

that degrades 8-oxo-rGTP (19).

This effect of a well-known DNA replication fidelity enzyme on transcriptional fidelity may be the tip of the iceberg. The rNTP and dNTP pools and single-stranded RNA are probably more susceptible than double-stranded DNA to free radical attack and other chemical modifications, a proposition used to explain why the DNA world has replaced the RNA world (20). The fidelity of RNA, a molecule that can be viewed as the disposable soma of genetic information—whereas DNA would be the heritable germ line—has been much less studied than that of DNA. So far, the only other example of a mutant affecting transcriptional fidelity is an RNA polymerase mutant that results in a three- to fourfold decrease in fidelity (21). There may be RNA repair activities still undiscovered. Such RNA repair activities might affect other important RNA transactions such as editing and splicing.

The enhanced variability of RNA and the consequent effect on proteins may provide an increased spectrum of enzymatic activities available to a given genome and, perhaps, facilitate adaptive mutagenesis (22–25). Possible reverse transcription of RNA errors into DNA, or generation of transient mutators (26, 27), could be a significant source of variations in DNA-based genomes, whereas a change in RNA fidelity could influence the evolution of RNA viruses. Although DNA fidelity may be most important in avoiding malignant cell transformation in dividing cells, RNA fidelity may be essential for the functional maintenance of cells, and in particular of nondividing cells such as heart muscle, neurons, and other predominantly quiescent (G_1/G_0 phase) cells.

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12. Other specific mutations are probably not responsible for the observed increase in β -galactosidase activity. For instance, an interesting candidate, suggested by one of the referees, is a T-to-G mutation on the transcribed strand leading to a Ser at position 461. Such a mutant has a β -galactosidase activity of 30 Miller units [C. G. Cupples and J. H. Miller, *Genetics* **120**, 637 (1988)] and could therefore contrib-

ute to the observed leakiness. However, the formula could be modified to estimate the effect of Ser⁴⁶¹ mutants: $M(\text{pop}) = M(\text{Ser})f(\text{Ser}) + M(\text{lac}^-)f(\text{lac}^-)$, where $M(\text{Ser})$ and $f(\text{Ser})$ are the Miller units and frequencies of Ser⁴⁶¹ mutants. $f(\text{Ser})$ would need to constitute >1% of each liquid culture to account for the increase in β -galactosidase concentration. Such a high mutation frequency at a single site seems highly unlikely. By the same basic logic, mutations induced by lack of MutT activity cannot easily explain the extent of the observed increase in leakiness. However, it is difficult to eliminate the possibility that a variety of mutants are responsible for some of the observed effects, especially if such mutants are not colony forming.

13. This is different from the effect of the *mutT* mutation on frequencies of mutation, which are similar in rich medium under anaerobiosis and aerobiosis [R. G. Fowler, J. A. Erickson, R. J. Isbell, *J. Bacteriol.* **176**, 7727 (1994); F. Taddei *et al.*, unpublished observations]. The fact that, under anaerobiosis, we can separate the effect of MutT on DNA and RNA fidelity is consistent with the idea that *mutT*-mediated mutations are not responsible for the observed increase in β -galactosidase activity in a *mutT*- aerobic culture.
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30. We thank D. Touati and S. Stambuk for providing strains, R. Wagner for rewriting the initial manuscript, and R. D'Ari for revising the final version. Supported by grants from the Ministry of Education, Science and Culture of Japan and from the French agencies Association de la Recherche contre le Cancer and Actions Concertées Coordonnées Sciences du Vivant du Ministère de l'Enseignement Supérieur et de la Recherche and Groupement de Recherche et d'Etudes sur les Génomes.

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Functional Dynamics of GABAergic Inhibition in the Thalamus

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The inhibitory γ -aminobutyric acid-containing (GABAergic) neurons of the thalamic reticular and perigeniculate nuclei are involved in the generation of normal and abnormal synchronized activity in thalamocortical networks. An important factor controlling the generation of activity in this system is the amplitude and duration of inhibitory postsynaptic potentials (IPSPs) in thalamocortical cells, which depend on the pattern of activity generated in thalamic reticular and perigeniculate cells. Activation of single ferret perigeniculate neurons generated three distinct patterns of GABAergic IPSPs in thalamocortical neurons of the dorsal lateral geniculate nucleus: Low-frequency tonic discharge resulted in small-amplitude IPSPs mediated by GABA_A receptors, burst firing resulted in large-amplitude GABA_A IPSPs, and prolonged burst firing activated IPSPs mediated by GABA_A and GABA_B receptors. These functional properties of GABAergic inhibition can reconfigure the operations of thalamocortical networks into patterns of activity associated with waking, slow-wave sleep, and generalized seizures.

GABAergic neurons are the major inhibitory cell type in the mammalian brain and exhibit a wide variety of morphological and physiological properties (1). The activation of GABAergic neurons can inhibit postsynaptic target cells through increases in Cl⁻ conductance, mediated by the GABA_A receptor, and increases in K⁺ conductance, mediated by the GABA_B receptor (2), although it remains unclear whether these different receptors are activated by the same

or different subgroups of GABAergic neurons (3–5). In addition, some types of GABAergic neuron can generate high-frequency burst discharges, and the functional influence of these on the postsynaptic GABA response has not yet been determined (6, 7). The functional properties of IPSPs mediated by the GABA_A and GABA_B receptors are intimately involved in the operation of thalamic and cortical networks (8).

Thalamocortical neurons in the dorsal lateral geniculate nucleus (LGNd) are densely innervated by the GABAergic neurons of the perigeniculate nucleus (PGN) (9) in a manner that is equivalent to the

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innervation of other regions of the thalamus by the thalamic reticular nucleus. The activation of thalamic reticular or PGN neurons results in the activation of both GABA_A- and GABA_B-receptor-mediated IPSPs in thalamocortical cells (6, 10, 11), although it is not known whether these are activated by the same or different GABAergic neurons, nor is it precisely known how the action potential activity of the presynaptic neuron affects the functional properties of these IPSPs.

To answer these questions, dual intracellular recordings were obtained in vitro from monosynaptically connected pairs (*n* = 41 pairs) of PGN GABAergic neurons and thalamocortical cells in the LGNd of a ferret (12). Intracellular injection of a depolarizing current into the GABAergic neuron, either as steady current or as a pulse, resulted in the generation of trains of single action potentials (Fig. 1A). These single action potentials evoked small-amplitude (0.2 to 1.6 mV) IPSPs in thalamocortical neurons (Fig. 1A). Hyperpolarization of PGN neurons to membrane potentials of -65 to -90 mV resulted in the removal of inactivation of the low-threshold Ca²⁺ current and therefore the generation of low-threshold Ca²⁺ spike-mediated bursts of action potentials in response to depolarization (Fig. 1B). These bursts consisted of 5 to 15 action potentials at frequencies of 250 to 550 Hz and were typical of those generated by PGN and thalamic reticular cells during slow-wave sleep in vivo (13). Burst, or high-frequency tonic, discharges in PGN neurons resulted in large-amplitude (up to 10 mV) IPSPs in postsynaptic thalamocortical cells (Fig. 1, B and C); these IPSPs were effective in generating rebound low-threshold Ca²⁺ spikes in thalamocortical cells (Fig. 1B) (14). Interestingly, close examination of the individual IPSPs revealed that not only did each action potential in the presynaptic neuron result in an IPSP, despite the high frequency of discharge, but also that the amplitude of the second to approximately fourth unitary IPSPs increased markedly during the generation of the burst discharge (Fig. 1Bb; *n* = 7 out of 7 cases analyzed). This frequency-dependent facilitation also occurred when the PGN neuron generated tonic trains of action potentials (Fig. 1, C and D). Interestingly, the facilitation of unitary IPSP amplitude was frequency dependent, increasing with increases in frequency from about 100 to 500 Hz with a significant average correlation of 0.62 (*P* < 0.001; Fig. 1Db). This facilitation only occurred during the first two to four action potentials. The IPSPs generated by additional action potentials in the train became progressively smaller in amplitude (Fig. 1C).

In normal bathing medium, the interaction of PGN and thalamocortical neurons resulted in the generation of spindle waves (Fig. 2B) (6, 15). Burst firing in PGN neurons resulted in the generation of rebound low-threshold Ca²⁺ spikes and bursts of Na⁺-dependent action potentials in thal-

amocortical neurons, which in turn re-excited the PGN neurons through the generation of excitatory postsynaptic potentials (EPSPs) in these cells. Bath (20 to 100 μM; *n* = 6) or local (1 mM in micropipette; *n* = 2) application of the GABA_A receptor antagonist bicuculline methiodide resulted in

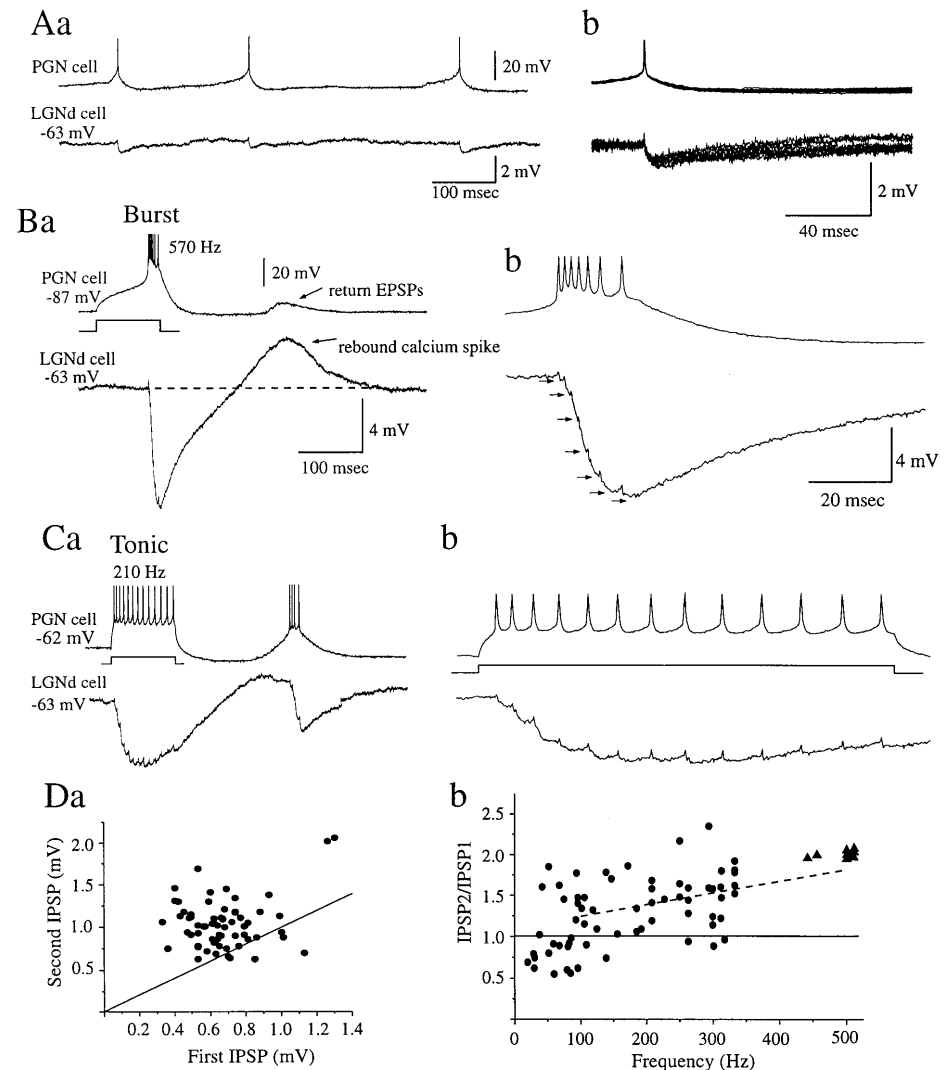


Fig. 1. Inhibitory postsynaptic potentials facilitate when activated at high frequencies between single PGN and LGNd neurons. **(Aa)** Dual intracellular recording from a PGN GABAergic neuron and an LGNd thalamocortical cell. Single action potentials activate IPSPs that are about 0.8 mV in amplitude. **(Ab)** Ten action potentials and the corresponding IPSPs are overlain on the recording from **(Aa)**. **(Ba)** Activation of a low-threshold Ca²⁺ spike in the PGN neuron results in a high-frequency burst of seven action potentials. This burst of action potentials results in the activation of a 9-mV-amplitude compound IPSP in the LGNd cell and is large enough to result in the generation of a small rebound low-threshold Ca²⁺ spike. **(Bb)** Expansion of the induced IPSP reveals the individual IPSPs. Their amplitudes increase and then decrease during the generation of the burst. **(Ca)** Similarly, inducing the PGN neuron to discharge at a high rate (average of 210 Hz) results in the generation of a large compound IPSP in the postsynaptic cell. **(Cb)** Expansion of the IPSP barrage in **(Ca)**. **(Da)** Plot of the amplitude of the second unitary IPSP versus that of the first that occur in response to tonic firing in the PGN neuron at frequencies greater than 100 Hz. Each dot represents a single trial. **(Db)** Relation between the frequency of discharge of the PGN neuron and the ratio of the amplitude of the second to the first IPSPs. The triangles represent data obtained from low-threshold Ca²⁺-spike-mediated bursts of action potentials [see **(B)**], and the dots were obtained with tonic discharge [see **(C)**]. The dashed line represents the best-fit linear regression (*r* = 0.62). Each symbol represents a single trial. All illustrated data in this figure are from the same PGN and thalamocortical cell pair, the morphology of which is illustrated in Fig. 4.

a transformation of these normal spindle waves into abnormal, "paroxysmal" events (Fig. 2F) that resembled the activity in some animal models of generalized absence seizures (16). These abnormal network oscillations are generated through the activation of large, slow GABA_B-receptor-mediated IPSPs in thalamocortical cells by PGN neurons (Fig. 2F) (6).

Close examination of the IPSPs generated in thalamocortical cells by a single PGN neuron revealed that the application

of bicuculline methiodide (*n* = 8) resulted in a complete block of IPSPs generated by low-frequency (<100 Hz) tonic trains of action potentials in the presynaptic neuron and a complete, or near complete, abolition of IPSPs generated by normal burst firing in PGN cells at normal discharge frequencies (250 to 350 Hz) (Fig. 2C), indicating that these events are mediated largely, if not exclusively, by the activation of GABA_A receptors. However, in the presence of GABA_A receptor antagonists, PGN neu-

rons also generated prolonged high-frequency burst discharges, particularly during the generation of "paroxysmal" activity (Fig. 2F), owing to disinhibition from other PGN cells (6, 17). In six out of eight cells, the activation of prolonged discharges in single PGN neurons resulted in the activation of slow bicuculline-resistant IPSPs in thalamocortical cells (Fig. 2E), whereas in the other two pairs, there was no detectable postsynaptic response. Bath application of the GABA_B receptor antagonist CGP 35348 (0.8 mM) or local application of CGP 54626A (200 μM in micropipette) resulted in an abolition of this slow IPSP (*n* = 2), confirming that it is mediated by GABA_B receptors, an effect that is reversible (Fig. 2, G and H). The activation of the PGN with local application of glutamate (0.5 mM in micropipette) in the presence of bicuculline also resulted in the generation of slow IPSPs (Fig. 3B), and these were completely blocked by local application of CGP 35348 (2 mM in micropipette; *n* = 12) or CGP 54626A (1 mM in micropipette; *n* = 4), confirming their mediation by GABA_B receptors (18).

In addition to their threshold for activation, IPSPs mediated by GABA_A and GABA_B receptors also differed significantly in their delay to onset. Bursts of action potentials in single PGN neurons resulted in the activation of GABA_A-receptor-mediated IPSPs with a delay to onset of <1 ms, whereas the activation of prolonged burst discharges resulted in the activation of GABA_B-receptor-mediated IPSPs at a delay of 30 to 42 ms (Fig. 3A). This delay to onset of GABA_B-receptor-mediated IPSPs was also observed when the PGN was activated by local application of glutamate (*n* = 17; Fig. 3, B and C). Activation of the PGN with glutamate resulted in IPSPs that contained both bicuculline-sensitive fast and CGP 35348-sensitive slow components (*n* = 12; Fig. 3, B and C). The GABA_B-receptor-mediated IPSPs or IPSCs (inhibitory postsynaptic currents) had a delay to onset that was on average 52.8 ms (±17.8 ms; *n* = 17) slower than that for activation of GABA_A-receptor-mediated IPSPs in the same neurons, and this delay did not shorten as the amplitude of the GABA_B response was increased by an increase in the dose of glutamate application (Fig. 3B). Interestingly, exogenous application of GABA (0.25 to 0.5 mM in micropipette) to thalamocortical cells resulted in GABA_B-receptor-mediated responses (blocked by CGP 54626A; 1 mM in micropipette) that had a delay to onset that was, on average, only 17.1 ms (±9.9 ms; *n* = 8) slower than the picrotoxin-sensitive GABA_A response in the same neurons (Fig. 3D).

Intracellular injection of biocytin suc-

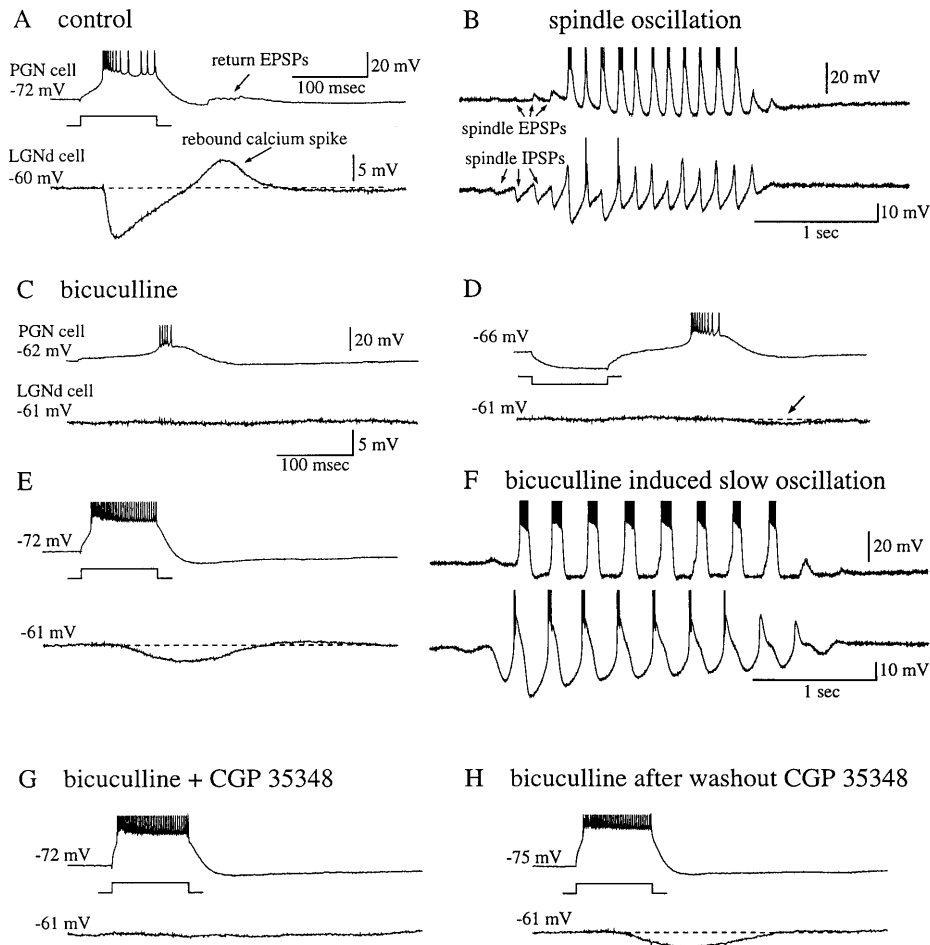


Fig. 2. Single PGN neurons can activate GABA_A and GABA_B receptors in LGNd thalamocortical cells. **(A)** Activation of a burst of action potentials in the presynaptic PGN neuron results in a large IPSP and small rebound Ca²⁺ spike in the thalamocortical cell. The burst of action potentials in the PGN cell is followed by "return EPSPs," which are generated by the rebound burst firing of inhibited thalamocortical neurons (74). **(B)** Spontaneous generation of a spindle wave is associated with repetitive burst firing in the PGN neuron and the occurrence of repetitive IPSPs in the LGNd cell. The IPSPs are generated by the activity of this PGN cell as well as others. Note the duration of the bursts of action potentials in the PGN cell. **(C)** Bath application of bicuculline (100 μM) results in the abolition of the response to a burst of five action potentials. **(D)** Induction of a burst of 11 action potentials results in a small (<1 mV) slow IPSP in the thalamocortical cell (arrow). **(E)** Increasing the discharge of the PGN neuron to 40 action potentials results in a 3-mV-amplitude slow IPSP in the thalamocortical cell. **(F)** After bath application of bicuculline, the geniculate slice spontaneously generates abnormal oscillations during which the PGN neuron generates prolonged high-frequency (30 to 50 action potentials) burst discharges, and the thalamocortical cell is hyperpolarized through the occurrence of large GABA_B-receptor-mediated IPSPs. **(G)** Bath application of the GABA_B receptor antagonist CGP 35348 (0.8 mM) abolishes the slow IPSP occurring in response to the activation of the PGN neuron, as well as the paroxysmal network oscillations. **(H)** This effect of CGP 35348 is reversible. Scales for recordings in (C), (D), (E), (G), and (H) are the same as that in (A).

cessfully revealed the axonal arbors and dendritic trees of five pairs of PGN and thalamocortical neurons (Fig. 4A). Recovery of the morphology of connected pairs of PGN and LGNd neurons revealed 11, 60, 62, 69, and 69 juxtapositions associated with presynaptic swellings between the axons of the recorded PGN neurons and the dendrites of the thalamocortical cells (Fig. 4, B through D). The number of these putative synaptic contacts was significantly correlated ($r = 0.99$; $P < 0.01$) with the amplitude of the burst-induced IPSPs recorded between these pairs of neurons (2.3, 7.1, 7.4, 8.7, and 8.8 mV, respectively). Plots of the distribution of these presumed synaptic terminals on the relative lengths of the thalamocortical cell dendrites revealed that the putative synapses are localized throughout the dendritic tree with their highest density in the intermediate portions (Fig. 4, E and F). Although these synapses were not examined with an electron microscope, previous correlations of GABAergic synapses with light and electron microscopes have demonstrated that about 80% of the putative contacts identified optically are confirmed to be synaptic junctions with the electron microscope (19).

Earlier studies of the influence of single GABAergic neurons on other neurons in the hippocampus and cerebral cortex have repeatedly demonstrated that GABAergic cells activate IPSPs by activating GABA_A receptors (20). In contrast, single GABAergic cells have not been pharmacologically demonstrated to activate GABA_B IPSPs (21), even though when multiple GABAergic neurons are activated strongly, or when GABA uptake is blocked, GABA_B-receptor-mediated IPSPs are prominent (22). On the basis of these findings, it has been suggested that distinct subpopulations of GABAergic neurons activate the GABA_A- and GABA_B-receptor-mediated IPSPs (3). An alternative hypothesis that has yet to be examined in these brain regions is that GABA_B-receptor-mediated IPSPs merely require a higher frequency and more prolonged discharge before their activation is functionally expressed (4).

Here we demonstrate that single GABAergic PGN neurons are responsible for the activation of IPSPs mediated by both GABA_A and GABA_B receptors and that the pattern of presynaptic discharge is critical in determining which of these two types of IPSP are activated. With single action potentials, we could not detect GABA_B-receptor-mediated IPSPs; the activation of these IPSPs required the generation of prolonged bursts of action potentials (Fig. 2). The delay to onset of these IPSPs may have multiple origins, including

the properties of G-protein-mediated events that are the intermediaries between receptor binding and K⁺ channel opening (23) or the location of receptors in relation to the synaptic terminals (4, 24, 25).

These functional properties of postsynaptic GABA_A- and GABA_B-receptor-mediated inhibition suggest that low-frequency (<100 Hz) tonic discharge of PGN neurons will predominately control the dendritic processing and probability of action-potential generation in thalamocortical neurons through the activation of GABA_A-receptor-mediated fast IPSPs. In contrast, the generation of high-frequency bursts of activity, such as occurs during slow-wave sleep, will result in the generation of large GABA_A-receptor-mediated IPSPs, owing to

facilitation as well as temporal summation of the unitary IPSPs. These large compound IPSPs are effective in activating rebound low-threshold Ca²⁺ spikes, which are critical to the generation of normal thalamocortical rhythms, such as spindle waves, during slow-wave sleep (15). Finally, the prolonged and high-frequency burst discharge of PGN neurons, or perhaps the simultaneous activation of a number of PGN cells, also results in the strong activation of GABA_B receptors (6, 10). The slow kinetics and prolonged time course of GABA_B-receptor-mediated IPSPs facilitate the generation of the slow synchronized oscillations such as those that may underlie absence seizures; these IPSPs may therefore play a critical role in such seizures (26). These results therefore em-

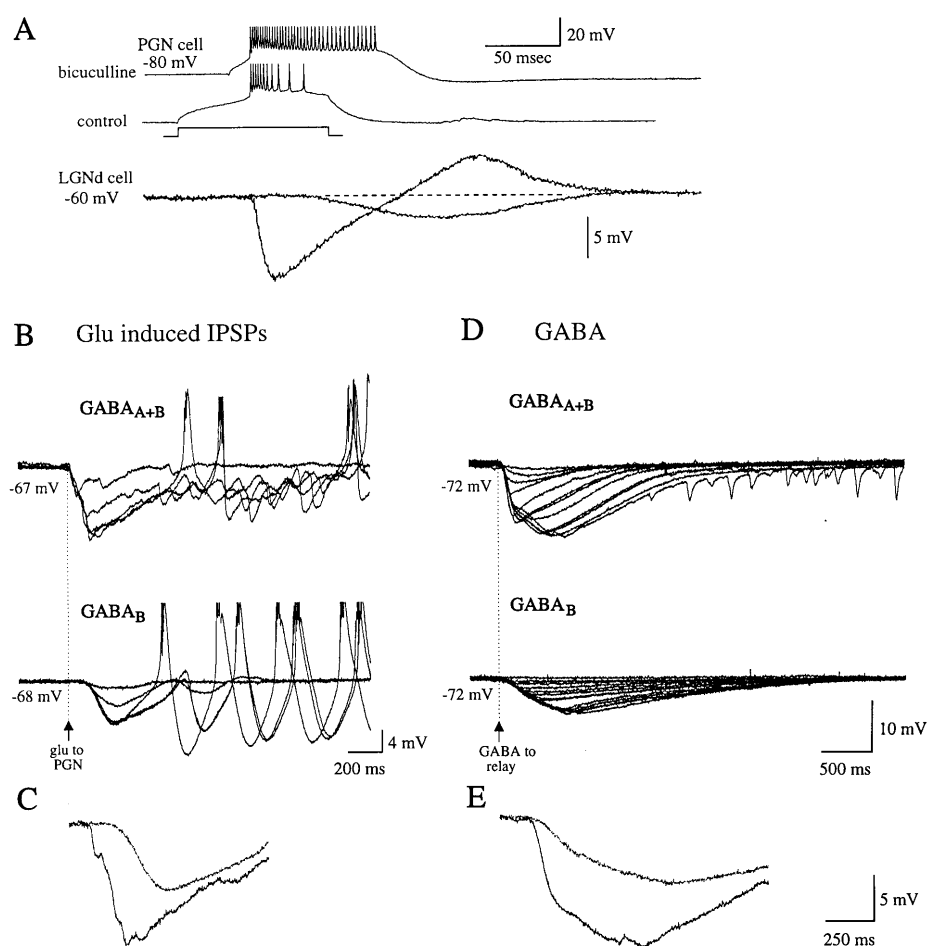
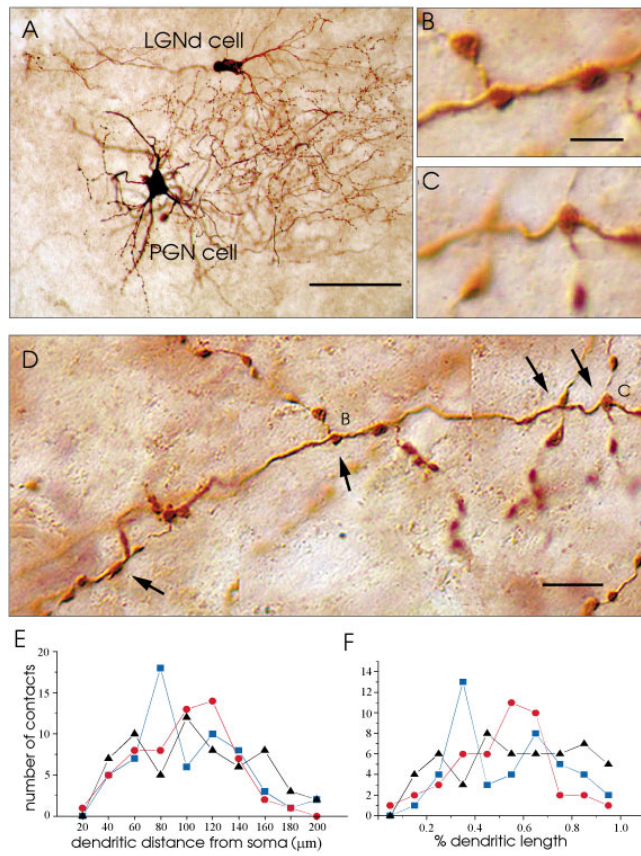


Fig. 3. The activation of GABA_B-receptor-mediated IPSPs is associated with a prolonged delay. **(A)** Activation of a burst of action potentials in the PGN cell of Fig. 2 before and after bath application of bicuculline reveals that the GABA_B-receptor-mediated slow IPSP has a prolonged delay of about 30 ms before onset of this event, whereas the GABA_A-receptor-mediated IPSP does not. **(B)** Similarly, large GABA_B-receptor-mediated IPSPs in thalamocortical cells that are generated in response to activation of the PGN with local application of glutamate also exhibit a prolonged delay [the response to increasing doses of glutamate in the PGN before and after the block of GABA_A receptors with local application of bicuculline (400 μ M in micropipette) is shown]. **(C)** Overlap of the glutamate-induced fast and slow IPSPs. Note differences in onset latency. **(D)** Responses of the thalamocortical neuron to local application of GABA before and after block of GABA_A receptors with local application of picrotoxin (200 μ M in micropipette). **(E)** Overlap of the fast and slow responses to GABA application reveal only a small difference in their onset latency (25).

Fig. 4. Morphological features of putative synaptic contacts between a single PGN GABAergic neuron and a LGNd thalamocortical cell. **(A)** Low-power photomicrograph of the biocytin-filled PGN and LGNd neurons demonstrating the extensive overlap in the axonal arbor of the PGN cell and the dendritic arbor of the LGNd neuron. Scale bar, 100 μm . **(B)** and **(C)** High-power photomicrographs of putative synaptic contacts between the PGN axon and the dendrite of the LGNd cell. Scale bar, 5 μm . **(D)** Lower power example of the overlap between the PGN axon and one of the dendrites of the LGNd cell. Scale bar, 10 μm . **(E)** Putative synaptic contacts between single PGN and LGNd cells are distributed throughout the dendritic arbor, with no putative synaptic contacts occurring either on the soma or within the first 20 μm of the dendrites. Data from three different pairs are shown. **(F)** Data in



(E) after normalization for total length of the dendrite upon which the putative synaptic contact is located. Activation of a burst of spikes in this PGN neuron resulted in the activation of an 8.7-mV fast bicuculline-sensitive IPSP and a 2.3-mV slow bicuculline-resistant IPSP at a membrane potential of about -60 mV in the identified thalamocortical cell.

phasize the critical role of the control of the excitability and discharge pattern of GABAergic neurons in the determination of the state of forebrain processing between waking, sleep, and epilepsy.

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- Standard in vitro slice and intracellular recording techniques were used. Sagittal (400 μm thick) slices of the ferret LGNd and PGN were formed on a vibratome and maintained at 34° to 35°C in an interface-style recording chamber. Bathing medium contained 124 mM NaCl, 2.5 mM KCl, 1.2 mM MgSO_4 , 1.25 mM NaPO_4 , 2 mM CaCl_2 , 26 mM NaHCO_3 , and 10 mM dextrose and was aerated with 95:5 mixture of O_2 : CO_2 to a final pH of 7.4. Dual intracellular recordings were obtained between a PGN neuron and a thalamocortical cell in the neighboring region of the A or A1 laminae with the use of beveled glass micropipettes (70 to 100 megohms) filled with 1.2 M potassium acetate and 2% biocytin. The pattern of action potential activity generated in the presynaptic neuron was controlled by adjusting the membrane potential through the injection of dc and by adjusting the amplitude and duration of the current pulse. The properties of IPSPs generated in postsynaptic neurons were examined at membrane potentials of -55 to -70 mV. About 5 to 10% of the pairs were monosynaptically connected in the direction from the PGN to LGNd. Capacitance coupling between the simultaneously recorded neurons was minimized by wrapping the microelectrodes in Paraffin (paraffin) and placing a metallic shield in between the two intracellular micropipettes. Biocytin-filled neurons were visualized by standard techniques (27). The presence and location of putative synaptic junctions between the PGN and thalamocortical neurons were examined with an $100\times$ oil immersion lens us-

ing differential interference contrast optics. CGP 35348 and CGP 54626A were kind gifts of Novartis (Switzerland). Ferrets were cared for and used in accordance with all appropriate regulatory guidelines.

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