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25. A series of 24 18-nucleotide primers with complete identity to the murine Btk cDNA sequence (GenBank L08967) were used to generate 380–base pair overlapping products spanning the entire *btk* coding sequence. Full sequence information is available from the authors.
26. Filter lifts containing 5×10^5 plaques from a 129/Sv murine genomic λ phage library were screened with a 0.6-kb unique region murine cDNA probe as described (*3*). Clones Btk-C and Btk-D were evaluated by restriction mapping, subcloned into pGEM7ZF(+), and partially sequenced by Sequenase (U.S. Biochemical) with T7, SP6, and *btk* unique region oligonucleotide primers.
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Cellular Mechanisms of a Synchronized Oscillation in the Thalamus

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Spindle waves are a prototypical example of synchronized oscillations, a common feature of neuronal activity in thalamic and cortical systems in sleeping and waking animals. Spontaneous spindle waves recorded from slices of the ferret lateral geniculate nucleus were generated by rebound burst firing in relay cells. This rebound burst firing resulted from inhibitory postsynaptic potentials arriving from the perigeniculate nucleus, the cells of which were activated by burst firing in relay neurons. Reduction of γ-aminobutyric acid$_A$ (GABA$_A$) receptor–mediated inhibition markedly enhanced GABA$_B$ inhibitory postsynaptic potentials in relay cells and subsequently generated a slowed and rhythmic population activity resembling that which occurs during an absence seizure. Pharmacological block of GABA$_B$ receptors abolished this seizure-like activity but not normal spindle waves, suggesting that GABA$_B$ antagonists may be useful in the treatment of absence seizures.

Synchronized neuronal oscillations have been observed in thalamocortical networks during slow wave sleep (*1*, *2*), sensory processing (*3*), and generalized seizures (*4*). Spindle waves are one example of these neuronal oscillations and occur during the early stages of slow wave sleep. They appear as 7- to 12-Hz oscillations that wax and wane in amplitude over a 2- to 4-s period and that reappear once every 3 to 10 s (*1*, *2*). These synchronized oscillations are generated in the thalamus and depend on activity in the GABA-containing neurons of the thalamic reticular nucleus (nRt) or perigeniculate nucleus (PGN), although their dependence on the activity of relay neurons is unclear (*2*). The cellular bases of spindle wave generation are not only important for understanding thalamic function and the generation of the electroencephalogram during sleep but are also relevant to the understanding of generalized epilepsy, because the cellular mechanisms that generate absence seizures appear to be similar to those that generate spindle oscillations (*4*). We have studied the cellular mechanisms of spindle wave generation in thalamic slices and the transformation of these oscillations by GABA$_A$ receptor antagonists into activity resembling that of absence seizures.

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Intracellular and extracellular multiple unit recordings from sagittal, coronal, or horizontal slices (*5*) from the dorsal lateral geniculate nucleus (LGNd) of ferrets revealed the spontaneous occurrence of spindle waves (Fig. 1, A and E). These in vitro spindle waves were recorded in the PGN and all three laminae of the LGNd (A, A1, and C). They recurred with a regularity of one every 3 to 30 s (typically 10 to 20 s) and were characterized by a within spindle frequency of 5 to 9 Hz (typically 6 to 7 Hz; Figs. 1B and 2A) (*6*).

Intracellular recordings from LGNd relay cells during spindle waves revealed a rhythmic occurrence of inhibitory postsynaptic potentials (IPSPs) (*7*), a few of which were followed by rebound bursts of action potentials (Fig. 1C; $n = 88$ cells). These

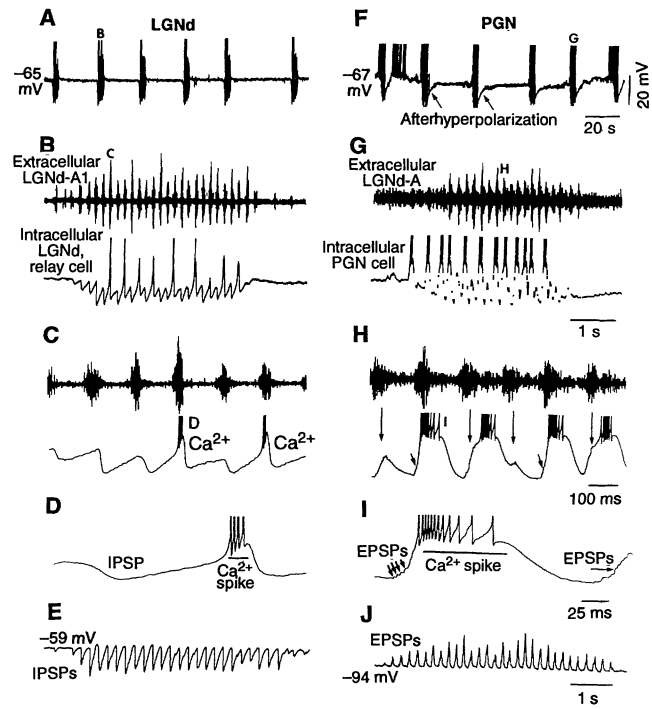

Fig. 1. Spindle oscillations in ferret geniculate slices. Letter labels in individual panels refer to a subsequent panel of higher temporal resolution. (**A**) Intracellular recording from an LGNd relay cell in lamina A revealed the recurrence of spindle waves approximately once every 20 s. (**B** to **D**) Detail of one spindle wave and parts of the spindle wave recorded both intracellularly in a relay neuron and locally as extracellular multiple unit activity in the LGNd. (**E**) Depolarization to −59 mV with intracellular injection of current completely inactivated the low threshold Ca^{2+} current and revealed the barrage of IPSPs associated with a spindle wave. (**F**) Intracellular recording from a morphologically identified PGN GABAergic cell during spindle wave generation. (**G**) Expansion of one spindle wave in the PGN cell and the simultaneously recorded multiple unit activity in lamina A. (**H**) Close examination of the simultaneous recordings from (G) revealed that each burst of activity in the relay lamina was associated with a barrage of EPSPs in the PGN cell. This barrage of EPSPs then activated, on occasion, a low threshold Ca^{2+} spike and a high-frequency burst discharge. (**I**) In some cases groups of three to five EPSPs arrived at the same frequency at which relay cells generated action potentials during a burst [compare (D) and (I)]. (**J**) Hyperpolarization of PGN cells to −94 mV prevented the activation of low threshold Ca^{2+} spikes and revealed the underlying barrages of EPSPs. Spindle oscillations were successfully recorded in at least one slice in 78 out of 85 experiments.

action potential bursts always occurred in synchrony with local multiple unit activity that was recorded extracellularly (Fig. 1, B and C). Thalamic relay neurons generate rebound bursts of action potentials by activating the low threshold Ca^{2+} current (8). Repetitive (6 to 10 Hz) injection of short-duration pulses (60 to 100 ms) of hyperpolarizing current similar in amplitude to the IPSPs occurring during spindle waves also resulted in rebound low threshold Ca^{2+} spikes. These spikes were identical to those occurring at the offset of IPSPs (n = 4 cells). Steady depolarization of relay neurons to near firing threshold with the intracellular injection of current abolished the rebound bursts occurring during spindle waves (Fig. 1E), which was to be expected if these events were mediated by the low threshold Ca^{2+} current (8). The arrival of IPSPs in relay neurons is associated with the removal of inactivation of the low threshold Ca^{2+} current. As the membrane potential repolarizes during the offset of the IPSP, a low threshold Ca^{2+} spike is generated, causing a burst discharge (Fig. 1D) (8).

Two possible sources of IPSPs in relay neurons are local GABAergic neurons within the laminae of the LGNd and the neurons of the PGN, which are GABAergic (9). Intracellular recordings from presumed local GABAergic interneurons (*10*) revealed a striking lack of activity during the generation of spindle waves (n = 3 cells). In contrast, intracellular recordings from PGN cells revealed strong barrages of excitatory postsynaptic potentials (EPSPs) arriving in synchrony with burst firing in the relay laminae (Fig. 1H; n = 29 cells). These barrages of EPSPs often activated a low threshold Ca^{2+} spike and a subsequent high-frequency burst discharge (Fig. 1, G to I). Hyperpolarization (Fig. 1J) or depolarization (Fig. 3A) of PGN cells with an intracellular injection of current prevented the activation of low threshold Ca^{2+} spikes and revealed the underlying barrages of EPSPs (*11*). Discernible EPSPs in PGN cells often arrived in groups of three to six at a frequency between 250 and 350 Hz, as would be expected if they were generated by burst firing in LGNd relay neurons (Fig. 1I, arrows).

In many PGN cells (n = 14 out of 29), spindle waves were also associated with a progressive hyperpolarization, a subsequent enhancement of burst firing resulting from the removal of inactivation of the low threshold Ca^{2+} (or T) current, and the generation of an afterhyperpolarization (Fig. 1, F and G). In contrast to the hyperpolarization of thalamic relay cells (Fig. 1B), the progressive hyperpolarization of PGN cells persisted after the generation of a spindle wave and resulted in an afterhyperpolarization (Fig. 1F). The hyperpolarization disappeared when the cell was manually depolarized to the tonic firing range to prevent the occurrence of burst discharges (Fig. 3A), and therefore the hyperpolarization presumably represents the activation of a Ca^{2+}-activated K^+ current by the low threshold Ca^{2+} spikes (*12*).

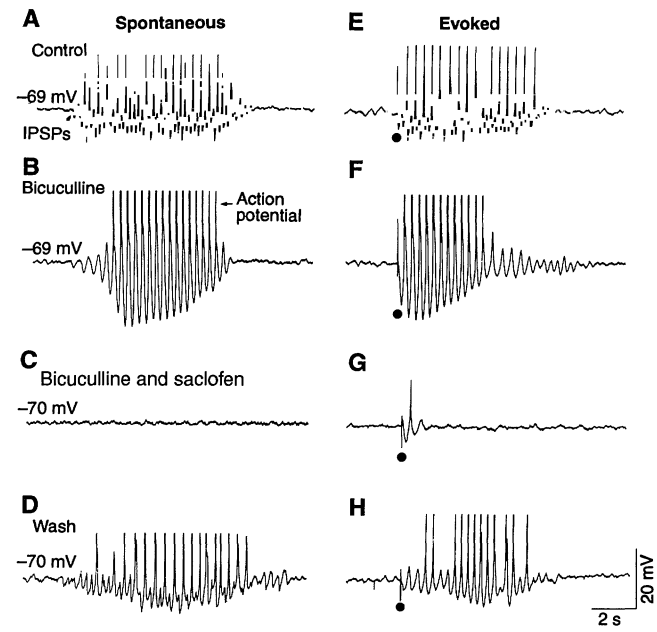

Fig. 2. Involvement of $GABA_A$ and $GABA_B$ receptors in spindle wave generation. (**A** and **E**) Intracellular recording from a relay cell during either spontaneous spindle waves (A) or spindle waves evoked by electrical stimulation of cortical inputs [(E), delivered at filled circles]. (**B** and **F**) Bath application of bicuculline (25 μM) slowed the oscillation to 2 to 4 Hz, markedly increased rebound burst firing, and increased the number of action potentials generated in each burst. (**C** and **G**) Local application by micropipette of the $GABA_B$ receptor antagonist 2-hydroxysaclofen (2 mM) abolished spontaneous oscillation and markedly reduced evoked oscillation. (**D** and **H**) These effects are reversible.

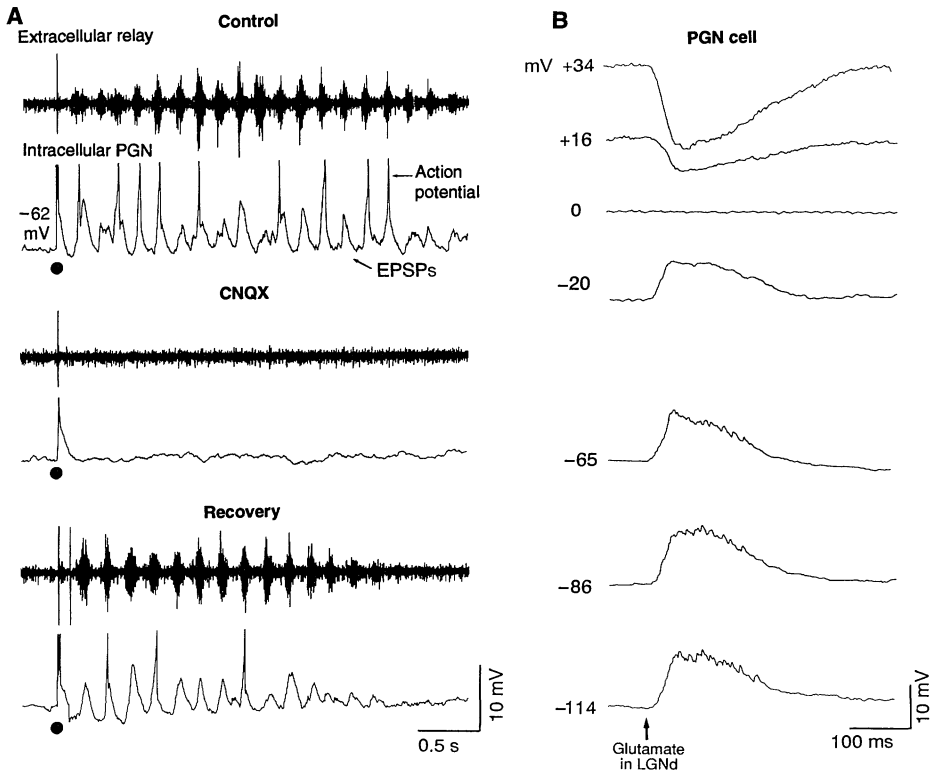

Fig. 3. Rhythmic burst firing in relay cells activates PGN cells by means of AMPA-kainate receptors. (**A**) Simultaneous extracellular recording from a relay lamina and intracellular recording from a PGN cell during a spindle wave evoked by electrical stimulation of the corticothalamic tract (filled circles) and the reversible abolition of this activity by locally applying CNQX (250 μM, by micropipette) to the region of the PGN cell. (**B**) Reversal potential of EPSPs recorded in a PGN cell in response to the application of glutamate (0.5 mM, arrow) in a relay lamina. The intracellular recording electrode contained QX-314 (50 mM) to block voltage-dependent Na^+ currents and Cs^+ (2 M cesium acetate) to block K^+ currents. Local application of bicuculline (250 μM) and saclofen (0.5 mM) blocked IPSPs in this cell.

In vivo investigations have led to the suggestion that the barrages of IPSPs arriving in thalamic relay neurons during spindle waves are mediated by burst firing in nRt and PGN neurons and the subsequent activation of a Cl^- conductance (*2*). In ferret LGNd relay cells, we reversed the rhythmic IPSPs to excitatory potentials by injecting Cl^- into the cell with recording microelectrodes which contained 3 M KCl. This result suggests an important role for $GABA_A$ receptors (n = 4 cells) (*13*). Local (100 to 250 μM) or bath (25 to 50 μM) application of the $GABA_A$ receptor antagonist bicuculline methiodide either abolished spontaneous spindle waves (n = 8 out of 25 cells) (*14*) or decreased the within-spindle frequency to 2 to 4 Hz and enhanced rebound burst firing in individual relay neurons (Fig. 2, A and B; n = 17 out of 25 cells). Subsequent local (1 to 3 mM) or bath (0.2 to 1 mM) application of the $GABA_B$ receptor antagonist 2-hydroxysaclofen abolished these large, slow IPSPs as well as the evoked or spontaneous slowed oscillations, indicating that they are mediated by $GABA_B$ receptors (Fig. 2, C and G; n = 8 cells). With $GABA_A$ receptors blocked, extracellular single unit and intracellular recordings from PGN cells revealed a marked increase in action potential discharge during each cycle of the spontaneous or evoked oscillation (n = 6 cells), probably resulting from the increased participation of LGNd relay cells in each cycle of the slowed spindle wave (*15*). This increased firing of PGN cells presumably enhances $GABA_B$ receptor activation in relay neurons and subsequently enhances rebound burst firing by increased removal of inactivation of the low threshold Ca^{2+} current (Fig. 2, A, B, E, and F).

In contrast to the effects of blocking $GABA_A$ receptors in normal slices, blocking $GABA_B$ receptors with bath application of 2-hydroxysaclofen (0.5 to 1 mM; n = 5 experiments) did not abolish or markedly alter spontaneous or evoked spindle waves. These data indicate that the activation of $GABA_B$ receptors is not essential to the generation of these normal oscillations.

We investigated the dependence of spindle wave generation on excitatory transmission using bath or local applications of the excitatory amino acid receptor antagonists D-2-amino-5-phosphonovalerate (D-APV) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). Spindle waves were not abolished by bath application (25 to 50 μM) of the *N*-methyl-D-aspartate (NMDA) receptor antagonist D-APV, indicating that NMDA receptors do not need to be activated for spindle wave generation (n = 3 experiments). In contrast, when we applied the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)–kainate receptor antagonist CNQX either in the bath (25 μM; n = 2 cells) or locally to the PGN (250 μM; n = 2 cells), spontaneous and evoked spindle wave generation was completely abolished in both the relay laminae and PGN (Fig. 3A). This suggests that activation of AMPA-kainate receptors on PGN cells is critical to the generation of spindle waves. In addition, intracellular recording from PGN cells revealed that activating relay cells with a local pressure-pulse application of glutamate results in barrages of EPSPs that reverse polarity at 0 mV and exhibit a relation to membrane potential that suggests a significant contribution by AMPA-kainate receptors (Fig. 3B).

The importance of connections between the PGN and relay laminae in maintaining spindle wave oscillations was examined in slices cut with a small knife (1 mm in length). We found that severing the connections between a limited portion of the PGN and relay laminae eliminated spindle activity at the center of the disconnected regions, even though neuronal activity in these separated regions otherwise appeared normal (n = 7; Fig. 4A) (*16*). From the center of the disconnected region to the ends of the knife cut, spindle waves gradually increased in strength in both the relay laminae and PGN (Fig. 4A). These results suggest that the axonal connections between the PGN and relay laminae spread anterior to posterior and dorsal to ventral (or vice versa) within the sagittal slice. We confirmed this pattern of connections by visualization of the axonal projections from single thalamocortical relay and PGN cells intracellularly filled with biocytin (*17*).

These findings indicate that spindle waves are generated through a reciprocal interaction between the GABAergic cells of the nRt and PGN and the excitatory thalamocortical relay neurons (*1*), and that both intrinsic and circuit properties make essential contributions (Fig. 4B). On the basis of these data, we propose the following mechanism for the generation of spindle waves: Activation of a critical number of PGN GABAergic neurons results in widespread inhibition of thalamic relay cells. During these IPSPs, the inactivation of the low threshold Ca^{2+} spike is removed to a sufficient degree in a subset of relay cells to allow the generation of rebound Ca^{2+} spikes and associated bursts of action potentials at the offset of the IPSPs. These bursts of action potentials in relay cells then depolarize PGN cells, and this depolarization activates a low threshold Ca^{2+} spike–mediated burst of action potentials in PGN neurons (*18*). Progressive hyperpolarization of PGN cells through a presumed Ca^{2+}-sensitive K^+ current results in, first, an

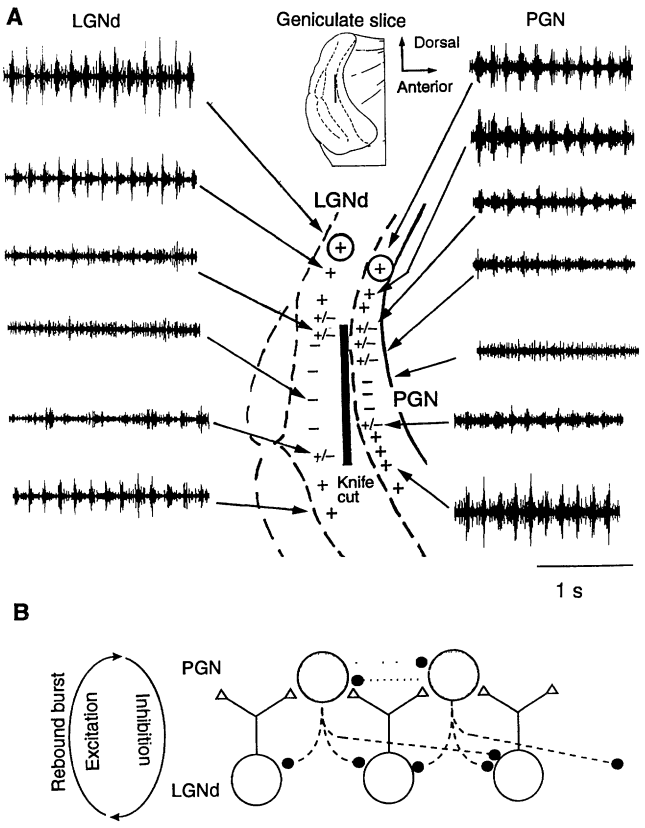

Fig. 4. Disconnection of LGNd and PGN abolishes spindle waves in both regions. (**A**) A small knife cut (1 mm, solid line) was made between the PGN and LGNd. Extracellular multiple unit recordings in both the PGN and LGNd revealed robust spindling (+) outside the region of the knife cut and the lack of spindling (−) anterior and posterior to the center of the knife cut. Near the ends of the cut, spindling was poor (+/−) but still synchronous with the adjacent portions of the slice. In contrast, spindling in the portion of the slice ventral to the knife cut occurred independently from that dorsal to the knife cut. The middle portion is a magnified view of the PGN and lamina A and the location of each recording. We obtained recordings from two electrodes: one was stationary at a reference site (circles), and the other was moved in 100- to 200-μm increments. (**B**) Schematic diagram of neuronal connections involved in spindling. Activation of PGN GABAergic cells inhibits (filled circles) a number of relay cells, a subset of which generates a rebound Ca^{2+} spike–mediated burst of action potentials that, in turn, excite (open triangles) once again the PGN neurons.

enhancement (by increased removal of inactivation of the low threshold Ca^{2+} current) and then a decrement (by hyperpolarization below the activation threshold of the Ca^{2+} current) of spindle waves, giving rise to the waxing and waning of these oscillations. We propose that synchronization of this oscillation between neighboring cells in either the PGN or relay laminae results from a large overlap in their afferent and efferent connections (Fig. 4B). Interestingly, if we block $GABA_A$ receptors, enhanced but slowed oscillations are generated which resemble those of absence seizures (*4*). These findings reinforce the hypothesis that abnormally strong activation of nRt or PGN cells may underlie the generation of generalized absence seizures, that the activation of $GABA_B$ receptors is critical to their generation, and suggest a pharmacological approach to treatment for this disorder (*4*, *19*).

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5. Male or female ferrets (2 to 12 months old) were deeply anesthetized with sodium pentobarbital (30 mg per kilogram of body weight intraperitoneally) and killed by decapitation in accordance with Yale University Medical School guidelines for the use of animals in research. We prepared sagittal slices (400 μm thick) on a vibratome and maintained them in an interface chamber at 34° to 35°C. The perfusion medium contained 126 mM NaCl, 2.5 mM KCl, 1.2 to 2.0 mM $MgSO_4$, 1.25 mM NaH_2PO_4, 2 mM $CaCl_2$, 26 mM $NaHCO_3$, and 10 mM dextrose. Solutions were aerated with 95% O_2 and 5% CO_2 to pH 7.4. Drugs were applied either locally with the pressure-pulse technique or in the bathing medium. We placed concentric stimulating electrodes in the optic radiation to activate corticothalamic fibers. Intracellular recording electrodes contained either 2 M potassium acetate and 2% biocytin or 4 M potassium acetate. During recording, the location of the PGN and each lamina of the LGNd were visible. Cells intracellularly labeled with biocytin were visualized with standard techniques. We performed immunocytochemical staining for GABA also with standard techniques to confirm the location of the PGN.
6. The rate of recurrence of spindle waves was dependent on temperature. Decreasing the temperature increased the rate of spindle generation. The in vitro recordings were performed at a temperature of 34° to 35.5°C to improve tissue viability and enhance the recurrence of spindling. Recordings of spindling in barbiturate-anesthetized ferrets with a rectal temperature of 35° to 36°C revealed within-spindle frequencies between 6 and 11 Hz (8.7 ± 1.4 Hz, SD; n = 136 spindles). These results were similar to those we obtained from slices.
7. We concluded that the hyperpolarizing events occurring in relay cells during spindle waves were IPSPs because these hyperpolarizing events reversed to depolarizing events when Cl^- was injected intracellularly. They were also blocked or modulated by the $GABA_A$ receptor antagonists bicuculline methiodide and picrotoxin (see text). In addition, the occurrence of hyperpolarizing events was highly correlated with neuronal activity that was simultaneously recorded in the PGN (n = 19 experiments).
8. The low threshold Ca^{2+} current is completely inactivated in thalamic relay neurons at membrane potentials positive to approximately −65 mV. Hyperpolarization negative to −65 mV removed some portion of this inactivation. Subsequent repolarization of the membrane potential (such as during the offset of an IPSP) activated this Ca^{2+} current and generated a low threshold Ca^{2+} spike [see H. Jahnsen and R. R. Llinás, *J. Physiol.* (*London*) **349**, 227 (1984)]. Because of the kinetics and voltage dependence of activation, inactivation and removal of inactivation of this current, and the presence of other ionic currents, relay neurons tend to rebound burst at a slower frequency (0.5 to 4 Hz) than the IPSPs arriving during spindle waves [R. Curró Dossi, A. Nuñez, M. Steriade, *J. Physiol.* (*London*) **447**, 215 (1992); J. R. Huguenard and D. A. McCormick, *J. Neurophysiol.* **68**, 1373 (1992); (*20*)].
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11. We compared the amplitude–time course and the voltage dependence of the depolarizing potentials underlying the bursts of action potentials in PGN neurons during spindles and in PGN neurons that were injected intracellularly with current pulses. This comparison revealed the burst of action potentials to be generated by low threshold Ca^{2+} spikes mediated by the low threshold Ca^{2+} current (*12*). These low threshold Ca^{2+} spikes were typically activated by the arrival of barrages of EPSPs.
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13. The reversal potential of normal IPSPs recorded with potassium acetate electrodes could not be determined accurately because of large decreases in apparent input resistance on hyperpolarization. This, presumably, was a consequence of the activation of a hyperpolarization-activated cation current (*20*) and the distal location of PGN synapses on relay cell dendrites [J. Cucchiaro, D. J. Uhlrich, S. M. Sherman, *J. Comp. Neurol.* **31**, 316 (1991)].
14. In slices in which application of bicuculline abolished spontaneous spindle waves, electrical stimulation to cortical afferents still evoked synchronized oscillations, even though the intraspindle frequency was decreased (see Fig. 2F). Also, in the presence of $GABA_A$ receptor antagonists IPSPs were markedly slowed in their time-to-peak amplitude (compare Fig. 2, A and B), as would be expected with the different kinetics of $GABA_A$- and $GABA_B$-mediated IPSPs.
15. Short-duration (milliseconds) IPSPs occurred in PGN neurons intermixed with EPSPs during the generation of spindle waves, and the IPSPs presumably arose from the activation of neighboring PGN neurons. Therefore, it is likely that disinhibition of PGN cells also contributed to an increased discharge of action potentials from these neurons in bicuculline.
16. Electrical activation of cortical afferents or local application of glutamate resulted in a burst of neuronal activity in regions not generating spindle waves that was similar to that found in normal (uncut) slices.
17. Reconstruction of single, biocytin-filled PGN neurons revealed dense axonal innervations of all relay laminae (n = 8 cells). Reconstruction of single relay cells often revealed an axon collateral that ran in a dorsal-ventral direction within the PGN (n = 7 cells).
18. A study has suggested that a more intact nRt may generate spindle-like oscillations autonomously [M. Steriade, L. Domich, G. Oakson, M. Deschênes, *J. Neurophysiol.* **57**, 260 (1987)].
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