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 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 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.

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 barrage 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 barrage 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. 1) 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, arrow).

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 B) Intracellular recording from a relay cell during either spontaneous spindle waves (A) or spindle waves evoked by electrical stimulation of cortical inputs (B, 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\(_{A}\) 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 DX-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_A 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_A 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 Ca²⁺ 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 a slice 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 Ca²⁺ spike is removed to a sufficient degree in a subset of relay cells to allow the generation of rebound Ca²⁺ 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 Ca²⁺–mediated burst of action potentials in PGN neurons (18). Progressive hyperpolarization of PGN cells through a presumed Ca²⁺–sensitive K⁺ current results in, first, an

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**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 spindle (+) 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 Ca²⁺ 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 Ca2+ current) and then a decrement (by hyperpolarization below the activation threshold of the Ca2+ 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_A receptors is critical to their generation, and suggest a pharmacological approach to treatment for this disorder (4, 19).

REFERENCES AND NOTES

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 main-