geniculate nucleus (Steriade & Deschênes, 1984; Steriade, Deschênes, Domich & Mulle, 1985; Steriade, Domich & Oakson, 1987; Steriade, McCormick & Sejnowski, 1993; von Krosigk *et al.* 1993). The mechanisms by which nRt or PGN neurons generate the bursts of action potentials that give rise to these IPSPs have been unclear. The structure of the bursts of action potentials recorded both intracellularly and extracellularly *in vivo* during the generation of spindle waves suggests that they are generated through the activation of low threshold Ca^{2+} spikes (Domich, Oakson & Steriade, 1986; Mulle, Madariage & Deschênes, 1986) that are different in some aspects from those occurring in thalamic relay cells (e.g. see Huguenard & Prince, 1992). These low threshold Ca^{2+} spikes have been proposed to be generated either in response to the arrival of excitatory postsynaptic potentials generated by burst firing in thalamic relay neurons or in response to relief of hyperpolarization generated by the inhibitory postsynaptic potentials arriving from other nRt/PGN neurons (Andersen & Andersson, 1968; Deschênes, Madagaria-Domich & Steriade, 1985; Mulle *et al.* 1986; Steriade *et al.* 1987, 1993; Buzsáki, 1991; von Krosigk *et al.* 1993). Intracellular recordings from nRt cells *in vivo* have revealed short duration depolarizing events that arrive at a frequency similar to burst firing in thalamic relay neurons, suggesting that these events are EPSPs (Mulle *et al.* 1986). These presumed EPSPs may underlie the activation of low threshold Ca^{2+} spikes in nRt/PGN neurons during spindle wave generation. However, surgical isolation of the rostral aspects of the nRt from the rest of the thalamus results in islands of nRt neurons that still generate brief periods of oscillation in the upper frequency range of spindles (Steriade *et al.* 1987), suggesting that the nRt may be capable of generating spindle waves autonomously from the rest of the thalamus. Indeed, detailed studies of the electrophysiological properties of nRt neurons *in vitro* demonstrate that these cells have the intrinsic ability to generate short (< 1 s) periods of rhythmic oscillation, mediated by an interaction between the low threshold Ca^{2+} current and a Ca^{2+}-activated K^+ current (Avanzini, de Curtis, Panzica & Spreafico, 1989; Bal & McCormick, 1993).

Absence seizures in humans are periods of highly synchronized oscillation, which exhibit a rapid onset and offset and a characteristic frequency of 3 Hz (Niedermeyer, 1990). Investigation of animal models of absence seizures have demonstrated that these epileptic events are generated as an abnormal oscillation in thalamocortical systems using cellular mechanisms similar to those underlying the genesis of spindle waves (see Kostopoulos, Gloor, Pellegrini & Gotman, 1981*a*; Kostopoulos, Gloor, Pellegrini & Siatitsas, 1981*b*; Avoli & Gloor, 1982; Avoli, Gloor, Kostopoulos & Naquet, 1990; Buzsáki, Smith, Berger, Fisher & Gage, 1990; Steriade *et al.* 1993). In particular, the recent demonstration that systemic, intraventricular, or intrathalamic application of specific antagonists to $GABA_B$ receptors can block absence (or spike-and-wave) seizures in rodent models has focused attention on both the normal and abnormal mechanisms of $GABA_B$ receptor-mediated oscillation in thalamic networks (Hosford *et al.* 1992; Liu, Vergnes, Depaulis & Marescaux, 1992; Snead, 1992).

Recently we have described an *in vitro* slice preparation of ferret lateral geniculate nucleus that generates spindle rhythms, and we have shown that these spindle waves can be modified by block of $GABA_A$ receptors into paroxysmal events that resemble in some aspects absence seizures and that are critically dependent upon the activation of $GABA_B$ receptors (von Krosigk *et al.* 1993; Bal *et al.* 1995). Here we use this novel slice preparation to investigate further the cellular mechanisms by which the GABAergic neurons of the PGN participate in the generation of spindle waves and absence seizure-like oscillations *in vitro*.

METHODS

Procedures for preparation of slices and intracellular recordings are presented in the accompanying paper. The location of the perigeniculate nucleus in the ferret was positively identified by immunocytochemical staining for GABA, using standard procedures (Schwartz & Meineke, 1992). Extracellular single and multiple unit recordings of spindle waves in the perigeniculate nucleus were recorded *in vivo* in ferrets anaesthetized with sodium pentobarbitone (30–40 mg kg^{-1} I.P.) and maintained at a core (rectal) body temperature of 36–37 °C. Putative perigeniculate neurons were identified *in vivo* by their unusually short duration action potentials (McCormick & Prince, 1986; McCormick & Wang, 1991), generation of characteristic burst discharges (Domich *et al.* 1986) and location just anteromedial to the body of the LGNd. Spindle waves were recorded in the PGN after block of retinal activity by infusion of tetrodotoxin (100 μM) using a 31 gauge needle briefly inserted into each eye. Upon completion of extracellular recordings *in vivo*, the animals were overdosed with sodium pentobarbitone and perfused through the heart with 4% phosphate-buffered paraformaldehyde. Reconstruction of electrode tracts gave positive identification of recording sites in the PGN.

Intracellular recordings *in vitro* were obtained using bevelled microelectrodes (Sutter Instruments beveller, BV-10, Novato, CA, USA) formed from 1B100F omega-dot glass (World Precision Instruments) and filled with either 4 M potassium acetate or 1 M potassium acetate and 2% biocytin. We restricted our analysis to only those perigeniculate cells exhibiting a stable resting membrane potential below firing threshold and a relatively high input impedance (> 75 MΩ) since it was these cells that behaved in a manner similar to extracellularly recorded PGN cells during spindle wave generation.

RESULTS

Extracellular multiple ($n = 4$) and single unit ($n = 5$) recordings from neurons of the perigeniculate nucleus (PGN) of barbiturate-anaesthetized ferrets revealed that these neurons discharge in rhythmic bursts during the generation of spindle waves (Fig. 1), as has been reported in other species (Steriade & Deschênes, 1984; Buzsáki, Bickford, Ponomareff, Thal, Mandel & Gage, 1988). Measurement of these burst discharges revealed ten to twenty action potentials per burst at frequencies of up to 400–700 Hz and a characteristic pattern in which the interspike interval first shortens and then lengthens during the burst (Fig. 1*B*, expanded traces), as observed previously (Domich *et al.* 1986). Perigeniculate neurons participated in spindle waves with interburst discharge

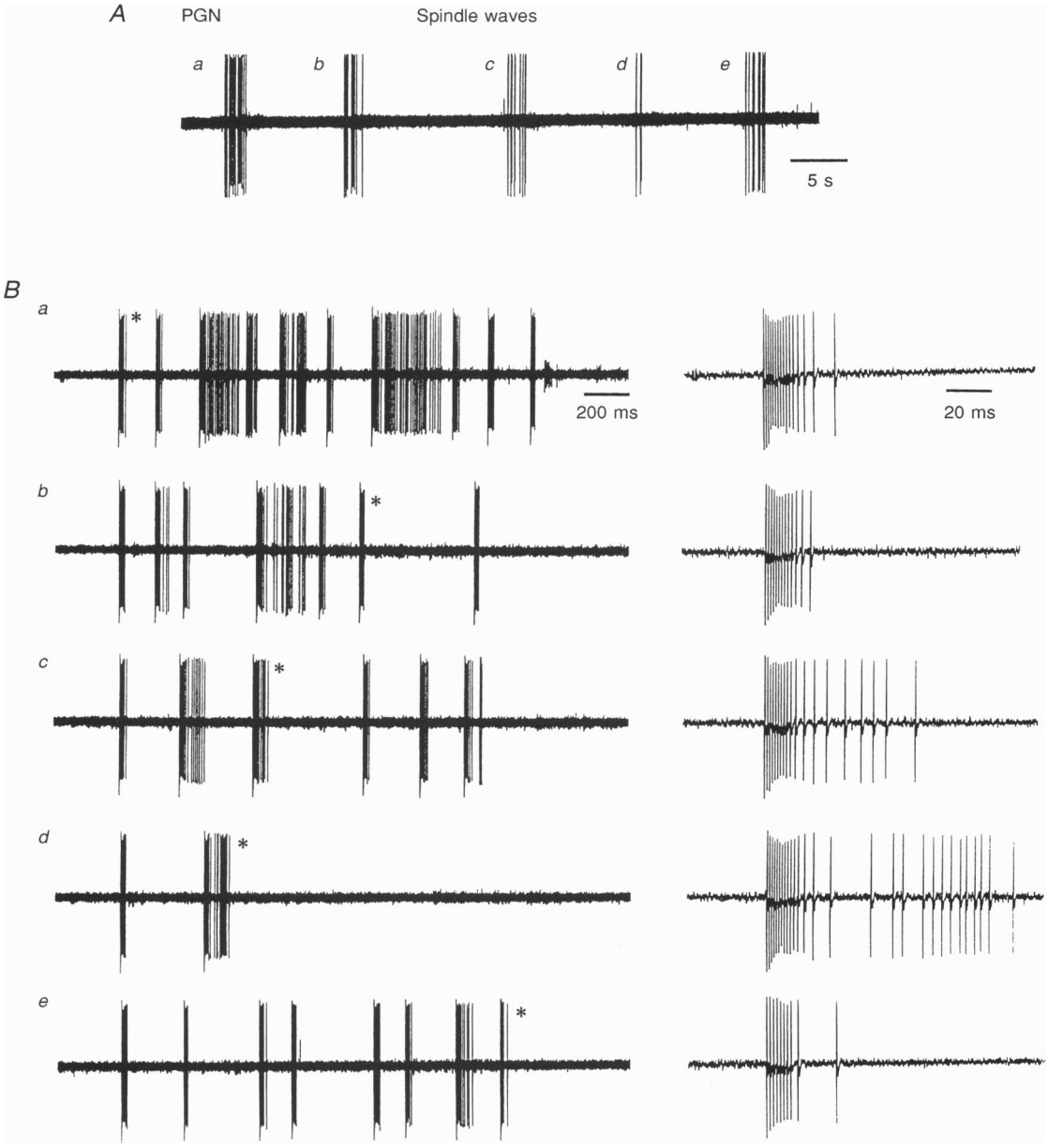

Figure 1. Extracellular recording of a perigeniculate neuron *in vivo* during the generation of spindle waves

A, extracellular recording at slow time base illustrating the activity of a single PGN neuron during the generation of five spindle waves. *B*, expansion of the indicated spindle waves illustrates the repetitive generation of high frequency burst discharges in this neuron during spindle wave generation. The burst discharges indicated by the asterisk are expanded for detail on the right. Note the high frequency of action potential generation of up to 700 Hz. Each burst discharge consists of 12–20 action potentials and sometimes is followed by a tonic 'tail' of activity (*Ba*, *c* and *d*).

frequencies of 2–7 Hz (Fig. 1*B*) and these cells therefore discharge during a higher percentage of cycles of the oscillation than is observed in thalamic relay neurons (see Bal *et al.* 1995), although this varied from spindle wave to spindle wave (Fig. 1*B*) and from PGN neuron to PGN neuron. The high frequency bursts of action potentials in PGN neurons were occasionally followed by a tonic tail of activity (see Fig. 1*Ba*, *Bc* and *Bd*), as seen in nRt neurons recorded *in vivo* (Steriade, Domich & Oakson, 1986).

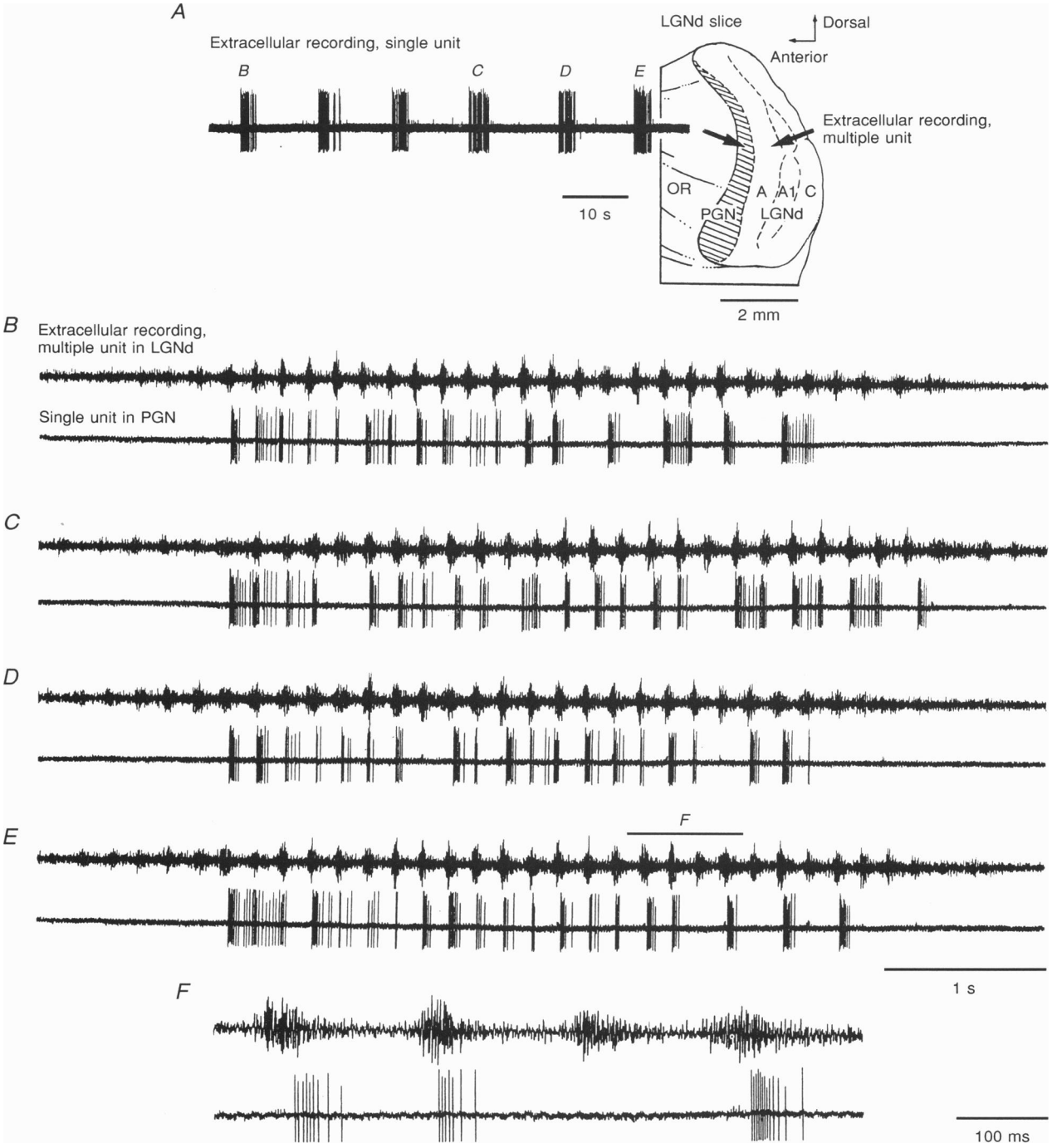

Figure 2. Extracellular single and multiple unit activity recorded from relay and PGN neurons during spindle wave generation *in vitro*

A, extracellular single unit recording in the PGN at a slow time base illustrates six spindle waves. Drawing illustrates recording arrangement. *B–E*, expansion of the indicated spindle waves illustrates the repetitive burst firing in the PGN cell. Each burst of action potentials followed a burst of activity in the relay lamina A. *F*, expansion of part of trace in *E* for detail.

Extracellular multiple and single unit recordings in the perigeniculate nucleus (PGN) of the ferret lateral geniculate maintained *in vitro* revealed a similar pattern of activity to that observed in the PGN *in vivo* during spindle wave generation (Fig. 2). Perigeniculate neurons recorded *in vitro* generated strong bursts of four to thirteen action potentials of up to 500 Hz during spindle waves with an interburst frequency of 2–9 Hz (Fig. 2). Burst discharges in individual PGN neurons were synchronous with the group activity of neighbouring PGN neurons as well as that of the relay cells of the A laminae immediately posterior to the PGN. On average, the onset of each burst of action potentials in the PGN tended to be delayed with respect to those in the LGNd (Figs 2 and 4). In general, the probability of burst discharges in single PGN neurons was highest in the middle of the spindle wave and lower near the end (Fig. 2).

Intracellular recordings were obtained from ninety-three PGN neurons during the generation of spindle waves *in vitro*. Of these neurons, fifteen were morphologically identified by the intracellular injection of biocytin. Examination of these neurons on the light microscopic level revealed extensive axonal arborization within the A and C laminae of the LGNd (not shown). Intracellular recordings from PGN neurons revealed that these cells generate repetitive bursts of action potentials and undergo a progressive hyperpolarization during the generation of spindle waves (Fig. 3*D* and *E*). The persistence of this hyperpolarization resulted in the generation of a 5–10 s duration after-hyperpolarization following each spindle wave (Fig. 3*D* and *E*; the duration of the after-hyperpolarization was measured from the last burst discharge in the spindle to the return to baseline membrane potential). In PGN neurons in which the resting membrane potential was positive to the burst firing mode, this progressive hyperpolarization could result in the hyperpolarization of these cells into the burst firing mode (not shown).

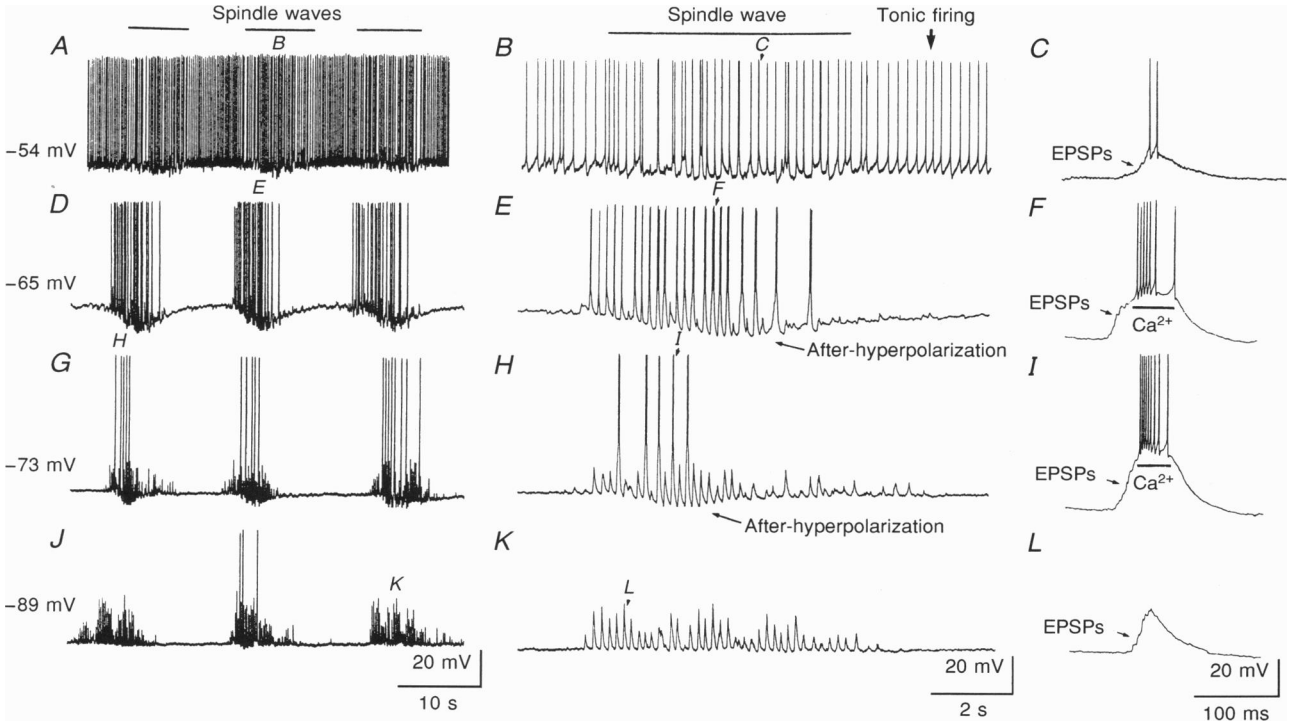

Figure 3. Voltage dependence of activity in PGN cells during spindle wave generation

The response of this PGN neuron during the generation of spindle waves while the neuron is depolarized or hyperpolarized to different membrane potentials with intracellular injection of current is illustrated. At normal resting membrane potential (−65 mV) this PGN neuron generated repetitive bursts of action potentials followed by a prolonged after-hyperpolarization (*D* and *E*). Each burst of action potentials was generated by a low threshold Ca^{2+} spike preceded by a barrage of presumed EPSPs (*F*). Depolarization of the PGN neuron to −54 mV with current injection inhibited both the generation of repetitive bursts and the prolonged after-hyperpolarization (*A–C*). Hyperpolarization of the PGN neuron to −73 mV reduced the percentage of EPSP barrages that activated low threshold Ca^{2+} spikes and reduced the amplitude of the slow after-hyperpolarization (*G* and *H*). Further hyperpolarization to −89 mV brought the membrane potential below threshold for generation of low threshold Ca^{2+} spikes (*J* and *K*). At this membrane potential, repetitive barrages of EPSPs were seen in isolation (*L*).

The bursts of action potentials exibited two distinct components: an underlying slow depolarizing spike and barrages of putative postsynaptic potentials (PSPs; Fig. 3*F* and *I*). Examination of the voltage dependence, as well as the amplitude time course, of the slow depolarizing event underlying the burst of action potentials revealed it to be mediated by a low threshold Ca^{2+} spike. For example, comparison of the slow spike underlying burst discharges during spindle waves with that generated on the offset of a hyperpolarizing current pulse, which is known to be generated by a low threshold Ca^{2+} spike (Huguenard & Prince, 1992; Bal & McCormick, 1993), revealed a marked similarity in amplitude, time course, and voltage dependence (i.e. enhanced with moderate hyperpolarization and inactivated with depolarization) between the intrinsic and spindle-associated slow spikes. Each low threshold Ca^{2+} spike occurring during spindle waves was preceded by a barrage of EPSPs which, on occasion, arrived in groups of three to six at frequencies of 250–400 Hz, which is similar to the frequencies of action

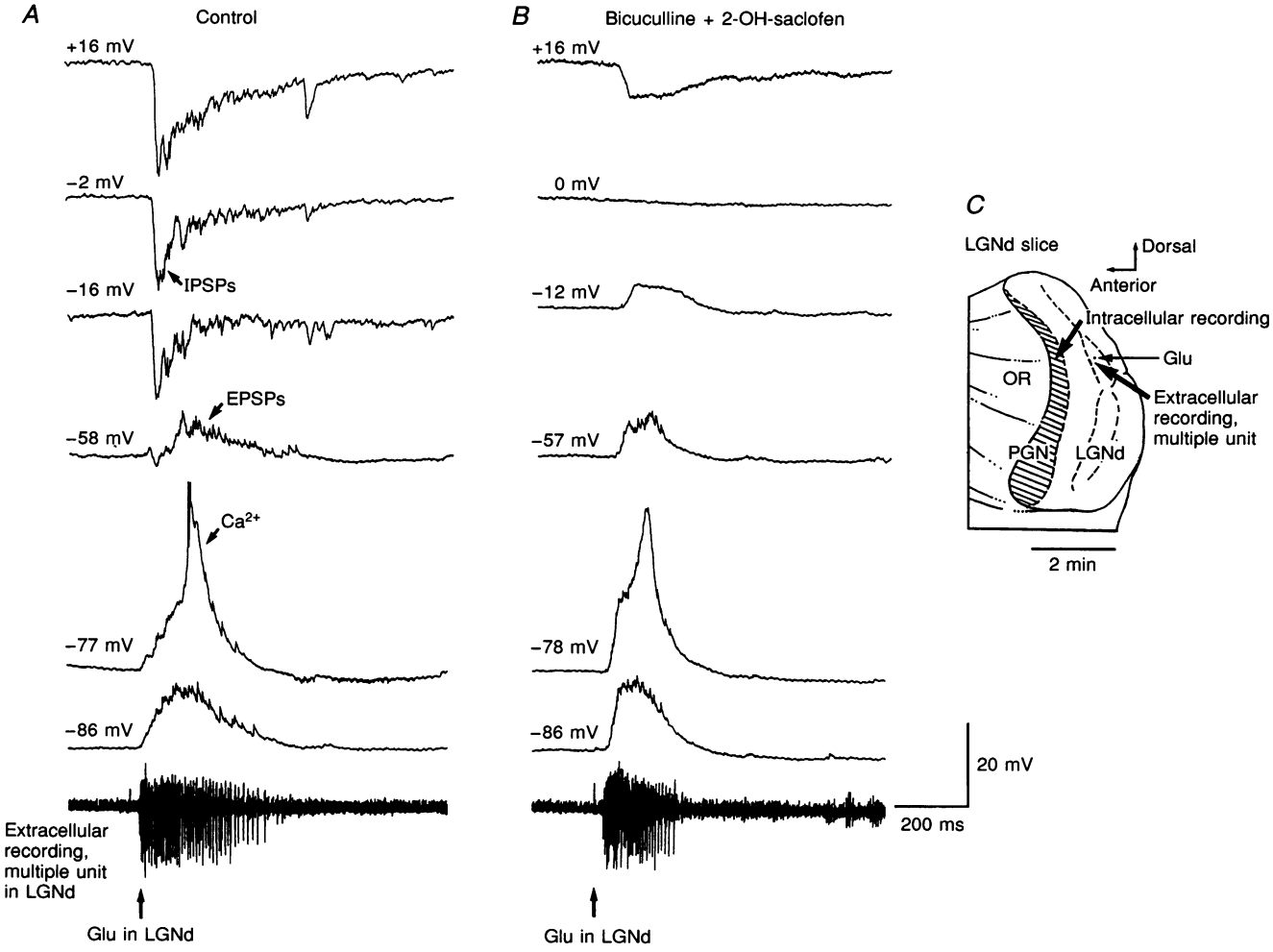

Figure 4. Reversal potential of PSP barrages in PGN neurons evoked by local application of glutamate in the relay lamina

A, brief application of glutamate (500 μM in micropipette) to the A1 lamina of the LGNd resulted in a brief (200–300 ms) barrage of action potentials in the local multiple unit recording. This activation of neurons in the A1 lamina was associated with a barrage of PSPs in the PGN neuron. Depolarization or hyperpolarization of the PGN neuron to different membrane potentials demonstrated that the PSP barrage was associated with both inhibitory and excitatory components (e.g. −58 mV). *B*, block of $GABA_A$ and $GABA_B$ receptors with local application of (−)-bicuculline methiodide (250 μM in micropipette) and 2-OH-saclofen (1 mM in micropipette) resulted in isolation of the EPSP components. Under these circumstances the EPSPs reversed polarity at 0 mV. *C*, illustration of recording and glutamate application arrangement. Intracellular recording microelectrode contained 2 M caesium acetate to reduce K^+ currents and 50 mM QX-314 to block voltage-dependent Na^+ currents.

potential generation in bursting relay neurons (see Bal *et al.* 1995). Hyperpolarization of PGN neurons with intracellular injection of current to −65 to −73 mV enhanced the amplitude and intensity of burst discharges (Fig. 3*D–I*). Further hyperpolarization of PGN neurons to −89 mV or more negative prevented these burst discharges and revealed underlying barrages of PSPs (Fig. 3*J–L*). The amplitude of these barrages of PSPs increased and then decreased during the generation of individual spindle waves (Fig. 3*J* and *K*). The PSP barrages were dominated at resting membrane potentials by EPSPs that consisted of a substantial number of individual components, which ranged widely in amplitude from 0·4 to 13 mV (see Figs 3, 4 and 7).

Depolarization of PGN neurons to approximately −54 mV or more positive during the generation of spindle waves inhibited the occurrence of low threshold Ca^{2+} spikes and moved the neurons into the single spike mode of action potential generation (Fig. 3*A*). At these membrane potentials, PGN neurons generated increased activity during spindle waves, but failed to generate high frequency burst discharges, as expected from the voltage dependence of the low threshold Ca^{2+} current (Fig. 3*B* and *C*).

The origin of the putative synaptic potentials occurring during the generation of spindle waves was examined using simultaneous recording of LGNd and PGN activity, and by examining the voltage dependence, pharmacology and reversal potential of these events. Simultaneous extracellular multiple unit recordings of LGNd activity in the A lamina adjacent to an intracellularly recorded PGN neuron revealed that each barrage of PSPs occurring in PGN cells is associated with a burst of activity in the LGNd, even if the PGN neuron did not generate a low threshold Ca^{2+} spike (see von Krosigk *et al.* 1993). Indeed, extracellular application of glutamate to circumscribed regions of the A laminae resulted in barrages of PSPs in PGN neurons similar or identical to those occurring during spindle waves (Fig. 4). To examine the voltage dependence of the PSPs, PGN neurons were recorded with microelectrodes containing 2 M caesium acetate to reduce K^+ conductances and 50 mM lidocaine *N*-ethyl bromide quarternary salt (QX-314) to reduce active Na^+ conductances. Depolarization of PGN neurons results in a substantial decrease in the amplitude of the barrages of PSPs arriving during the spontaneous generation of spindle waves or to those activated by brief pulses of glutamate in the A laminae (Figs 4*A* and 5). Under these conditions, the reversal potential of these barrages of PSPs varied from cell to cell from −30 to 0 mV ($n = 12$; Figs 4*A* and 5). The PSP barrages occasionally exhibited short duration (6–13 ms) individual events that had a rapid hyperpolarizing phase, suggesting that the PSP barrages may represent a mixture of EPSPs and IPSPs (Figs 4*A* and 5). Indeed, tracing of the processes of biocytin-labelled PGN cells revealed putative axonal collaterals within the PGN, suggesting that PGN cells may contact one another, as previously demonstrated in the cat (Yen, Conley, Hendry & Jones, 1985; Uhlrich, Cucchiaro, Humphrey & Sherman, 1991).

In order to block the influence of GABAergic inhibition in the PGN, the $GABA_A$ receptor antagonist (−)-bicuculline methiodide was applied either locally with a broken micropipette (200–250 μM) or in the bathing medium (20–25 μM). Under these conditions, the PSP barrages evoked by brief application of glutamate in the A laminae exhibited a reversal potential of around 0 mV, indicating that these barrages represent EPSPs (Fig. 4*B*). The voltage dependence of these EPSP barrages suggested that the activation of non-NMDA receptors makes a strong contribution to their generation (i.e. they did not exhibit a region of 'negative slope conductance'; Fig. 4*B*). Application of the $GABA_B$ receptor antagonist 2-OH-saclofen (0·2–1 mM) did not result in any observable effects on the EPSPs, IPSPs or reversal potential of PSP barrages in PGN neurons ($n = 2$).

Inhibitory events were also clearly evident in some PGN neurons during the generation of spindle waves, especially at depolarized membrane potentials (Figs 5 and 6). Interestingly, these PSP barrages appeared to be dominated by EPSPs followed by IPSPs, giving a spike-like appearance at some membrane potentials (e.g. Fig. 5*B* and *C*). This result is consistent with the idea that the EPSPs arise from the burst firing of LGN cells and the IPSPs arise from the subsequent burst firing of neighbouring PGN neurons surrounding the recorded PGN cell. If the PSP barrages that exhibited inhibitory components are overlaid with ones that did not, or ones that were recorded at or negative to the equilibrium potential for Cl^-, substantial overlap is revealed between these PSP events (Fig. 5*B–D*, parts *a* and *b*, and *c* and *d*). The hyperpolarizing phase of the PSP barrages often exhibited individual hyperpolarizing components occurring at the same frequency with which PGN cells burst (350–500 Hz), as if the PGN neuron was being inhibited by a burst of action potentials in a neighbouring PGN neuron (Fig. 5*E*).

The rapid rate of rise and short duration of the IPSPs occurring in PGN neurons during spindle wave generation suggested that these events may be mediated through the activation of $GABA_A$ receptors. Local application of (−)-bicuculline methiodide (200 μM in micropipette) to the PGN resulted in a marked suppression of these IPSPs (Fig. 6). Comparison of the PSP barrages before and after application of bicuculline revealed that the IPSPs and EPSPs overlap substantially in time, with the EPSP barrages becoming substantially

larger in amplitude after suppression of $GABA_A$ receptor-mediated IPSPs (Fig. 6*D*). Even though we observed IPSPs occurring in PGN neurons during spindle wave generation, these IPSPs were not followed by the rebound low threshold Ca^{2+} spikes that underlie the spike bursts responsible for spindle wave generation. Instead, close examination of the events immediately preceding the low threshold Ca^{2+} spikes during spindle wave generation in the ninety-three intracellularly recorded PGN neurons revealed that the Ca^{2+} spikes were triggered by barrages of depolarizing PSPs. The reasons why IPSPs occurring in PGN neurons did not generate rebound bursts of action

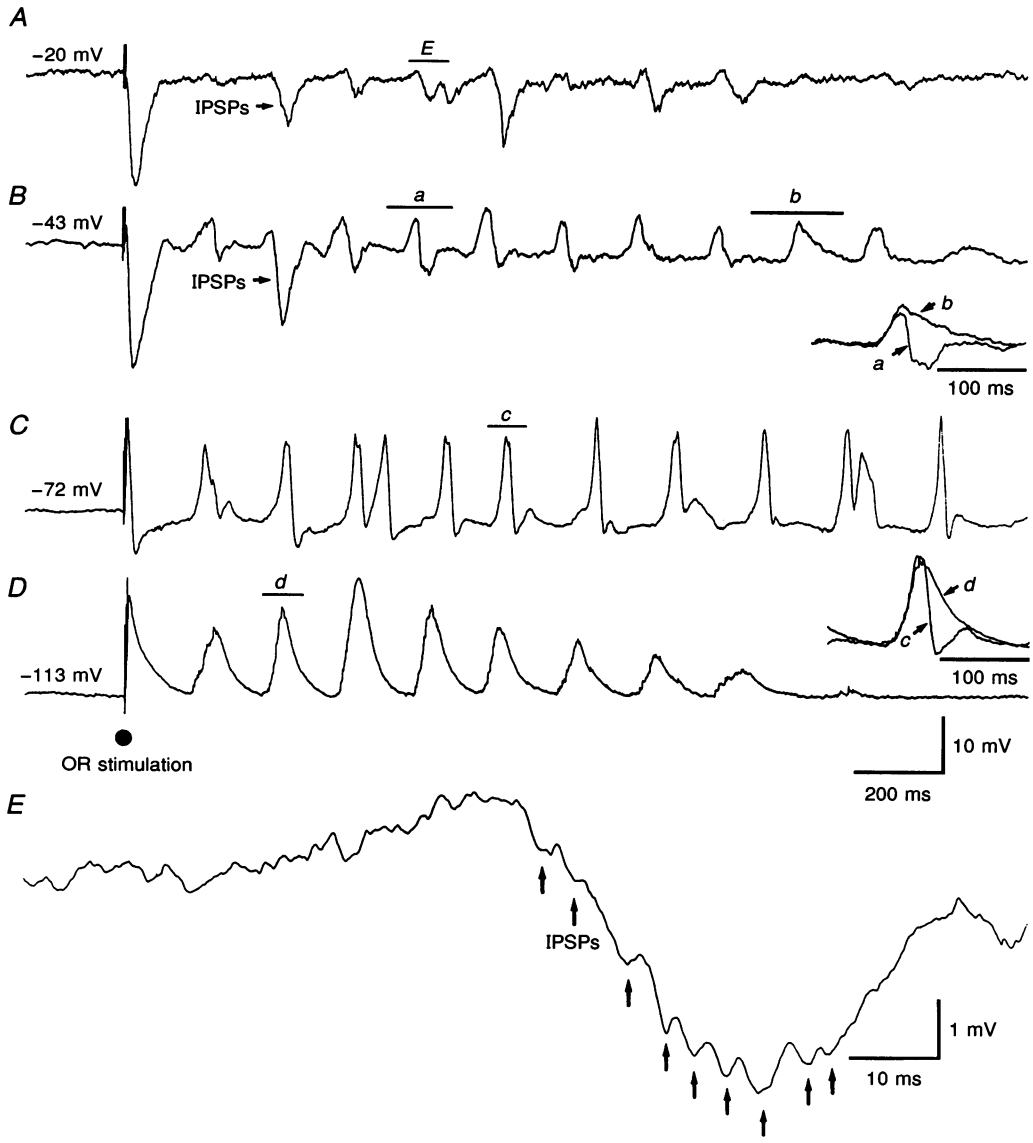

Figure 5. Inhibitory postsynaptic potentials are intermixed with EPSPs in PGN neurons during spindle wave generation

Intracellular recording of a PGN neuron at different membrane potentials during spindle wave generation. At −72 mV the PSP barrages arriving with each cycle of the spindle wave are found to have first a depolarizing, followed by a hyperpolarizing component. Evoking spindle waves at different membrane potentials illustrates the voltage dependence of these different components. Overlapping PSP barrages that either do or do not exhibit clear hyperpolarizing components illustrate the temporal overlap of these (*Ba* and *b*). Similarly, overlapping PSP barrages before and after reversal of the hyperpolarizing component with current injection are illustrated in *D* (traces *c* and *d*). *E*, often the hyperpolarizing components of the PSP barrages are associated with repetitive IPSPs (arrows) arriving at a frequency similar to action potential generation during burst firing in PGN neurons. Intracellular recording microelectrode contained QX-314 (50 mM) and caesium acetate (2 mM). Each spindle wave in this cell was evoked by stimulation of the optic radiation (OR) containing corticothalamic fibres (denoted by filled circle and consisting of 5 shocks at 200 Hz).

potentials are not yet known, although their short duration and intermixing with barrages of EPSPs may have contributed.

Intracellular recording of PGN neurons with caesium acetate- ($n = 4$) and potassium acetate-filled ($n = 4$) microelectrodes revealed on several occasions reciprocal functional connections between the PGN and excitatory inputs, presumably arising from relay cells. Intracellular injection of a depolarizing current pulse into the PGN neuron generated a low threshold Ca^{2+} spike and associated high frequency burst discharge. This burst discharge was followed at a latency of 140–150 ms by a barrage of EPSPs (Fig. 7*B*), presumably arising from a burst of action potentials in one or more relay cells. Indeed, the EPSP barrages were identical in structure to burst discharges typical of LGNd relay cells, in that there were three to five EPSPs per barrage occurring at a frequency of 250–350 Hz, with the inter-EPSP interval lengthening as the EPSP barrage occurred (Fig. 7*D–F*). At certain membrane potentials, these EPSP barrages could activate a low threshold Ca^{2+} spike and a high frequency burst of action potentials, although this was not followed by a subsequent barrage of EPSPs, presumably owing to the tendency of relay cells to

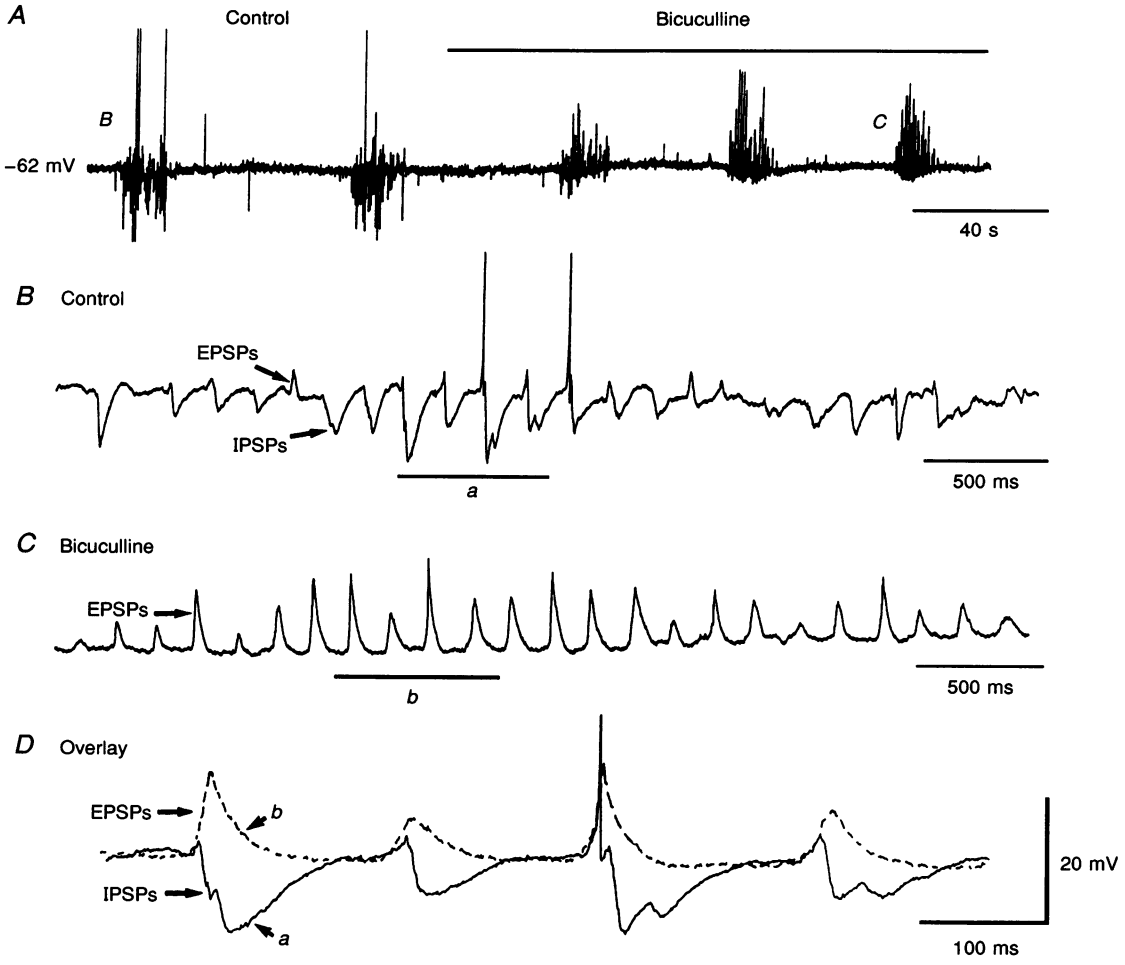

Figure 6. Inhibitory postsynaptic potentials in PGN neurons are blocked by local application of (−)-bicuculline methiodide

A, intracellular recording of synaptic barrages in a PGN neuron at resting membrane potential of −62 mV. This PGN neuron exhibited particularly pronounced inhibition during spindle wave generation. *B*, expansion of one spindle wave reveals both depolarizing (EPSPs) and hyperpolarizing (IPSPs) components. Local application of (−)-bicuculline methiodide (250 μM in micropipette) completely blocks the hyperpolarizing components and leaves the depolarizing EPSPs (*A* and *C*). *D*, overlaying the traces before and after application of (−)-bicuculline methiodide illustrates the influence of the hyperpolarizing PSPs in PGN neurons. Intracellular recording microelectrode contained QX-314 (50 mM) to block voltage-dependent Na^+ conductances and caesium acetate (1·5 M) and potassium acetate (1·5 M). Fast action potentials were considerably broadened in duration, shortened in amplitude, and occurred only irregularly, both before and after application of (−)-bicuculline methiodide (not shown).

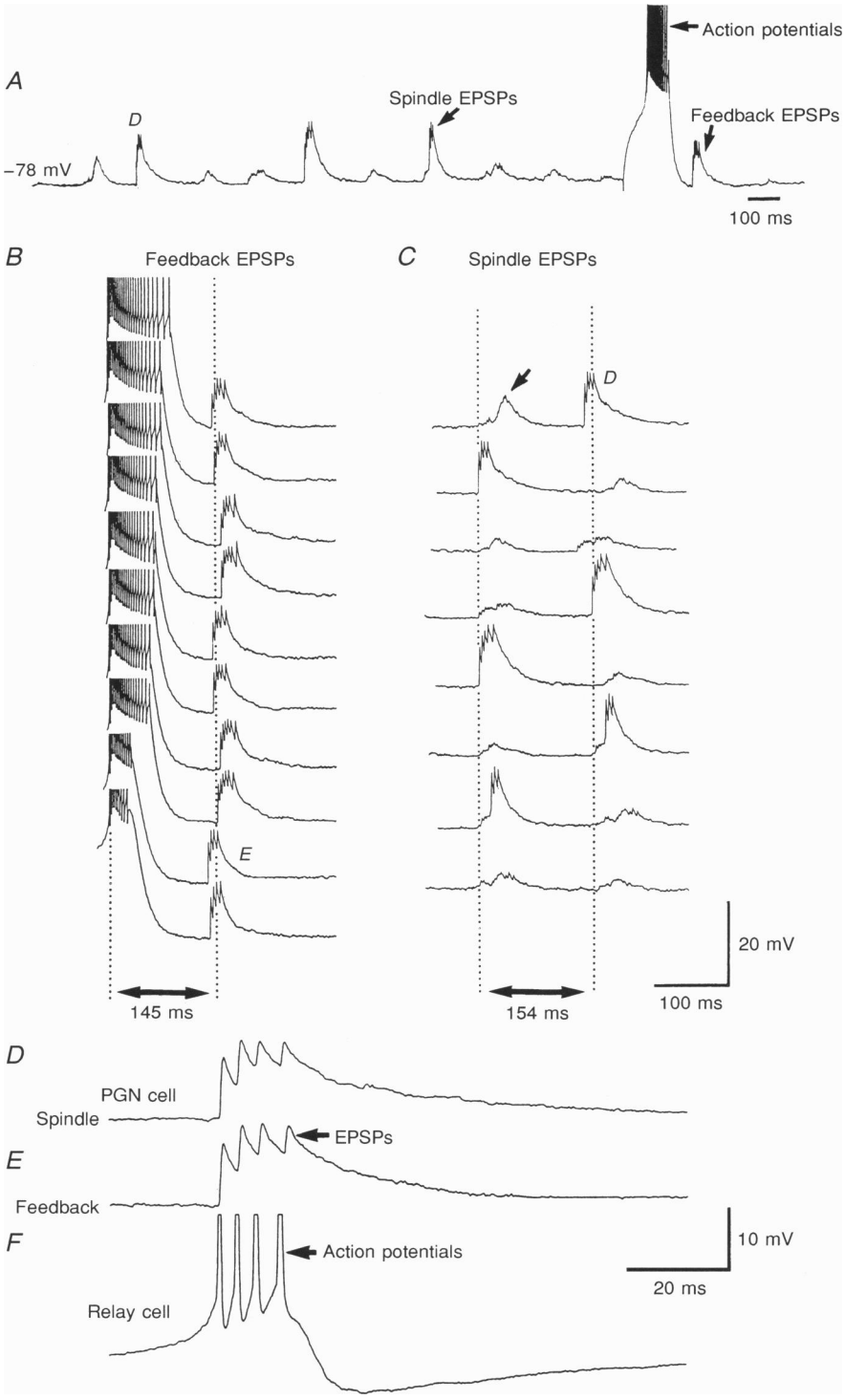

Figure 7. Activation of a PGN neuron results in a burst of feedback EPSPs

A, intracellular recording of a PGN neuron reveals barrages of EPSPs during the generation of a spindle wave. Intracellular injection of a depolarizing current pulse results in a burst of action potentials followed by a barrage of feedback EPSPs (*A*, right). *B*, illustration of feedback EPSPs associated with generation of low threshold Ca^{2+} spikes and bursts of high frequency action potentials in this PGN neuron. The dotted vertical line represents the average latency in this cell of 145 ± 3 ms (S.D.) from the first action potential to the beginning of the barrage of EPSPs. Examination of the interval between barrages of EPSPs in the same neuron during spindle waves revealed an average interval of

generate rebound burst discharges only once every 250–1000 ms (Bal *et al.* 1995). The latency from the first action potential generated in the PGN cell to the occurrence of the EPSP barrage varied on a trial-by-trial basis and ranged in the cell of Fig. 7 from 140 to 150 ms (Fig. 7*B*). Examination of the PSPs occurring in the same PGN cells during spontaneous spindle waves revealed barrages of EPSPs similar to those occurring after generation of a current pulse-induced burst discharge in these cells (cf. Fig. 7*D* and *E*). However, during spindle wave generation, these EPSPs often occurred without the presence of a burst of action potentials in the recorded cell during the previous barrage of EPSPs (Fig. 7*A*), indicating that the recorded neuron is not the only PGN neuron innervating the relay cell giving rise to these EPSPs. The interval between arrival of EPSP barrages occurring during spontaneous spindle waves (154 ms; $n = 75$ intervals) was similar to that associated with a burst of spikes in the single PGN cell (145 ms; $n = 33$ intervals; compare Fig. 7*B* and *C*), although the standard deviation in the interval between EPSP barrages during spindle waves (23 ms) was greater than that occurring in response to burst discharge in the single neuron (3 ms). These data suggest that the intraspindle frequency of 6–9 Hz is determined by the time required for a burst of activity in the PGN to inhibit relay cells and generate a rebound burst of action potentials and subsequently to excite neurons in the PGN again.

Presumably, these recurrent EPSPs are generated through inhibition of a number of relay cells, one or more of which rebound bursts following this inhibition, as seen during generation of spindle waves (see Bal *et al.* 1995). Indeed, we found that this functional connection between a single PGN neuron and relay cells was remarkably strong, with the activation of a burst discharge in a single PGN neuron being capable of initiating a spindle wave in the slice (not shown). In the cell of Fig. 8, for example, injection of variable duration depolarizing current pulses so as to result in the generation of different numbers of action potentials revealed that only two action potentials in the PGN neuron are required for feedback EPSPs to occur (Fig. 8, traces labelled 2). Increasing the number of action potentials from two to eleven, which is approximately the number of action potentials occurring during a normal burst discharge, increased the intensity of feedback EPSPs such that they were capable of reaching firing threshold and generating a series of action potentials. These action potentials were not followed by another barrage of EPSPs, presumably owing to the tendency of thalamic relay cells to burst only once every 250–1000 ms (Bal *et al.* 1995). Occasionally, trains of action potentials induced by depolarizing current pulses were followed by a depolarizing after-potential (Fig. 8, arrows in traces 4, 5 and 6) that may represent the activation of the Ca^{2+}-activated non-selective cation current known to be prevalent in these cells (Bal & McCormick, 1993).

The voltage dependence and amplitude time course of the EPSPs arriving in PGN neurons occurring during spindle waves suggest that they are mediated, as least in part, by non-NMDA receptors. Indeed, bath application of the NMDA receptor antagonist AP-5 or D-AP-5 ((±)- or D-(−)-2-amino-5-phosphonopentanoic acid, respectively; 60–100 μM) did not block the generation of spindle waves ($n = 7$; Fig. 9*A* and *B*). In contrast, bath application of the AMPA–kainate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 25 μM) resulted in a complete block of both spontaneous and optic radiation stimulation-evoked spindle waves in both the PGN and relay lamina (Fig. 9*B* and *C*; $n = 14$ experiments). Similarly, local application of CNQX into the PGN also resulted in a complete block of spindle waves ($n = 4$). This block of spindle waves presumably results from the block of synaptic transmission between relay neurons and PGN neurons. Indeed, local application of CNQX (250 μM) to the PGN greatly reduced the amplitude of feedback EPSPs following a current pulse-induced burst of action potentials in these GABAergic neurons (Fig. 9*D*). These results also indicate that AMPA–kainate receptors make a critical contribution to the generation of these feedback EPSPs (Fig. 9*D*).

Measurements of the durations of the different components of spindle waves in a representative PGN neuron revealed that the average interval from the first spike in the burst discharge to the onset of the EPSPs in the next cycle was 127 ± 8·9 ms (all values are ± S.D.; $n = 29$ intervals; Fig. 10). There was subsequently an average interval of 26 ± 7·0 ms ($n = 36$ measurements) between the beginning of the EPSPs and the generation of the first action potential of the next burst discharge

154 ± 23 ms (S.D.). Some of the EPSP barrages, both during spindle wave generation and as feedback EPSPs, exhibited 3–6 distinct peaks that occurred in a temporal manner typical for burst generation in relay neurons (*D–F*). Other PSP barrages exhibited individual EPSPs that were much smaller in amplitude (see traces in *C*). Data collected in between spindle wave generation after hyperpolarization to −78 mV. Postsynaptic potentials were identified as excitatory by their ability to activate action potentials at depolarized levels.

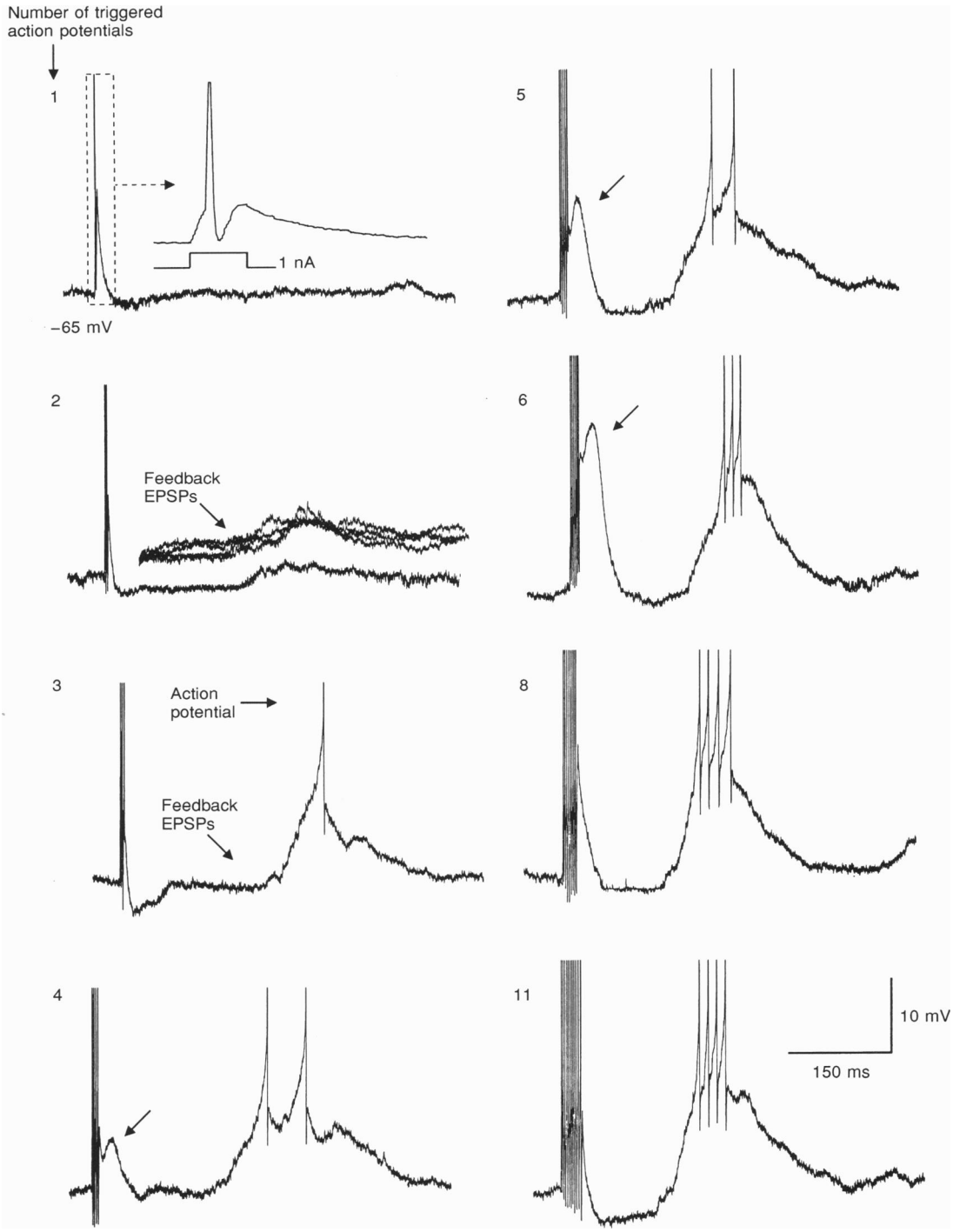

Figure 8. Dependence of feedback EPSPs on the number of action potentials generated by a PGN neuron

Intracellular injection of a depolarizing current pulse into a PGN neuron was used to activate a variable number of action potentials and to observe the resulting recurrent barrage of PSPs. Activation of a single action potential (see expanded trace) in the PGN neuron was not followed by recurrent PSPs, although increasing the duration of the current pulse in order to increase the number of action potentials to two was associated with feedback PSPs (traces labelled 2). Five examples of the recurrent PSPs are illustrated. Increasing the number of action potentials generated by the PGN neuron from 3 to 11 increases the amplitude of the recurrent PSPs such that they come to generate a burst of action potentials in the recorded PGN neuron. All records were obtained at the membrane potential of −65 mV.

(Fig. 10). Burst discharges in this PGN neuron averaged 28·2 ± 9·3 ms ($n = 36$) in duration. Thus, the longest component of the intraspindle interval was the time between action potential generation in the PGN neuron and the subsequent arrival of EPSPs (127 ms), although the latency from arrival of EPSPs and generation of the burst of action potentials in this neuron (26 ms) was also significant. These intervals are compared with the duration of IPSPs and burst discharges of a typical relay neuron in Fig. 10 (see Bal *et al.* 1995). In the accompanying study (Bal *et al.* 1995) we found that a burst of action potentials in a single relay neuron results in burst firing in a single PGN neuron at a substantially longer latency than found here during normal spindle wave generation (52 *vs.* 26 ms). The shortening of the interval during normal spindle wave generation presumably results from the additivity of convergent inputs from a number of relay cells onto each PGN neuron.

Bicuculline-induced slowed oscillation

As reported in the accompanying paper (Bal *et al.* 1995), bath application of (−)-bicuculline methiodide (5–50 μM) resulted in a marked slowing of the intraspindle frequency from 6–9 to 2·5–4 Hz. Extracellular single and multiple unit and intracellular recording from PGN neurons during the bath application of bicuculline revealed these cells to greatly increase both the number and frequency of action potentials generated per burst during the synchronized oscillations. In the cell of Fig. 11, for example, normal spindle waves were associated with a progressive hyperpolarization of PGN neurons and a subsequent increase in the number of action potentials generated in each burst discharge from two to

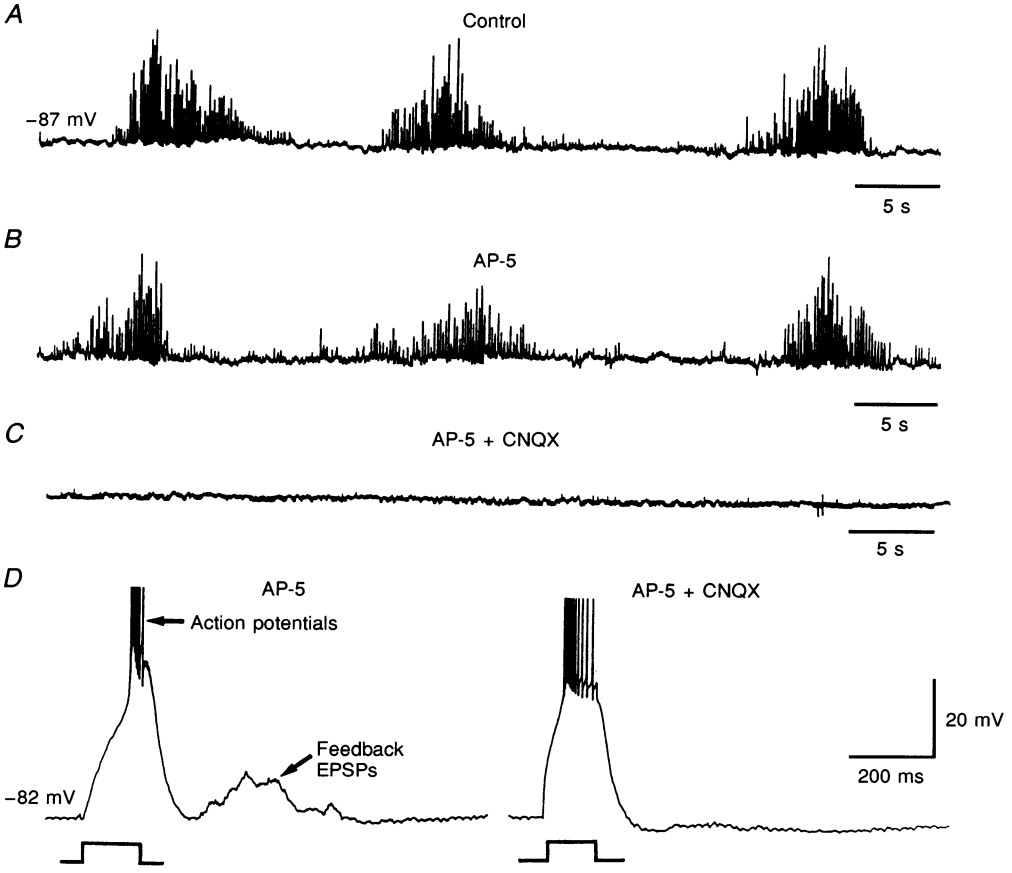

Figure 9. Activation of non-NMDA receptors underlies feedback EPSPs and spindle wave generation in the PGN

A, intracellular recording of a PGN neuron illustrating barrages of PSPs associated with spindle wave generation, after hyperpolarization to −87 mV with intracellular current injection to inhibit burst firing. *B*, bath application of the NMDA receptor antagonist AP-5 (60–100 μM) did not abolish spindle wave generation or the barrages of PSPs, nor did it consistently affect the interspindle period (not shown). *C*, in contrast, local application of the non-NMDA receptor antagonist CNQX (250 μM in micropipette) resulted in abolition of PSP barrages and spindle wave generation. *D*, similarly, bath application of AP-5 did not clearly affect feedback PSPs, while local application of CNQX greatly reduced their amplitude, indicating that these PSPs are mediated largely through activation of non-NMDA excitatory amino acid receptors.

approximately seven (Fig. 11*B* and *C*). In contrast, after bath application of (−)-bicuculline methiodide (25 μM), the burst discharges were significantly stronger, with even the first burst in the oscillation being composed of thirteen action potentials and later bursts increasing up to thirty action potentials (Fig. 11*F* and *G*; up to 60 action potentials were recorded in other cells). These increases in burst discharges were due in part to the increased amplitude and duration of the EPSP barrages (Fig. 11*D* and *H*) and a small hyperpolarization of the PGN neuron (Fig. 11*A* and *E*). Bath application of 2-OH-saclofen (250 μM) completely blocked these slowed oscillations, indicating that they were mediated through the activation of $GABA_B$ receptors ($n = 15$). The induction of repetitive burst discharges through the intracellular injection of current pulses was still associated with a slow after-hyperpolarization, indicating that this potential is not mediated through the activation of $GABA_A$ or $GABA_B$ receptors. Interestingly, the early portions of this progressive hyperpolarization were associated with an

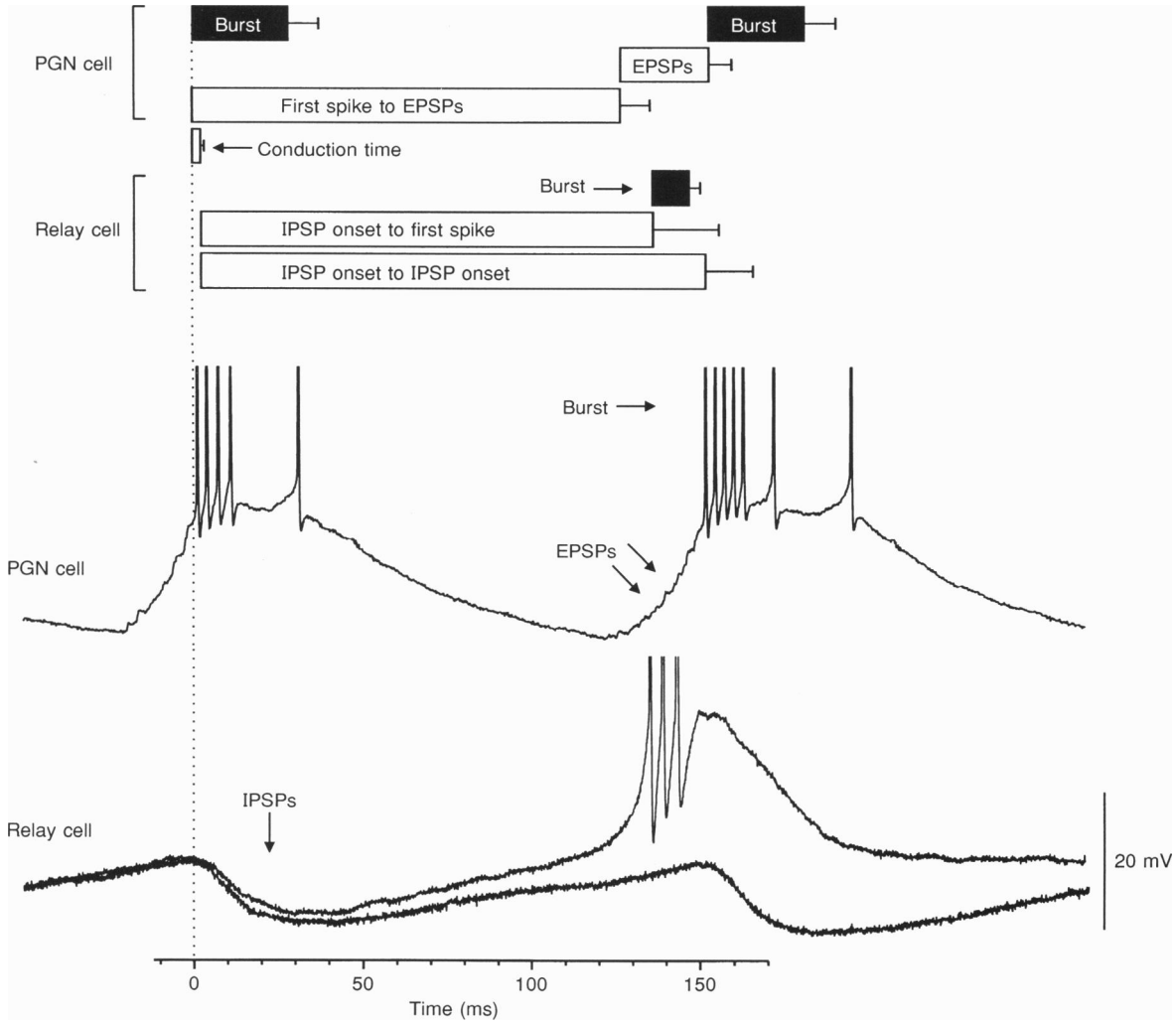

Figure 10. Temporal characteristics of the different components of spindle wave generation in a representative PGN and LGNd relay neuron

The burst of action potentials in this PGN neuron lasted an average of 28 ms, and was followed by EPSPs at a latency of 127 ms. The EPSPs averaged 26 ms in duration before another burst of action potentials was generated. In the relay neuron, the IPSP associated with spindle wave generation was 134 ms in duration if it was followed by a rebound Ca^{2+} spike, and 149·5 ms in duration if it was not. The burst of action potentials in this relay neuron was an average of 11 ms in duration. The conduction time between the PGN and relay was measured from simultaneous extracellular and intracellular recordings (see Bal *et al.* 1995). The EPSPs begin in the PGN cell before the average onset of the burst of action potentials in the relay neuron owing to the variation in onset of these action potential bursts and the convergence of several relay neurons onto the PGN cell. These illustrated recordings are from two separate experiments. Means with one standard deviation are illustrated.

increase in intensity of burst discharges, while the burst discharges became progressively smaller and even failed to occur as the hyperpolarization of the membrane potential continued to increase (Fig. 11*J*). This later effect presumably results from the current pulse being subthreshold for activation of the low threshold Ca^{2+} current.

The importance of the PGN in the generation of the bicuculline-induced slowed oscillation was examined through the local application of CNQX in the PGN ($n = 3$). Local application of CNQX (250 μM in micropipette) completely blocked the generation of the slowed oscillation in both the PGN and LGNd immediately adjacent to these manipulations, as we have previously reported for spindle wave generation (von Krosigk *et al.* 1993). These results confirm the critical role of the PGN in the generation of the slowed oscillation. However, PGN neurons may not be the only GABAergic neurons participating in the slow oscillation. Intracellular recordings from an interlaminar neuron during the bath application of (−)-bicuculline methiodide (20 μM) revealed a marked increase in the amplitude of PSP barrages and bursts of action potentials in this neuron (not shown), in a manner similar to that of PGN neurons (Bal *et al.* 1995).

DISCUSSION

The nucleus reticularis thalami is a shell-shaped structure in which nearly all, if not all, neurons are GABAergic (Houser, Vaughan, Barber & Roberts, 1980; Jones, 1985). Nucleus reticularis neurons are innervated by axon collaterals of corticothalamic and thalamocortical fibres and innervate thalamic relay neurons in a topographic manner (Friedlander, Lin, Stanford & Sherman, 1981; Ohara & Lieberman, 1985; Harris, 1987; Crabtree &

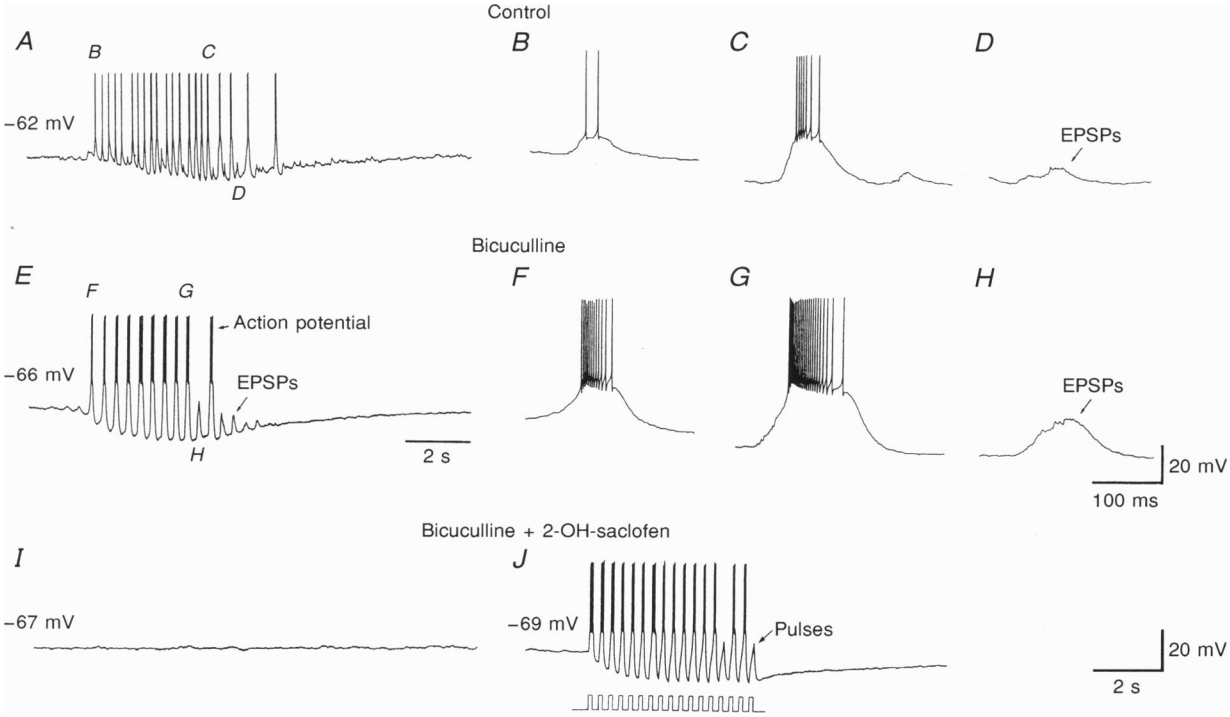

Figure 11. Bicuculline enhances and slows the frequency of repetitive burst firing in PGN neurons during the generation of synchronized oscillations

A, intracellular recording of a PGN neuron at resting membrane potential (−62 mV). During the generation of a spindle wave, the PGN neuron progressively hyperpolarizes, which enhances repetitive burst firing in this neuron (compare *B* and *C*). At the end of the spindle wave, the PSP barrages fail to activate a low threshold Ca^{2+} spike (*A*, PSPs expanded in *D* for detail). Bath application of the $GABA_A$ receptor antagonist (−)-bicuculline methiodide (25 μM) results in a slowing of the frequency of network oscillation and markedly enhances burst firing in the PGN neuron (*E–G*). Again, the EPSP barrages fail to activate low threshold Ca^{2+} spikes at the end of the synchronized oscillation (*E*). The underlying EPSP barrages are larger in amplitude than those before bicuculline application. Bath application of the $GABA_B$ antagonist 2-OH-saclofen results in abolition of this slowed oscillation (*I*). Intracellular injection of short duration depolarizing current pulses generated repetitive bursts of action potentials and a prolonged after-hyperpolarization. The progressive hyperpolarization brought the response to the depolarizing current pulses below threshold for generation of low threshold Ca^{2+} spikes (*J*).

Killackey, 1989; Uhlrich *et al.* 1991). The perigeniculate nucleus (PGN) is similar to the nucleus reticularis thalami and is intimately interconnected with the dorsal lateral geniculate nucleus. In the ferret, the PGN is especially pronounced, forming a thick inner shell of GABAergic neurons that lies inside the LGNd and is reticulated by corticothalamic and thalamocortical fibres.

Previous investigations of the cellular basis of spindle wave generation have revealed the nRt to contribute critically to this synchronized activity. Extracellular and intracellular recordings of nRt neurons have demonstrated that these cells discharge in high frequency bursts of action potentials during the generation of spindle waves (Mulle *et al.* 1986; Steriade *et al.* 1986). These action potential bursts are mediated in part through the activation of a low threshold Ca^{2+} spike and occur at a rate identical to that of the occurrence of IPSPs in thalamic relay neurons (Deschênes *et al.* 1984). Chemical lesions of the nRt, or physical separation of the nRt from the rest of the thalamus, results in abolition of spindle wave generation in both the thalamus and cerebral cortex (Steriade *et al.* 1985; Buzsáki *et al.* 1988), although isolation of the nRt from the body of the thalamus has been reported to leave local spots within the nRt that still spontaneously generate brief periods of synchronized activity in the upper frequency range of spindle waves (12–15 Hz; Steriade *et al.* 1987). Based upon this, and other data, two possible mechanisms of generation of spindle waves have been proposed: (1) the interaction of GABAergic neurons of the nRt in which the dendrodendritic and axonal connections between these neurons generate a synchronous oscillation, perhaps through GABAergic IPSPs and removal of inactivation of low threshold Ca^{2+} spikes (Deschênes *et al.* 1985; Steriade *et al.* 1987); and (2) as an interaction between nRt and relay neurons in which the nRt neurons are activated by EPSPs generated by burst firing in relay neurons of the thalamus (Mulle *et al.* 1986; Buzsáki, 1991). These two hypotheses are not mutually exclusive, and both mechanisms may be involved (see Steriade *et al.* 1990, 1993). Our present results indicate that the second hypothesis contributes strongly to the generation of spindle waves in the ferret LGNd *in vitro*. Intracellular recordings from PGN neurons during the generation of spindle waves reveal that the large majority of bursts of action potentials are mediated by low threshold Ca^{2+} spikes activated by the arrival of barrages of PSPs. That the PSP barrages are typically dominated by EPSPs generated by burst firing in thalamic relay neurons is indicated by several findings. First, the PSP barrages occurring during spindle waves display a reversal potential that is often rather positive to the reversal potential of IPSPs. Second, the spindle wave-associated barrages of PSPs are similar to those evoked by activation of relay cells through direct glutamate application in the LGNd. Third, the internal structure of the EPSP barrages occurring during spindle waves was often similar to the pattern of action potential generation in bursting relay neurons. Fourth, there is a high degree of synchrony of arrival of these EPSPs and action potential activity in the neighbouring A laminae (von Krosigk *et al.* 1993). Finally, the PSP barrages are blocked by bath or local application of the AMPA–kainate receptor antagonist CNQX.

The reversal potential of the PSP barrages is often negative to the expected reversal potential for pure EPSPs. This reversal potential is typically brought to more positive levels by local application of bicuculline, indicating that IPSPs are intermixed with the EPSPs. These IPSPs presumably arrive from burst firing in neighbouring PGN neurons. Indeed, depolarization of many PGN neurons with the intracellular injection of current revealed depolarizing followed by hyperpolarizing barrages of PSPs. These results are most consistent with the initial stages of the PSP barrages being dominated by burst firing in thalamic relay neurons and the subsequent burst of action potentials in PGN cells inducing IPSPs in neighbouring PGN neurons through local axonal, and perhaps dendrodendritic, synaptic contacts. Thus, the activity of individual PGN neurons is determined by a complex balance not only of extra-PGN afferents, but also by the pattern of activity generated within the PGN itself.

Intracellular investigation of the electrophysiological properties of nRt and PGN neurons has demonstrated that these cells have the intrinsic propensity to generate rhythmic bursts of action potentials in the frequency range of 1–12 Hz, with the lower frequencies being generated at more hyperpolarized membrane potentials (Avanzini *et al.* 1989; McCormick & Wang, 1991; Bal & McCormick, 1993). The ability of nRt and PGN cells to generate rhythmic burst firing in the frequency range of spindle waves allows these cells to participate in a relatively high percentage of cycles within each spindle wave, since the intrinsic frequency of oscillation preferred by the nRt/PGN cell matches that generated by the circuit (e.g. 6–10 Hz). This ability to follow each cycle of the spindle waves by single nRt/PGN neurons suggests that the majority of nRt or PGN cells generate burst firing during each phase of the spindle wave (at least at the height of the spindle wave), thereby giving rise to strong convergence of inhibitory input onto thalamic relay cells.

During the generation of spindle waves in PGN neurons *in vitro*, a majority of these cells are found to hyperpolarize progressively, resulting in a prominent after-hyperpolarization following the spindle wave activity. We suggest that this progressive hyperpolarization of PGN neurons during spindle wave generation may modulate the occurrence of low threshold Ca^{2+} spikes such that the Ca^{2+} spikes are at first progressively enhanced

through the removal of inactivation of the low threshold Ca^{2+} current, thereby facilitating the growth or 'waxing' of the spindle wave. Additional mechanisms involved in the growth of spindle waves are likely to involve the progressive recruitment of neighbouring PGN and relay neurons into the oscillation through the divergence of axonal connections. Indeed, we have recently found through simultaneous extracellular recordings from multiple ($n = 2$ to 8) locations in the LGNd slice that spindle waves travel along the long axis (dorsal–ventral) of the slice as a wave of activity and disappear from the slice in between spindle waves (Kim, Bal & McCormick, 1994). This result indicates that spindle waves grow in strength as more and more neurons are recruited into this oscillation as the spindle wave passes the point of intracellular recording. At the end of the spindle wave, the hyperpolarization becomes larger and reduces the ability of the PSP barrages to reach threshold for generation of low threshold Ca^{2+} spikes and lowering the intrinsic frequency at which PGN cells prefer to generate burst discharges (Bal & McCormick, 1993). These two features may lead to a decrease in participation of PGN neurons in the spindle wave, a decrease in the IPSPs generated in thalamic relay neurons, and presumably contribute to the failure or 'waning' of the spindle wave. Other potential contributors to the end of the spindle wave include changes in GABAergic PGN–relay cell synaptic transmission, such as changes in Cl^- equilibrium potential (e.g. Thompson & Gähwiler, 1989), decreases in excitatory transmission between relay cells and PGN neurons, and changes intrinsic to the participating neurons, such as Ca^{2+}-dependent alterations in ionic currents (see Destexhe, McCormick & Sejnowski, 1993). Indeed, we have recently found that the ability of bursts of action potentials in single PGN neurons to generate return EPSPs is compromised immediately following the generation of a spindle wave (T. Bal & D. A. McCormick, unpublished observations), suggesting that the waning of spindle waves is associated with failure of the PGN–relay cell–PGN loop. These, and other, possibilities can be best examined through dual intracellular recordings from synaptically coupled pairs of relay and PGN neurons during the generation of spindle waves.

Previous intracellular recordings of nRt activity in the cat *in vivo* during spindle wave generation have yielded results that in many ways are similar to those found in the ferret LGNd *in vitro*, and in some ways different. In contrast to the hyperpolarization observed here in PGN neurons, *in vivo* intracellular recordings often show a steady depolarization of nRt neurons during the first half of the spindle wave followed by a steady repolarization back to the prespindle membrane potential during the second half of the spindle wave (Mulle *et al.* 1986), although small hyperpolarizing components during evoked spindle waves have also been found (Contreras, Curró Dossi & Steriade, 1993). In addition, extracellular recordings from nRt neurons during natural slow wave sleep reveal that these cells often end a spindle wave with the generation of a tonic discharge of action potentials, suggesting membrane depolarization (Steriade *et al.* 1986). Possible explanations for these differences include: (1) a difference in state of the GABAergic nRt/PGN neurons in the anaesthetized cat, the unanaesthetized cat, and the isolated ferret LGNd *in vivo* and *in vitro* such that neurons express different ionic conductances under the different conditions; (2) the higher frequencies (7–14 Hz) of spindle wave generation in the cat nRt and the more intact network *in vivo* results in temporal summation of EPSPs that is not prominent at the lower frequencies (6–8 Hz) of spindle wave generation in the ferret LGNd *in vitro*; (3) a lack of observation of the slow hyperpolarization with intracellular recordings *in vivo* is the result of a slightly compromised integrity of membrane input resistance (see Results); and (4) a difference in the status of afferent inputs (e.g. cortical, brainstem). Additional experiments clearly are needed to address this issue. One possible way to test the hypothesis that nRt/PGN neurons progressively hyperpolarize *in vivo* without recording intracellularly in these neurons would be to examine carefully extracellular recordings of these cells in naturally sleeping animals to see if the bursts of action potentials become progressively larger and then smaller during spindle wave generation (but see Steriade *et al.* 1986). The performance of these and other experiments will be required to explain these differences in the *in vivo* and *in vitro* observations.

Our data suggest that PGN neurons exert a substantial inhibitory influence on one another through the activation of $GABA_A$ receptor increases in Cl^- conductance through their axon collaterals. These inhibitory potentials may also arise from the activity of interlaminar interneurons (Bal *et al.* 1995), although an axonal projection from these cells to the PGN has not been demonstrated. One attractive hypothesis is that the IPSPs help to repolarize the membrane potential of the PGN cell in between bursts of action potentials in order to prepare the cell for the next arrival of EPSPs from the relay cells and the next generation of a low threshold Ca^{2+} spike. In this manner, both the GABAergic interactions between nRt/PGN cells and the activation of these cells by glutamatergic inputs from the relay cells, as well as the intrinsic membrane properties of the nRt/PGN cells, are important in the generation of spindle oscillations. In this manner, we envision a sheet of interconnected nRt/PGN neurons in which the axonal and dendrodendritic interconnections facilitate the generation of spindle waves through the regulation of the falling phase of the EPSP barrages and low threshold Ca^{2+} spike-mediated bursts of action potentials. Indeed, although normally EPSP barrages drive Ca^{2+} spikes in nRt cells, removal of the PSP barrages may allow the nRt neurons to continue to generate rhythmic oscillations through the interaction

between intrinsic membrane properties of each cell (Avanzini *et al.* 1989; Bal & McCormick, 1993) and the interconnections of these cells in a large network. In this interpretation, our findings that block of glutamatergic excitation, or knife cuts between the PGN and LGNd, results in abolition of spindle waves in both the LGNd and PGN (von Krosigk *et al.* 1993) may represent the lack of a large enough network of interconnected GABAergic neurons to generate the spindle-like rhythm that is observed in the isolated nRt *in vivo* (Steriade *et al.* 1987). However, even if this were the case, all other physiological data suggest that under normal conditions, the GABAergic neurons of the nRt/PGN are driven to generate bursts of action potentials by barrages of EPSPs arriving from bursts of action potentials in thalamic relay neurons (Mulle *et al.* 1986; present paper). In this manner, the ability of nRt neurons to generate spindle-like activity at the single cell and local circuit level may facilitate the generation of spindle waves in the intact thalamus by preparing these cells to respond to the intra-spindle rate of arrival of PSP barrages from thalamic relay cells.

An additional role for GABAergic inhibition between PGN neurons may be in the regulation of neuronal responsiveness to the EPSP barrages such that the block of $GABA_A$ receptors results in a marked enhancement of the discharge of PGN cells to each EPSP barrage (e.g. Figs 5, 6 and 12). Indeed, bath application of the $GABA_A$ receptor antagonist bicuculline methiodide results in a marked increase in the discharge of PGN neurons during the generation of each oscillation. This large increase in action potential activity in PGN cells presumably results from disinhibition from neighbouring PGN neurons and a large increase in the intensity of burst discharges in thalamic relay neurons (Bal *et al.* 1995). The increase in PGN discharge presumably contributes to the enhanced activation of $GABA_B$ receptors observed in thalamic relay neurons observed under these conditions. Soltesz, Lightowler, Leresche & Crunelli (1989) have previously demonstrated that block of $GABA_A$ receptors in the absence of the PGN also results in a strong increase in the amplitude of the $GABA_B$ receptor-mediated IPSP. These investigators hypothesized that this effect may be due to disinhibition of intrageniculate interneurons (Crunelli, Haby, Jassick-Gerschenfeld, Leresche & Pirchio, 1988; Soltesz *et al.* 1989). Analysis of synaptic profiles on both intralaminar and interlaminar GABAergic neurons demonstrates that these cells receive GABAergic innervation, and we have recently found that intralaminar interneurons are inhibited by GABA acting through $GABA_A$ receptors (H.-C. Pape & D. A. McCormick, unpublished observation). As far as a non-PGN contribution to activation of $GABA_B$ receptors is concerned, our results suggest that interlaminar, rather than intralaminar, GABAergic neurons are more likely to contribute during the slowed oscillation, since the interlaminar neurons appear to be strongly activating in a manner similar to PGN neurons. However, even if the interlaminar interneurons contributed to the generation of the slowed oscillation, the ability of local antagonism of non-NMDA receptors in the PGN to block the generation of the slowed oscillation indicates that the PGN is critically involved in the generation of this paroxysmal event.

Cellular mechanisms for the generation of spindle waves: a speculative scenario

To illustrate our hypothesis for the generation of spindle waves in thalamocortical circuits, we would like to suggest the following speculative scenario. The transition to slow wave sleep is associated with a decrease in release of neurotransmitters from brainstem and hypothalamic systems, including acetylcholine, norepinephrine, serotonin and histamine (reviewed by Steriade & McCarley, 1990; McCormick, 1992). The combined effect of decreased release of these transmitter substances allows nRt/PGN, thalamic relay and some cortical neurons to hyperpolarize progressively into the burst firing mode of action potential generation. Upon activation of a critical number of inputs into the thalamus, either from the cerebral cortex or from the retina, or as a result of coincidence of spontaneous activity in a critical number of relay or nRt/PGN neurons (which may be a burst of action potentials in only one PGN neuron), a spindle wave is initiated. It is likely that the GABAergic neurons of the nucleus reticularis will be the first to discharge, owing to their sensitivity to afferent fibre activation in the burst firing mode and their large dendritic fields (e.g. Spreafico, Battaglia & Frassoni, 1991). Burst firing in the nRt/PGN will result in the hyperpolarization of a number of relay cells, some of which may rebound burst in response to the depolarizing phase of the IPSP. The total time required for one cycle (PGN to relay and back to PGN) is approximately 100–150 ms and is dominated by the kinetics of the IPSPs generated in relay neurons (Bal *et al.* 1995). As the relay neurons are inhibited, the PGN cells that burst during the first cycle are also being hyperpolarized from the activation of a Ca^{2+}-activated K^+ current (Bal & McCormick, 1993). The rebound burst of action potentials in relay neurons and the subsequent barrage of EPSPs arriving in the nRt/PGN neurons now generates an enhanced burst discharge owing to the increased removal of inactivation of the low threshold Ca^{2+} current. In addition, the divergence of axonal connections from the LGNd to the PGN results in the activation of additional PGN cells that were not activated during the first, initiating burst (Fig. 12, spindle wave). Burst firing in the PGN again hyperpolarizes the relay cells hyperpolarized during the first cycle. However, again, the divergence of axonal connections from the PGN to the LGNd results in additional relay cells also being hyperpolarized, and consequently rebound bursts in a greater number of cells. In this manner, the spindle wave is envisioned to grow in intensity and to travel through

the slice through recruitment of new elements as a 6–10 Hz wave of activity (see also Kim *et al.* 1994).

As the hyperpolarization of nRt/PGN cells progresses during the generation of the spindle wave, neurons begin to fail to respond to the barrages of EPSPs. The decrease in number of PGN cells bursting results in a decrease in the amplitude of the IPSP induced in relay cells and the number of relay cells hyperpolarized. Subsequently fewer relay cells rebound burst on the next cycle, and again even fewer PGN neurons are activated during the subsequent cycle. In this manner, the spindle wave winds down (wanes). The persistence of the after-hyperpolarization following the spindle wave may contribute to the generation of the interspindle period. Other possible factors contributing to the waning of spindle waves include a decrease in ability of burst firing in PGN neurons to generate rebound bursts of action potentials in thalamocortical relay cells (T. Bal & D. A. McCormick, unpublished observations).

Block of $GABA_A$ receptors is envisioned to disinhibit PGN and some types of intra-LGNd interneurons, resulting in pronounced discharges in these cells in response to barrages of EPSPs from relay neurons. These prolonged bursts of action potentials result in the strong activation of $GABA_B$ receptors on relay neurons and a subsequent increase in the amplitude of the rebound burst discharge, which itself leads to an even stronger burst of action potentials in the PGN neurons on the next cycle, thus leading to the generation of a paroxysmal event (Fig. 12, bicuculline-induced slowed oscillation). We suggest,

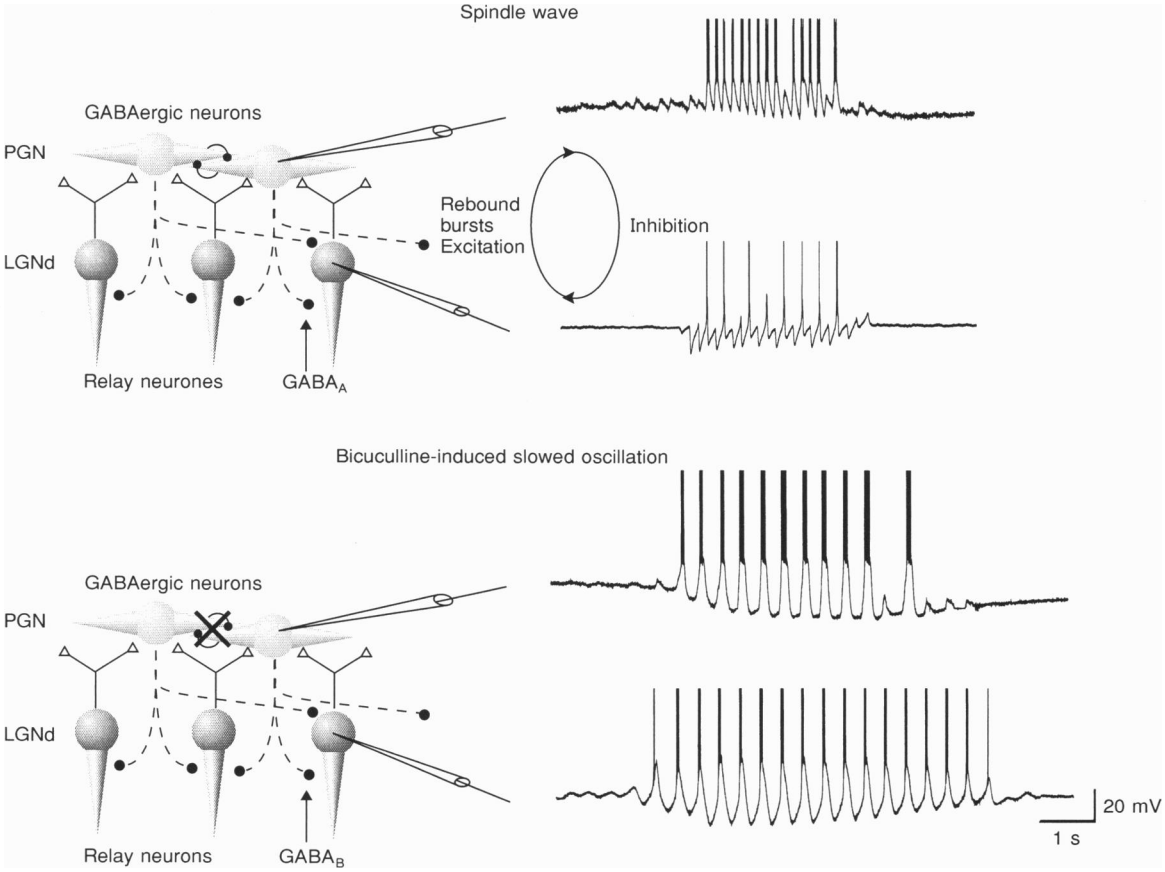

Figure 12. Summary diagram of the circuit properties involved in generation of spindle waves and the bicuculline-induced slowed oscillation

Spindle waves are generated as an interaction between PGN neurons and relay neurons such that PGN neurons inhibit a number of relay neurons, some of which rebound burst following the IPSP. These rebound bursts activate low threshold Ca^{2+} spikes and action potential bursts in PGN neurons. During the spindle wave, PGN neurons progressively hyperpolarize, which increases and then decreases their participation in spindle wave generation. Block of $GABA_A$ receptors with (−)-bicuculline methiodide results in the disinhibition of PGN neurons from one another, increases the intensity of action potential bursts in PGN neurons, increases the activation of $GABA_B$ receptors on relay cells, and subsequently increases the bursts of action potentials in relay neurons. This increase in relay neuron discharge also increases the activation of PGN neurons. In this manner, block of $GABA_A$ receptors results in the generation of a slowed paroxysmal discharge which is critically dependent upon $GABA_B$ receptors for its generation.

therefore, that a key event in the generation of the abnormal thalamocortical discharges associated with absence (spike-and-wave) seizures is the abnormally strong discharge in intrathalamic GABAergic neurons, particularly PGN/nRt cells. This abnormally strong discharge may arise from a number of different factors, including a strong increase in corticothalamic discharge through disinhibition of the cerebral cortex (Gloor, Quesney & Zumstein, 1977; Avoli & Kostopoulos, 1982), strong increases in relay or PGN/nRt neuronal discharge through hyperpolarization or changes in intrinsic properties, and finally, as suggested here, through disinhibition of intrathalamic inhibitory mechanisms. A thorough investigation of all possibilities is required before the precise cellular mechanisms of absence seizure generation will be known.

Andersen, P. & Andersson, S. A. (1968). *Physiological Basis of the Alpha Rhythm.* Appleton-Century-Crofts, New York.

Avanzini, G., de Curtis, M., Panzica, F. & Spreafico, R. (1989). Intrinsic properties of nucleus reticularis thalami neurones of the rat studied *in vitro*. *Journal of Physiology* **416**, 111–122.

Avoli, M. & Gloor, P. (1982). Interaction of cortex and thalamus in spike and wave discharges of feline generalized penicillin epilepsy. *Experimental Neurology* **76**, 196–217.

Avoli, M., Gloor, P., Kostopoulos, G. & Naquet, R. (ed.) (1990). *Generalized Epilepsy. Neurobiological Approaches.* Birkhauser, Boston, MA, USA.

Avoli, M. & Kostopoulos, G. (1982). Participation of corticothalamic cells in penicillin-induced generalized spike and wave discharges. *Brain Research* **247**, 159–163.

Bal, T. & McCormick, D. A. (1993). Mechanisms of oscillatory activity in guinea-pig nucleus reticularis thalami *in vitro*: a mammalian pacemaker. *Journal of Physiology* **468**, 669–691.

Bal, T., von Krosigk, M. & McCormick, D. A. (1995). Synaptic and membrane properties underlying synchronized oscillations in the ferret lateral geniculate nucleus *in vitro*. *Journal of Physiology* **483**, 641–663.

Buzsáki, G. (1991). The thalamic clock: emergent network properties. *Neuroscience* **41**, 351–364.

Buzsáki, G., Bickford, R. G., Ponomareff, G., Thal, L. J., Mandel, R. & Gage, F. H. (1988). Nucleus basalis and thalamic control of neocortical activity in the freely moving rat. *Journal of Neuroscience* **8**, 4007–4026.

Buzsáki, G., Smith, A., Berger, S., Fisher, L. J. & Gage, F. H. (1990). Petit mal epilepsy and Parkinsonian tremor: hypothesis of a common pacemaker. *Neuroscience* **36**, 1–14.

Contreras, D., Curró Dossi, R. & Steriade, M. (1993). Electrophysiological properties of cat reticular thalamic neurones *in vivo*. *Journal of Physiology* **470**, 273–294.

Crabtree, J. W. & Killackey, H. P. (1989). The topographic organization and axis of projection within the visual sector of the rabbit's thalamic reticular nucleus. *European Journal of Neuroscience* **1**, 94–109.

Crunelli, V., Haby, M., Jassik-Gerschenfeld, D., Leresche, N. & Pirchio, M. (1988). Cl^-- and K^+-dependent inhibitory postsynaptic potentials evoked by interneurones of the rat lateral geniculate nucleus. *Journal of Physiology* **399**, 153–176.

Deschênes, M., Madagaria-Domich, A. & Steriade, M. (1985). Dendrodendritic synapses in cat reticularis thalami nucleus: a structural basis for thalamic spindle synchronization. *Brain Research* **334**, 169–171.

Deschênes, M., Paradis, M., Roy, J. P. & Steriade, M. (1984). Electrophysiology of neurons of lateral thalamic nuclei in cat: resting membrane properties and burst discharges. *Journal of Neurophysiology* **51**, 1196–1219.

Destexhe, A., McCormick, D. A. & Sejnowski, T. J. (1993). A model for 8–10 Hz spindling in interconnected thalamic relay and reticularis neurons. *Biophysical Journal* **65**, 2473–2477.

Domich, L., Oakson, G. & Steriade, M. (1986). Thalamic burst patterns in the naturally sleeping cat: A comparison between cortically projecting and reticularis neurones. *Journal of Physiology* **379**, 429–449.

Friedlander, M. J., Lin, C.-S., Stanford, L. R. & Sherman, S. M. (1981). Morphology of functionally identified neurons in lateral geniculate nucleus of the cat. *Journal of Neurophysiology* **46**, 80–129.

Gloor, P., Quesney, L. F. & Zumstein, H. (1977). Pathophysiology of generalized penicillin epilepsy in the cat: The role of cortical and subcortical structures. II. Topical application of penicillin to the cerebral cortex and to subcortical structures. *Electroencephalography and Clinical Neurophysiology* **43**, 79–94.

Harris, R. M. (1987). Axon collaterals in the thalamic reticular nucleus from thalamocortical neurons in the rat ventrobasal thalamus. *Journal of Comparative Neurology* **258**, 397–406.

Hosford, D. A., Clark, S., Cao, Z., Wilson, W. A., Lin, F.-h., Morisett, R. A. & Huin, A. (1992). The role of GABA-B receptor activation in absence seizures of lethargic (lh/lh) mice. *Science* **257**, 398–401.

Houser, C. R., Vaughn, J. E., Barber, R. P. & Roberts, E. (1980). GABA neurons are the major cell type of the nucleus reticularis thalami. *Brain Research* **200**, 341–354.

Huguenard, J. R. & Prince, D. A. (1992). A novel T-type current underlies prolonged Ca^{2+}-dependent burst firing in GABAergic neurons of the rat thalamic reticular nucleus. *Journal of Neuroscience* **12**, 3804–3817.

Jahnsen, H. & Llinás, R. (1984*a*). Electrophysiological properties of guinea-pig thalamic neurons: an *in vitro* study. *Journal of Physiology* **349**, 205–226.

Jahnsen, H. & Llinás, R. (1984*b*). Ionic basis for the electroresponsiveness and oscillatory properties of guinea-pig thalamic neurons *in vitro*. *Journal of Physiology* **349**, 227–247.

Jones, E. G. (1985). *The Thalamus.* Plenum Press, New York.

Kim, U., Bal, T. & McCormick, D. A. (1994). Spindle oscillations in the ferret LGNd are traveling waves. *Society for Neuroscience Abstracts* **20**, 133.

Kostopoulos, G., Gloor, P., Pellegrini, A. & Gotman, J. (1981*a*). A study of the transition from spindles to spike and wave discharge in feline generalized penicillin epilepsy: Microphysiological features. *Experimental Neurology* **73**, 55–77.

Kostopoulos, G., Gloor, P., Pellegrini, A. & Siatitsas, I. (1981*b*). A study of the transition from spindles to spike and wave discharge in feline generalized penicillin epilepsy: EEG features. *Experimental Neurology* **73**, 43–54.

Liu, Z., Vergnes, M., Depaulis, A. & Marescaux, C. (1992). Involvement of intrathalamic $GABA_B$ neurotransmission in the control of absence seizures in the rat. *Neuroscience* **48**, 87–93.

McCormick, D. A. (1992). Neurotransmitter actions in the thalamus and cerebral cortex and their role in neuromodulation of thalamocortical activity. *Progress in Neurobiology* **39**, 337–388.

McCormick, D. A. & Prince, D. A. (1986). Acetylcholine induces burst firing in thalamic reticular neurones by activating a potassium conductance. *Nature* **319**, 402–405.

McCormick, D. A. & Wang, Z. (1991). Serotonin and noradrenaline excite GABAergic neurones of the guinea-pig and cat nucleus reticularis thalami. *Journal of Physiology* **442**, 235–255.

Mulle, C., Madariage, A. & Deschênes, M. (1986). Morphology and electrophysiological properties of reticularis thalami neurons in cat: *in vivo* study of a thalamic pacemaker. *Journal of Neuroscience* **6**, 2134–2145.

Niedermeyer, E. (1990). *The Epilepsies. Diagnosis and Management.* Urban and Schwarzenberg, Inc., Baltimore, MD, USA.

Ohara, P. T. & Lieberman, A. R. (1985). The thalamic reticular nucleus of the adult rat: experimental anatomical studies. *Journal of Neurocytology* **14**, 365–411.

Roy, J. P., Clercq, M., Steriade, M. & Deschênes, M. (1984). Electrophysiology of neurons of lateral thalamic nuclei in cat: Mechanisms of long-lasting hyperpolarizations. *Journal of Neurophysiology* **51**, 1220–1235.

Schwartz, M. L. & Meineke, D. L. (1992). Early expression of GABA-containing neurons in the prefrontal and visual cortices of rhesus monkeys. *Cerebral Cortex* **2**, 16–37.

Snead, O. C. (1992). Evidence for GABA-B mediated mechanisms in experimental generalized absence seizures. *European Journal of Pharmacology* **213**, 343–349.

Soltesz, I., Lightowler, S., Leresche, N. & Crunelli, V. (1989). On the properties and origin of the $GABA_B$ inhibitory postsynaptic potential recorded in morphologically identified projection cells of the cat dorsal lateral geniculate nucleus. *Neuroscience* **33**, 23–33.

Spreafico, R., Battaglia, G. & Frassoni, C. (1991). The reticular thalamic nucleus (RTN) of the rat: cytoarchitectural, Golgi, immunocytochemical, and horseradish peroxidase study. *Journal of Comparative Neurology* **304**, 478–490.

Steriade, M. & Deschênes, M. (1984). The thalamus as a neuronal oscillator. *Brain Research Reviews* **8**, 1–63.

Steriade, M., Deschênes, M., Domich, L. & Mulle, C. (1985). Abolition of spindle oscillations in thalamic neurons disconnected from nucleus reticularis thalami. *Journal of Neurophysiology* **54**, 1473–1497.

Steriade, M., Domich, L. & Oakson, G. (1986). Reticularis thalami neurons revisited: Activity changes during shifts in states of vigilance. *Journal of Neuroscience* **6**, 68–81.

Steriade, M., Domich, L. & Oakson, G. (1987). The deafferented reticular thalamic nucleus generates spindle rhythmicity. *Journal of Neurophysiology* **57**, 260–273.

Steriade, M. & McCarley, R. W. (1990). *Brainstem Control of Wakefulness and Sleep.* Plenum Press, New York.

Steriade, M., McCormick, D. A. & Sejnowski, T. J. (1993). Thalamocortical oscillations in the sleeping and aroused brain. *Science* **262**, 679–685.

Thompson, S. M. & Gähwiler, B. H. (1989). Activity-dependent disinhibition. I. Repetitive stimulation reduces IPSP driving force and conductance in the hippocampus *in vitro*. *Journal of Neurophysiology* **61**, 501–511.

Uhlrich, D. J., Cucchiaro, J. B., Humphrey, A. L. & Sherman, S. M. (1991). Morphology and axonal projection patterns of individual neurons in the cat perigeniculate nucleus. *Journal of Neurophysiology* **65**, 1528–1541.

von Krosigk, M., Bal, T. & McCormick, D. A. (1993). Cellular mechanisms of a synchronized oscillation in the thalamus. *Science* **261**, 361–364.

Yen, C. T., Conley, M., Hendry, S. H. C. & Jones, E. G. (1985). The morphology of physiologically identified GABAergic neurons in the somatic sensory part of the thalamic reticular nucleus in the cat. *Journal of Neuroscience* **5**, 2254–2268.

Acknowledgements

We thank Françoise Bal for technical assistance and Francine Trent for assistance with the *in vivo* recordings. This work was supported by the NIH, the Klingenstein Fund and the Sloan Foundation.

Received 11 April 1994; accepted 25 July 1994.