$GABA_B$ and NMDA Receptors Contribute to Spindle-Like Oscillations in Rat Thalamus In Vitro

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Jacobsen, Richard B., Daniel Ulrich, and John R. Huguenard. $GABA_B$ and NMDA receptors contribute to spindle-like oscillations in rat thalamus in vitro. *J Neurophysiol* 86: 1365–1375, 2001. Thalamic slice preparations, in which intrathalamic connectivity between the reticular nucleus and relay nuclei is maintained, are capable of sustaining rhythmic burst firing activity in rodents and ferret. These in vitro oscillations occur spontaneously in the ferret and have frequencies (6–10 Hz) within the range of sleep spindles observed in vivo. In the rat, mainly lower frequency (2–4 Hz) oscillations, evoked under conditions of low bath $[Mg^{2+}]$ and/or $GABA_A$ receptor blockade, have been described. Here we show that faster rhythms in the range of 4–9 Hz can be evoked in rat thalamic slices by electrical stimulation of the internal capsule and also occur spontaneously. When bath $[Mg^{2+}]$ was 2 mM, these spindle-like oscillations were most common in a brief developmental time window, peaking at *postnatal day 12* (*P12*). The oscillations were almost completely blocked by the $GABA_A$ receptor antagonist picrotoxin, and, in some cases, the frequency of oscillations was increased by the $GABA_B$ receptor antagonist CGP-35348. The selective blockade of *N*-methyl-D-aspartate (NMDA) or α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors by the antagonists 2-amino-5-phosphonovaleric acid or 1,2,3,4-Tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulfonamide (NBQX), respectively, significantly shortened oscillations but did not completely block them. A combination of the two drugs was necessary to abolish oscillatory activity. The barbituate pentobarbital, which enhances $GABA_AR$ responses, initially slowed and synchronized oscillations before completely blocking them. When bath $[Mg^{2+}]$ was reduced from 2 to 0.65 mM, evoked oscillations became more robust and were often accompanied by spontaneously arising oscillations. Under these conditions, $GABA_A$ receptor blockade no longer inhibited oscillations, but instead converted them into the slow, synchronous rhythms that have been observed in other studies. The effects of $GABA_B$ or NMDA receptor blockade were more pronounced in 0.65 mM than in 2 mM external $[Mg^{2+}]$. Thus spindle-like oscillations occur in rat thalamic slices in vitro, and we find that, in addition to the previously demonstrated contributions of $GABA_A$ and AMPA receptors to these oscillations, NMDA and $GABA_B$ receptors are also involved. The strong influence of external $[Mg^{2+}]$ on GABAergic pharmacology and a contribution of NMDA receptors during oscillations suggest a link between the excitability of NMDA receptors and the activation of $GABA_BR$-mediated inhibitory postsynaptic currents.

INTRODUCTION

A well-established property of the thalamus is the generation of transient 7- to 14-Hz oscillations that arise in a periodic manner during the early stages of slow-wave sleep (Steriade and Deschênes 1984; Steriade and Llinás 1988). These oscillations have been termed sleep spindles and are one of several rhythms arising in the thalamus and cortex during non–rapid eye movement (non-REM) sleep (McCormick and Bal 1997; Steriade and Deschênes 1984; Steriade et al. 1993). The extensive reciprocal connectivity between thalamic and cortical neurons results in propagation of spindles through the cortex (Steriade and Llinás 1988), but the inability of cortex to produce these rhythms when disconnected from thalamus (Burns 1950), and their persistence in decorticated thalamus (Morison and Basset 1945), indicate a thalamic origin for spindle oscillations.

While the function of sleep spindles remains a mystery, the cellular mechanisms leading to their generation have been intensively studied and largely elucidated over the past several decades. Nearly 20 yr ago, electrophysiological recordings from thalamic neurons in vitro (Llinás and Jahnsen 1982) and in vivo (Deschênes et al. 1982; Roy et al. 1984) provided the basis for a mechanistic understanding of the state-dependent oscillatory behavior of the thalamus. Reciprocal connections between the inhibitory, GABAergic nucleus reticularis (nRt) and excitatory relay nuclei appear to provide the essential circuitry for spindle generation in the thalamus (von Krosigk et al. 1993; but see Steriade et al. 1987). During sleep onset, thalamic neurons undergo a membrane hyperpolarization (Hirsch et al. 1983; Steriade et al. 1986) that fundamentally alters their firing properties, primarily through the deinactivation of a low-threshold calcium current (Coulter et al. 1989; Crunelli et al. 1989; Hernandez-Cruz and Pape 1989; Jahnsen and Llinás 1984; Suzuki and Rogawski 1989). The biophysical properties of this calcium current in nRt and relay nuclei (Huguenard and Prince 1992) are such that it can be activated by incoming excitatory potentials in the former and on repolarization following inhibitory potentials in the latter (Bal et al. 1995a,b; Huguenard and Prince 1994a; Warren et al. 1994). In both cases, activation leads to a regenerative calcium spike that depolarizes the cells above Na^+-channel threshold long enough to cause a high-frequency burst of action potentials (Deschênes et al. 1982; Llinás and Jahnsen 1982). As a result of the feedback circuitry between nRt and relay nuclei, bursts in relay neurons produce glutamate receptor–dependent bursts in nRt neurons, which in turn inhibit relay cells. The relay neurons do not fire until they have recovered from the inhibi-

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tory input of nRt cells, meaning that the inter-burst duration, and thus the frequency of spindles, is largely dependent on the duration of inhibitory postsynaptic currents (IPSCs) in relay neurons (Bal et al. 1995a).

The generation of spindles is strongly influenced by inhibitory connections between nRt neurons, as shown by studies in which these predominantly $GABA_A$R-mediated connections (Ulrich and Huguenard 1996) are removed from the circuit either through pharmacological blockade (Bal et al. 1995a,b; Huguenard and Prince 1994a; Sanchez-Vives and McCormick 1997; Sanchez-Vives et al. 1997; von Krosigk et al. 1993) or genetic manipulation (Huntsman et al. 1999). The findings show that the absence of intra-nRt inhibition can enhance the output of nRt (Huguenard and Prince 1994b; Sanchez-Vives and McCormick 1997) and result in hyper-synchronous oscillatory activity (Huntsman et al. 1999). The slow, paroxysmal oscillations that result from $GABA_A$R blockade in ferret (Steriade et al. 1993; von Krosigk et al. 1993) and rat (Huguenard and Prince 1994a) thalamic slices resemble those seen during some forms of absence epilepsy.

The recent development of thalamic brain slice preparations in which spindle-like oscillations can be recorded has been an essential tool for working out the detailed molecular mechanisms responsible for oscillatory activity in the thalamus (reviewed in McCormick and Bal 1997). Thalamic slices from a number of species including rat (Huguenard and Prince 1994a; Leresche et al. 1991), mouse (Warren et al. 1994), and ferret (Bal et al. 1995a,b; von Krosigk et al. 1993) have demonstrated oscillatory behavior. The basic mechanisms responsible for spindle-like oscillations appear to be similar, although some differences between preparations are also apparent. Fast (5–10 Hz), spindle-like oscillations occur spontaneously in ferret slices under normal physiological conditions ($\sim$1.2 mM bath $[Mg^{2+}]$) (Bal et al. 1995a,b). In contrast, the pro-oscillatory conditions of $GABA_A$ receptor blockade and/or low bath $[Mg^{2+}]$ (0.2 mM) have been necessary to observe robust evoked oscillations in thalamic slices from rat (Cox et al. 1997; Huguenard and Prince 1994a; Ulrich and Huguenard 1995). Consistent with findings in ferret, oscillations evoked in rat slices in the presence of $GABA_A$R antagonism are slower (2–4 Hz). Here we show that oscillations with frequencies in a range closer to that expected for spindles can be evoked in rat slices, even under physiological conditions that would not be considered pro-oscillatory. The spindle-like oscillations in rat differ from those described in ferret (Bal et al. 1995a,b; von Krosigk et al. 1993) and mouse (Warren et al. 1994) in being sensitive to pharmacological blockade of $GABA_B$ and *N*-methyl-D-aspartate (NMDA) receptors.

METHODS

Slice preparation and recording

Thalamic slices were prepared as previously described (Huguenard and Prince 1994a). Briefly, rat pups of either sex, 11–15 days old were anesthetized (50 mg/kg ip pentobarbital sodium) and decapitated. The brain was removed and transferred into ice-cold slicing solution containing (in mM) 234 sucrose, 11 glucose, 24 $NaHCO_3$, 2.5 KCl, 1.25 NaH_2PO_4, 10 $MgSO_4$, and 0.5 $CaCl_2$ bubbled with 95% O_2-5% CO_2. The whole brain was glued onto a cover slip, and 400-μm horizontal slices were made using a vibratome (TPI, St. Louis, MO). The thalamus and parts of the adjacent striatum were dissected out and incubated in artificial cerebrospinal fluid (ACSF) containing (in mM) 126 NaCl, 26 $NaHCO_3$, 2.5 KCl, 1.25 $NaHPO_4$, 2 $MgCl_2$, 2 $CaCl_2$, and 10 glucose at 32°C for 1 h prior to recording. The solution was continuously bubbled with 95% O_2-5% CO_2, and temperature was allowed to return to room temperature after the initial hour.

For recordings, slices were placed in an interface type recording chamber and perfused with ACSF (described above) at 34°C. In reduced $[Mg^{2+}]$ experiments, the $[MgCl_2]$ in the perfusion solution was reduced to 0.65 mM. Intra-thalamic oscillations were evoked by a 20- to 100-V, 60- to 80-μs stimulus to the internal capsule (IC) through a bipolar tungsten electrode (FHC, Bowdoinham, ME) positioned at the border of the IC and the thalamic reticular nucleus (nRt). Stimulus interval was varied depending on the duration of an oscillation (to avoid inter-sweep variability presumably due to rundown) but was in the range of one stimulus every 15–60 s. Extracellular multi-unit activity was recorded using monopolar tungsten electrodes positioned in the ventrobasal (VB) somatosensory relay nucleus and/or nRt. The amplitude of action potentials ranged from 10 to 15 μV at threshold for spike detection (see *Data analysis*) to maximum potentials in the 100- to 200-μV range. Although only recordings from VB are presented in this study, spindle-like oscillations could be recorded simultaneously from VB and nRt, demonstrating that both nuclei were participating in oscillatory activity. Signals were band-pass filtered (10 Hz to 3 kHz) and recorded at different sample rates depending on the software used (1.7 kHz, Axotape, or 5–10 kHz, Axoscope, both from Axon Instruments, Foster City, CA).

Data analysis

A software Schmidt trigger was used to detect spikes. The "duration" of an oscillation was measured as the time from stimulus to last burst (defined as 4 spikes occurring within 50 ms). The "total spike activity" of an oscillation was defined as the number of spikes occurring between the time of stimulation and the end of the recording sweep. Autocorrelation analysis was performed on spike output, typically with a bin size of 5–10 ms, to estimate the synchrony of oscillations. The results from five consecutively evoked oscillations were summed for each plot presented in the results. Comparisons of synchrony were made using the height of the central peak relative to the adjacent valley of the autocorrelogram as an estimate of the proportion of synchronous versus asynchronous spike activity, respectively, during an oscillation. For comparisons of oscillation synchrony where total unit activity varied between pharmacological conditions within an experiment, autocorrelograms were normalized to the height of the central peak.

Fast Fourier transforms (FFTs) were also performed on spike output starting from the time of stimulation with bin sizes no larger than 20 ms. As summarized by the three-dimensional contour plots of FFT analysis presented in Figs. 1*B*, 2*B*, and 5*C*, FFTs were performed separately on each evoked spindle during an experiment. The oscillation frequency was calculated by determining the dominant spectral component between 2 and 10 Hz for each FFT spectra and averaging over at least five sweeps. Sweeps in which a dominant frequency could not be determined, defined as those in which the peak power of the dominant frequency comprised $<$40% of the summed peak power of the three most dominant frequencies, were not used.

Drugs

CGP-35348 (CGP, *p*-3-aminopropyl-p-diethoxymethyl phosphoric acid) was a gift from Ciba-Geigy (Basel). All other chemicals were from Sigma (St. Louis, MO). Picrotoxin, 1,2,3,4-Tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulfonamide (NBQX), and CGP stock solutions were made in DMSO such that the final concentration of DMSO in the extracellular recording solution was $\leq$0.5%. Unless

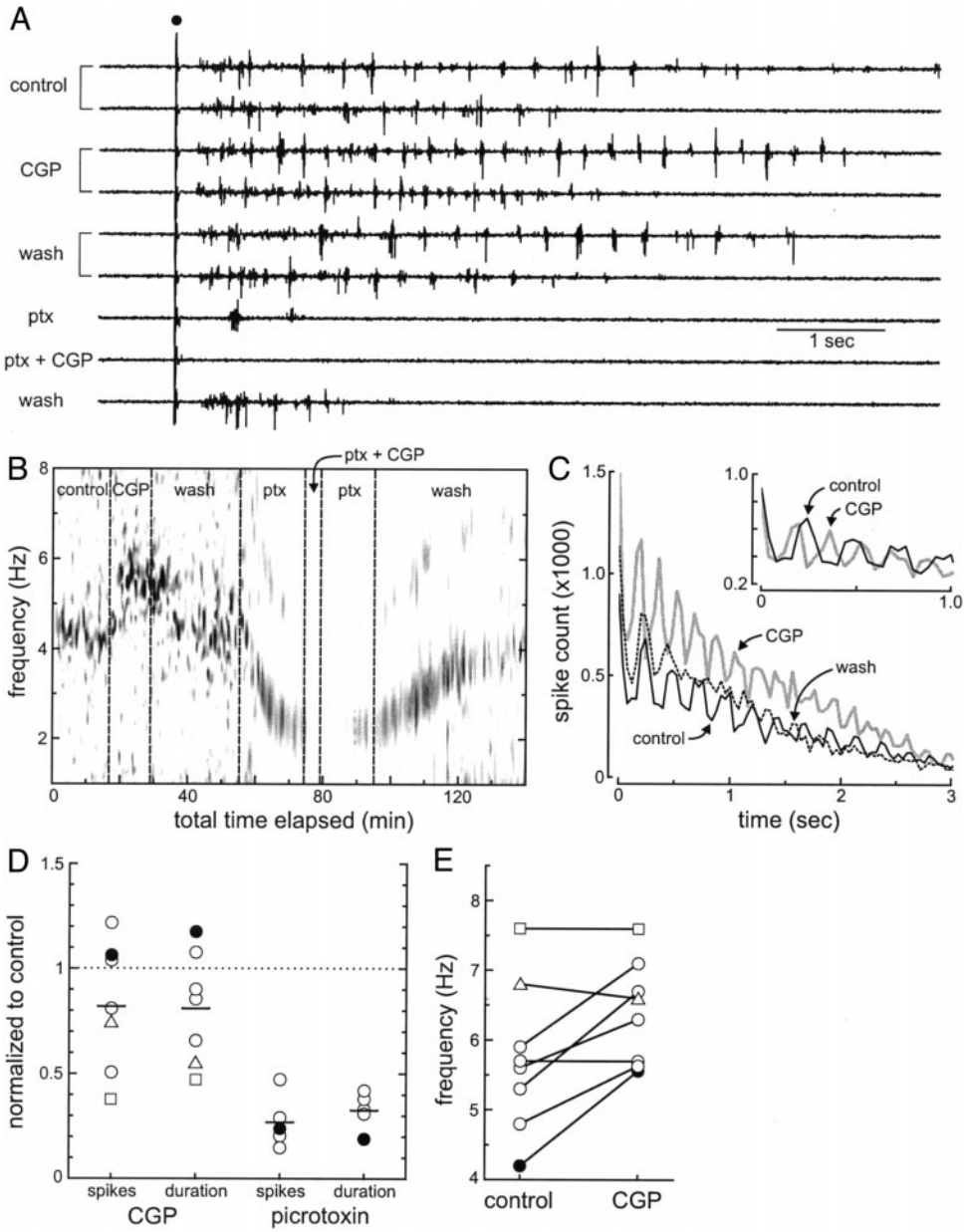

FIG. 1. GABAergic pharmacology of spindle-like oscillations (SLOs) recorded in 2 mM $[Mg^{2+}]_O$. *A*: extracellular, multi-unit recordings of oscillations in ventrobasal (VB) evoked by electrical stimulation of internal capsule (IC) at 40-s intervals. Two consecutive traces each are shown for oscillations evoked under control conditions, after bath application of 800 μM CGP-35348 (CGP) for ~10 min and following ~25 min of wash. The *last 3 traces* were recorded in the presence of 50 μM picrotoxin (ptx), a combination of ptx and CGP, and following ~50 min of wash, respectively. The dot at the *top left* indicates time of stimulation. *B*: the frequency components of each oscillation evoked during the experiment in *A* were determined by fast Fourier transform (FFT). The raw spectral power of the frequency components from each FFT is indicated by contour intensity, black being most intense. The dominant frequency of oscillations reversibly increased from 4–4.5 Hz under control conditions to 5–6 Hz in the presence of CGP. The addition of picrotoxin reversibly slowed oscillations to 2–2.5 Hz, and subsequent addition of CGP blocked them entirely. This effect was partially reversed during prolonged wash. *C*: autocorrelation (see METHODS) of spike activity from oscillations elicited under the conditions indicated on the plots. Blockade of $GABA_B$Rs increased the total number of spikes and the frequency of oscillations but did not influence the synchrony of spike activity, as shown by the normalized and expanded autocorrelograms in the *inset*. *D*: summary of CGP and picrotoxin effects on the total spike activity and duration (see METHODS) of SLOs for all experiments in 2 mM $[Mg^{2+}]_O$. Each point is the average of 5 consecutive evoked SLOs following drug application, normalized to the average response in control conditions. Horizontal bars are the average of all experiments. *E*: the effects of CGP on SLO frequency in 2 mM $[Mg^{2+}]_O$. Each point is the dominant frequency, as determined by FFT, averaged for 5 consecutively evoked SLOs (see METHODS). Error bars are not shown for clarity. Solid symbols in *D* and *E* represent the experiment shown above. △ and □, the 2 experiments in which CGP enhanced the synchrony of oscillations.

otherwise noted, drug concentrations were chosen to be 1–2 orders of magnitude greater than previously reported IC_{50}s.

RESULTS

Spindle-like oscillations recorded from rat thalamic slices

Previous studies have demonstrated that 2- to 4-Hz oscillatory activity can be evoked in rat thalamic slices using a combination of $GABA_A$ receptor ($GABA_A$R) blockade and reduced Mg^{2+} concentration in the extracellular recording solution ($[Mg^{2+}]_O$) (Cox et al. 1997; Huguenard and Prince 1994a; Ulrich and Huguenard 1995). We found that higher-frequency oscillations can occasionally be evoked in rat thalamic slices by electrical stimulation in the IC in the presence of 2 mM $[Mg^{2+}]_O$ [which is slightly higher than the ~1–1.3 mM found in physiological conditions (Hansen 1985)]. The frequency of these oscillations, usually above 4 Hz and as high as 9 Hz, is in the range observed for sleep spindles in vivo (Steriade and Llinás 1988) and in numerous in vitro studies using LGN slices from ferret (Bal et al. 1995a; Kim et al. 1995; von Krosigk et al. 1993). We thus refer to these 4- to 9-Hz oscillations in rat as "spindle-like oscillations," or SLOs.

Multi-unit, extracellular recordings of SLOs evoked by electrical stimulation of the internal capsule were made in the VB of rat thalamic slices, as detailed in METHODS. Our initial characterization of SLOs was done in 2 mM $[Mg^{2+}]_O$ to allow comparison with previous studies in rat in which this concentration was used (Cox et al. 1997; Huguenard and Prince 1994a). We found, however, that reducing $[Mg^{2+}]_O$ resulted in more robust spindle-like oscillations, as described below (see *Effects of $[Mg^{2+}]_O$ on SLOs*). Slices were selected for pharmacological manipulation based on the duration and frequency of evoked SLOs. Our selection criteria was for a minimum

duration of five resolvable cycles, or an oscillation lasting 1 s or more. Often, however, evoked oscillations were of much longer duration (up to 10 s). We found that the ability of a thalamic slice to support evoked SLOs in 2 mM $[Mg^{2+}]_O$ was strongly dependent on age. The proportion of preparations (each yielding 6 400-μm slices) that exhibited SLOs by age group was as follows: *postnatal day 11* (*P11*), 2/2; *P12*, 13/14; *P13*, 3/4; *P14*, 2/7. Rats younger than *P11* tended to have signal amplitudes that were near the threshold for resolving multi-unit extracellular activity, making the occurrence of SLOs difficult to assess. At age *P14* and older, this type of oscillation was very rarely observed in 2 mM $[Mg^{2+}]_O$. We focused our experiments on *P12* rats due to the ease of obtaining evoked SLOs at this age and the strength of the signal as compared with *P11* animals. For consistency, only data from this age group is presented in the remainder of this report.

A typical evoked SLO recorded in 2 mM $[Mg^{2+}]_O$ is shown in the *top two traces* of Fig. 1*A* (labeled "control"). The frequency of this activity was ~4.3 Hz, as determined by FFT of the unit activity that followed each stimulus (see METHODS). The FFT spectra for all control recording sweeps in this experiment are shown by the contour plot in Fig. 1*B* (portion labeled "control"). Note the cluster of darker contours (representing frequencies with the highest FFT power) in the 4- to 5-Hz range during this period.

Effects of $GABA_B$ receptor blockade on SLOs

Studies in mouse (Warren et al. 1994) and ferret (Bal et al. 1995a; von Krosigk et al. 1993) found that the blockade of $GABA_B$Rs did not affect evoked oscillatory activity in thalamic slices. We tested the contribution of $GABA_B$Rs to SLOs in rat by applying the antagonist CGP-35348 (CGP) to the bath solution during multi-unit, extracellular recording of evoked oscillations. Potential drug effects were quantified by measuring the total spike activity, duration, frequency, and synchrony of SLOs, as detailed in METHODS. An example of the effects of CGP is shown in Fig. 1. The effects were difficult to discern from raw recording traces (shown in Fig. 1*A*), but Fourier transform revealed a reversible increase (by ~25%) in the dominant frequency of oscillations following drug application (Fig. 1, *B* and *C*). An autocorrelation was performed on unit activity under control, CGP, and wash conditions to assess oscillation synchrony (see METHODS). The plots in Fig. 1*C* show a slight enhancement in the overall number of spikes detected during the oscillation in the presence of CGP relative to control and wash conditions. However, when the control and CGP autocorrelograms are normalized, there is no difference in the peak to valley ratio between the two plots (Fig. 1*C*, *inset*), suggesting no change in synchrony between these conditions.

In general, the effects of CGP on our measures of oscillation robustness (total spike activity and duration) were quite variable, ranging from a slight enhancement to a 50% reduction in these parameters (Fig. 1*D*). Although the population averages showed a slight suppression in both parameters, neither varied significantly between control and CGP conditions ($P = 0.18$, total spikes; $P = 0.11$, duration; 2-tailed *t*-test). In five of eight experiments, CGP caused a reversible increase in oscillation frequency (Fig. 1*E*). The magnitude of increase ranged from 10 to 30% of control and did not correlate with the frequency under control conditions. In two of eight experiments, CGP (800 μM) caused an increase in oscillation synchrony (based on autocorrelation analysis; see METHODS). In both of these experiments, CGP strongly suppressed the total spike activity and duration of oscillations while having little effect on frequency (Fig. 1, *D* and *E*, □ and △). An example of an experiment in which CGP application increased oscillation synchrony is presented in the section describing experiments with reduced $[Mg^{2+}]_O$.

Effects of pharmacological manipulation of $GABA_A$Rs on SLOs

The $GABA_A$R antagonist picrotoxin strongly suppressed SLOs recorded in 2 mM $[Mg^{2+}]_O$. An example is shown in the experiment in Fig. 1. The application of 50 μM picrotoxin reduced evoked activity from robust, high-frequency (~4.3 Hz) oscillations to brief, low-frequency (~2.3 Hz) events consisting of only one or two cycles (Fig. 1, *A* and *B*). A combination of picrotoxin and CGP abolished oscillatory activity (e.g., Fig. 1*A; n* = 3).

The suppressive effect of picrotoxin on SLOs is summarized for all experiments in Fig. 1*D*. In five of five experiments, the total spike activity and duration of oscillations in the presence of the drug was reduced by ~50–90%, differing significantly from control conditions across the population ($P < 0.001$, 2-tailed *t*-test). When more than one cycle of an oscillation remained after picrotoxin application, the frequency calculated from the inter-burst interval was greatly reduced (2.5 ± 0.2 Hz; mean ± SE, $n = 3$). These findings are consistent with observations made in young ferret, where spindles from animals up to *P39*, recorded in 1.2 mM $[Mg^{2+}]_O$, were largely blocked by the $GABA_A$R antagonist bicuculline (McCormick et al. 1995).

Effects of $[Mg^{2+}]_O$ on SLOs

Conditions of reduced extracellular magnesium can greatly enhance the ability of thalamic slices to support oscillations (Cox et al. 1997; Huguenard and Prince 1994a; Ulrich and Huguenard 1995). However, we found that decreasing $[Mg^{2+}]_O$ to 0.2 mM, the concentration used in these earlier studies, resulted in oscillations that were less stable over the extended periods of time required to perform the pharmacological manipulations described here. Instead, we adopted a protocol using an intermediate $[Mg^{2+}]_O$ of 0.65 mM. Reducing $[Mg^{2+}]_O$ from 2.0 to 0.65 mM markedly increased the occurrence of evoked and spontaneously arising SLOs in thalamic slices from *P12* rats (presented in this study) and in older animals (up to *P15*, data not shown). Figure 2*A* shows an example of the effects of reducing $[Mg^{2+}]_O$.

The effects of GABAergic blockade on SLOs evoked in 0.65 mM $[Mg^{2+}]_O$ were notably different from those seen in 2 mM $[Mg^{2+}]_O$. In reduced $[Mg^{2+}]_O$, the application of CGP caused either no change or an enhancement in the total spikes and duration of SLOs, as summarized in Fig. 2*D*. The example shown in Fig. 2 proved to be the most pronounced instance of an increase in these parameters. In addition, the influence of CGP on the frequency and synchrony of SLOs became more pronounced in 0.65 mM $[Mg^{2+}]_O$ such that clear effects were seen in every experiment. The application of CGP resulted in an increase in frequency (by 15–22%) in six of six experiments (as compared with 5/8 experiments in 2 mM $[Mg^{2+}]_O$), as

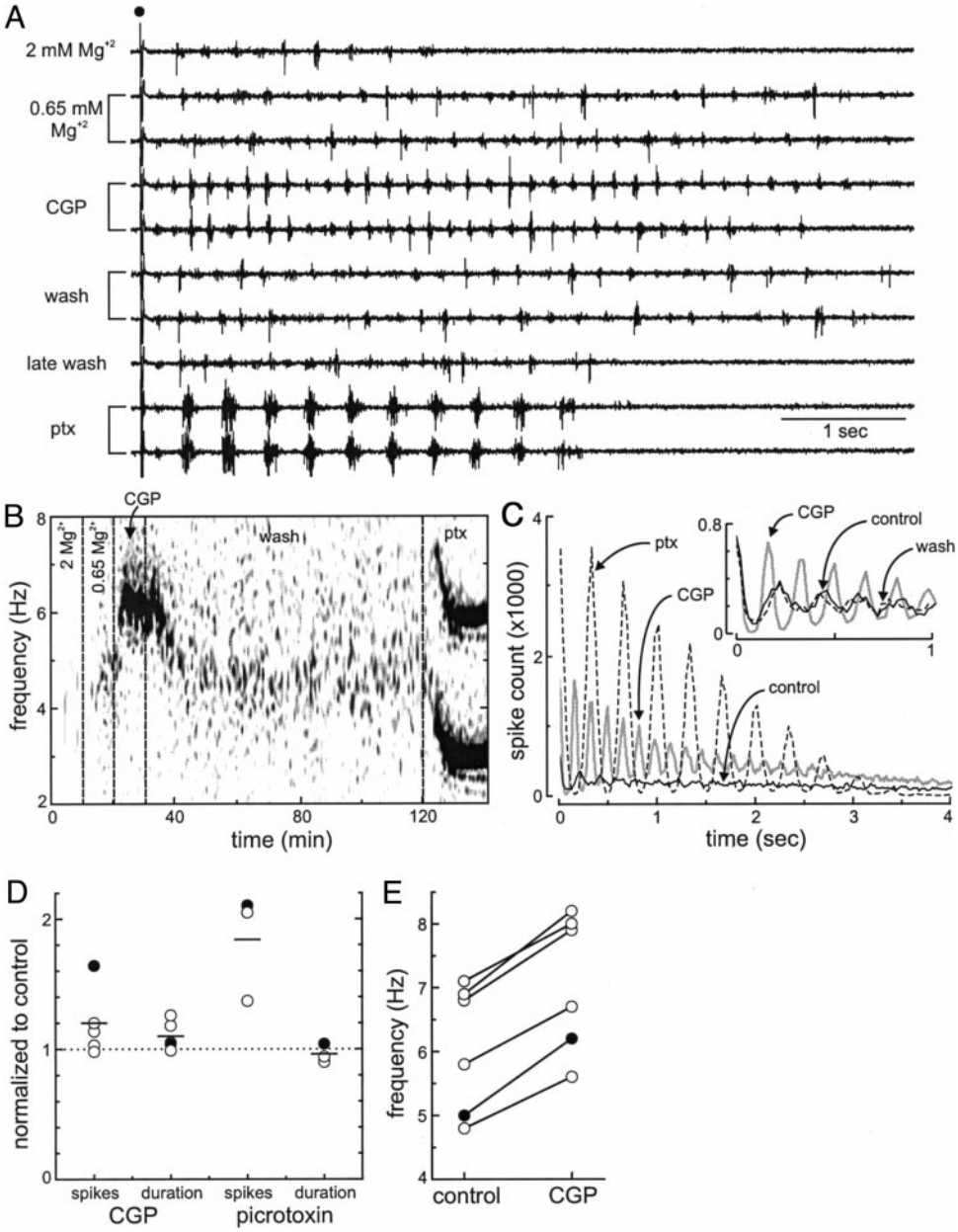

FIG. 2. GABAergic pharmacology of spindle-like oscillations recorded in 0.65 mM $[Mg^{2+}]_O$. *A*: extracellular, multi-unit recordings of oscillations in VB evoked by electrical stimulation of IC at 40-s intervals. The oscillations were greatly enhanced by reducing $[Mg^{2+}]_O$ from 2 mM (*top trace*) to 0.65 mM (*next 2 traces*). In reduced $[Mg^{2+}]_O$, the addition of 800 μM CGP-35348 (CGP) greatly increased the frequency and total spike activity of oscillations (*next 2 traces*). This effect was reversible following ~30 min of wash (*next 2 traces*). Following 90 min of wash the oscillation was attenuated (trace labeled "late wash"), but application of 50 μM picrotoxin (ptx) greatly increased both the synchrony and total spikes in the remaining oscillation (*last 2 traces*). *B*: the frequency components of each oscillation evoked during the experiment in *A* were determined by FFT. The raw spectral power of each frequency component of the FFT is indicated by contour intensity, black being most intense. The dominant frequency of oscillations reversibly increased from 4.5–5 Hz under control conditions to 6–6.5 Hz in the presence of CGP, and was slowed to 3 Hz in picrotoxin. This effect was partially reversed during prolonged wash. *C*: autocorrelation (see METHODS) of spike activity under the conditions indicated on the plots. In this experiment, blockade of $GABA_A$ or $GABA_B$ receptors increased the total spike activity and synchrony of the oscillations. The synchronizing effect of CGP application can be seen in the normalized and expanded plots in the *inset* (compare with Fig. 1*C*). *D*: the effects CGP or picrotoxin (ptx) on the total spike activity and duration of SLOs in 0.65 mM $[Mg^{2+}]_O$. Each point is the average of 5 consecutively evoked SLOs following drug application, normalized to the average response in control conditions. Horizontal bars are the average of all experiments. *E*: the effects of CGP on SLO frequency in 0.65 mM $[Mg^{2+}]_O$. Each point is the average of the dominant frequency, as determined by FFT, from 5 consecutively evoked SLOs (see METHODS). Solid symbols in *D* and *E* represent the experiment shown above.

shown by the example in Fig. 2 and population summary in Fig. 2*E*. Synchrony was also enhanced in every experiment (compared with only 2/8 experiments in 2 mM $[Mg^{2+}]_O$), as in Fig. 2*C*.

The most striking difference between oscillations recorded in high versus low $[Mg^{2+}]_O$ was in the response to $GABA_AR$ blockade by picrotoxin. In 2 mM $[Mg^{2+}]_O$, picrotoxin nearly abolished SLOs (see Fig. 1), while in 0.65 mM $[Mg^{2+}]_O$ oscillations became slow and hypersynchronous without being attenuated (Fig. 2, *A* and *B*). In the three experiments performed under these conditions, total spike activity showed a trend toward enhancement while duration remained unchanged (Fig. 2*D*, *right side*). The frequency of oscillations in the presence of picrotoxin was reduced to 2.7 ± 0.4 Hz (e.g., Fig. 2*B; n* = 3). These observations are consistent with results from earlier studies using older rats and 0.2 mM $[Mg^{2+}]_O$ (Cox et al. 1997; Huguenard and Prince 1994a; Ulrich and Huguenard 1995).

Contribution of NMDA receptors to oscillations

The enhancement of SLOs in reduced $[Mg^{2+}]_O$ raised the possibility that at least part of this effect might be explained by the inhibitory interaction of Mg^{2+} with NMDA receptors (NMDAR; Nowak et al. 1984). We tested this by applying the selective NMDAR antagonist 2-amino-5-phosphonovaleric acid (APV) to SLOs recorded in 2 mM $[Mg^{2+}]_O$. In eight of eight experiments, the application of APV attenuated both the total spike activity and the duration of SLOs by 15–75% (Fig. 3*C*). The reduction was significant across the population ($P < 0.001$, 2-tailed *t*-test). An example is provided in Fig. 3*A*. In this experiment, control oscillations were evoked by near-threshold (20 V) stimulation of the internal capsule, and bath application of 200 μM APV reduced the duration and total spike activity of oscillations by ~30%. A slight slowing of oscillation frequency was seen in the presence of APV in three of five experiments where frequency could be quantified before

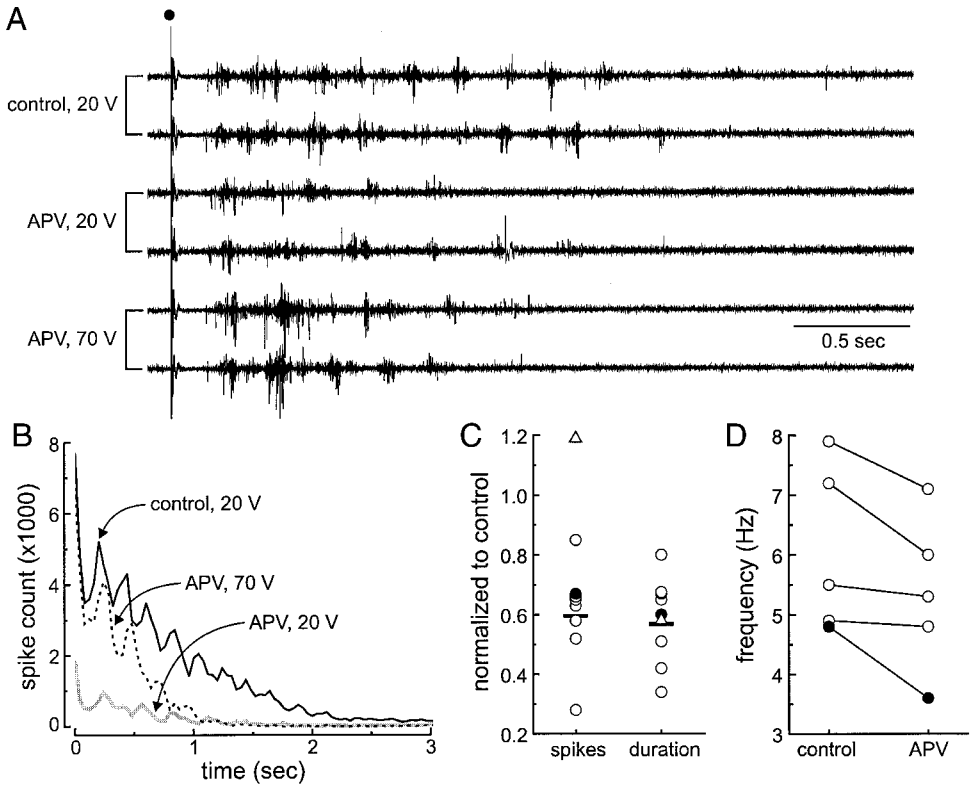

FIG. 3. The effects of *N*-methyl-D-aspartate (NMDA) receptor blockade on spindle-like oscillations. *A*: extracellular, multi-unit recording of oscillations in VB evoked by electrical stimulation in IC at 40-s intervals. Two consecutive traces each are shown for oscillations evoked under control conditions and, at the stimulus intensity indicated, following the application of 200 μM 2-amino-5-phosphonovaleric acid (APV) for ~10 min. An increase in stimulus intensity from 20 to 70 V in the presence of APV increased the number of spikes but not the duration of the oscillation (see *B*, below). *B*: autocorrelation (see METHODS) of spike activity under the 3 conditions shown above, as indicated on the plots. *C*: summary of the effects of APV on the total spike activity and duration of oscillations in all experiments. Each point is the average from 5 consecutive evoked oscillations following APV application normalized to the average response in control conditions. The experiment in *A* and *B* is represented by shaded circles (20-V stimulus) or triangles (70-V stimulus). Note the increase in total spikes, but not duration, when the stimulus intensity was increased from 20 to 70 V. Horizontal bars are the average of all experiments. *D*: the effects of APV on oscillation frequency for those experiments where it could be determined for both control and APV conditions. Each point is the average of dominant frequency from 5 evoked oscillations, as determined by FFT (see METHODS). Shaded circles represent the experiment in *A*.

and after drug application (e.g., Fig. 3, *A* and *B*, summarized in Fig. 3*D*).

The inhibitory actions of APV on evoked SLOs could occur at excitatory inputs activated by the initial stimulation of the internal capsule (at corticothalamic and/or thalamoreticular synapses) and/or at thalamoreticular synapses during the recurrent excitation of nRt cells necessary for the maintenance of oscillations. If NMDAR blockade only reduced the efficacy of the initial stimulus event, the effects of APV might be overcome by an increase in stimulus intensity. As demonstrated in Fig. 3, *A* and *B*, increasing stimulus intensity from 20 to 70 V greatly enhanced the total spike activity of oscillations in the presence of APV (to levels comparable with control) but was ineffective in recruiting additional cycles to the oscillation. Similar results were seen in two additional slices using stimulus intensities ranging from threshold to 100 V (not shown). While the correlation between increased unit activity and nRt output using this paradigm cannot be determined directly from extracellular recordings, these results are consistent with an influence of APV at thalamoreticular synapses during recurrent excitation, in addition to the likely effects on stimulus efficacy.

The inhibitory effect of APV was also tested in 0.65 mM $[Mg^{2+}]_O$ ($n = 3$, not shown). On average, the APV-induced reduction in total spike activity of SLOs was more pronounced in 0.65 mM than in 2 mM $[Mg^{2+}]_O$ (58 ± 4% vs. 39 ± 6% block, respectively). In addition, APV was slightly more effective at inhibiting the total spike activity of SLOs recorded in 0.65 mM $[Mg^{2+}]_O$ than was increasing $[Mg^{2+}]_O$ to 2 mM when the two conditions were tested sequentially in the same slice (58 ± 4% vs. 44 ± 4%, respectively, $n = 3$). These observations are consistent with a role for NMDAR excitability in explaining the strong influence of $[Mg^{2+}]_O$ on oscillatory activity in thalamic slices.

We next blocked α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate receptors using the selective antagonist NBQX and found that oscillations could still be evoked. The addition of a high concentration of NBQX (2 μM) altered the organization and reduced the duration of oscillations but had little effect on the total spike activity (see the example in Fig. 4). Oscillations evoked in the presence of NBQX could be completely blocked by the subsequent addition of 50 μM APV, confirming that they were mediated largely by NMDARs ($n = 3$, e.g., Fig. 4). In the example shown, NMDAR-mediated oscillations had a lower frequency than control oscillations (3.3 vs. 5.5 Hz), prolonged bursts and more synchronous burst activity (Fig. 4*B*). The frequency of NMDAR-mediated oscillations was dramatically reduced in three of four experiments using AMPA receptor (AMPAR) antagonists (Fig. 4*D*). In one of these experiments (indicated in Fig. 4, *C* and *D*, ■), the AMPA/kainate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) was tested on a rare SLO recorded from a *P15* animal with effects on frequency and burst structure similar to the example in Fig. 4.

Effects of pentobarbital on SLOs

We also tested the effects of the barbiturate pentobarbital (pb) on SLOs evoked in slices from *P12* rats. This drug has been shown to modulate $GABA_A$Rs by prolonging channel open times (Macdonald and Olsen 1994). As shown in Fig. 5, 100 μM pb reversibly abolished SLOs. During bath application of the drug, a progressive lengthening of inter-

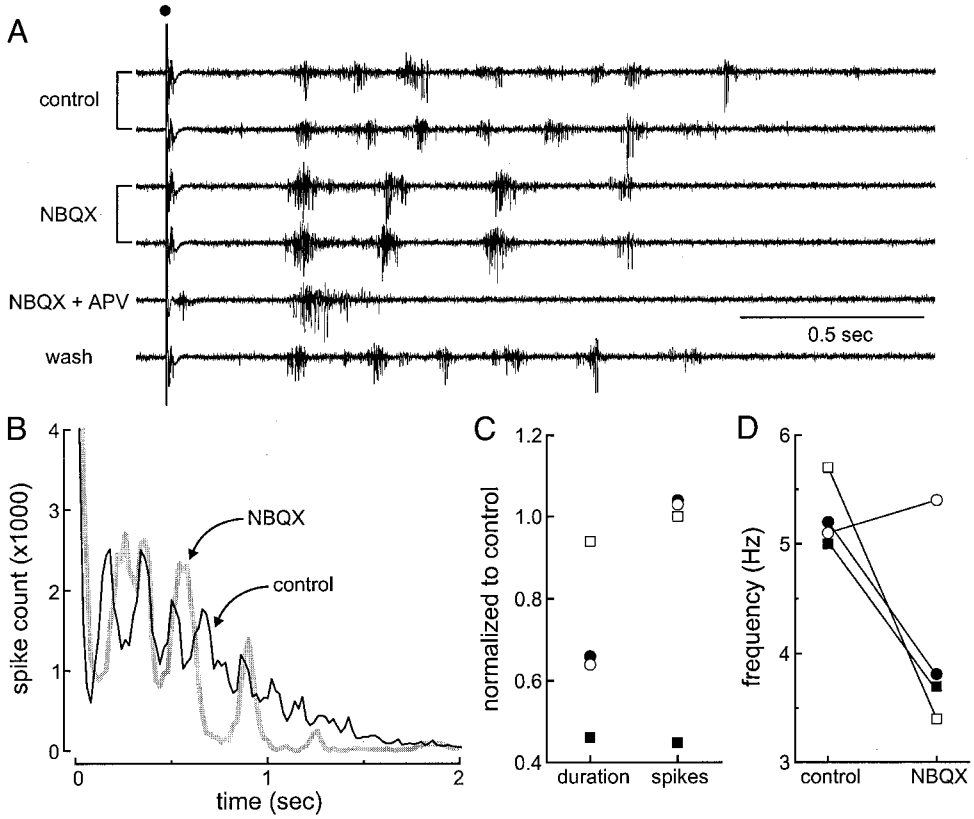

FIG. 4. The effects of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor blockade on spindle-like oscillations. *A*: extracellular, multi-unit recordings of oscillations in VB evoked by electrical stimulation in nucleus reticularis (nRt) at 30-s intervals. Two consecutive traces each are shown for control conditions and following bath application of 2 μM 1,2,3,4-Tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulfonamide (NBQX) for 30 min. The next trace was recorded in the presence of a combination of 50 μM APV and 2 μM NBQX, and the *last 2 traces* following 30 min of wash. The single delayed burst of spikes recorded after complete AMPA and NMDA receptor blockade was likely the result of direct excitation of nRt neurons by the stimulus, which triggered a rebound burst in VB neurons. *B*: autocorrelation (see METHODS) of spike activity under control and NBQX conditions, as indicated on the plots. *C*: summary of the effects on the total spike activity and duration of oscillations in 3 experiments using NBQX in *postnatal day 12* (*P12*) animals (●, ○, and □) and one using 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 20 μM) in a *P15* animal (■). Each point is the average from 5 consecutive evoked oscillations following drug application normalized to the average response under control conditions. *D*: the effects of NBQX on oscillation frequency (as determined by FFT, see METHODS) in the same experiments.

burst intervals gradually reduced the oscillation frequency from 6–7 Hz to ~4 Hz, accompanied by a pronounced synchronization of the remaining cycles. Eventually a complete loss of recurrent activity, including the initial rebound burst normally induced by the stimulus, was seen. The complete experiment is presented as a spike-rate contour plot in Fig. 5*B*. The progressive changes in oscillation frequency following pb application can be seen in the contour plot of FFTs in Fig. 5*C*. Similar effects, including the eventual loss of all oscillatory activity in the presence of 100 μM pb, were reproduced in two additional experiments (not shown).

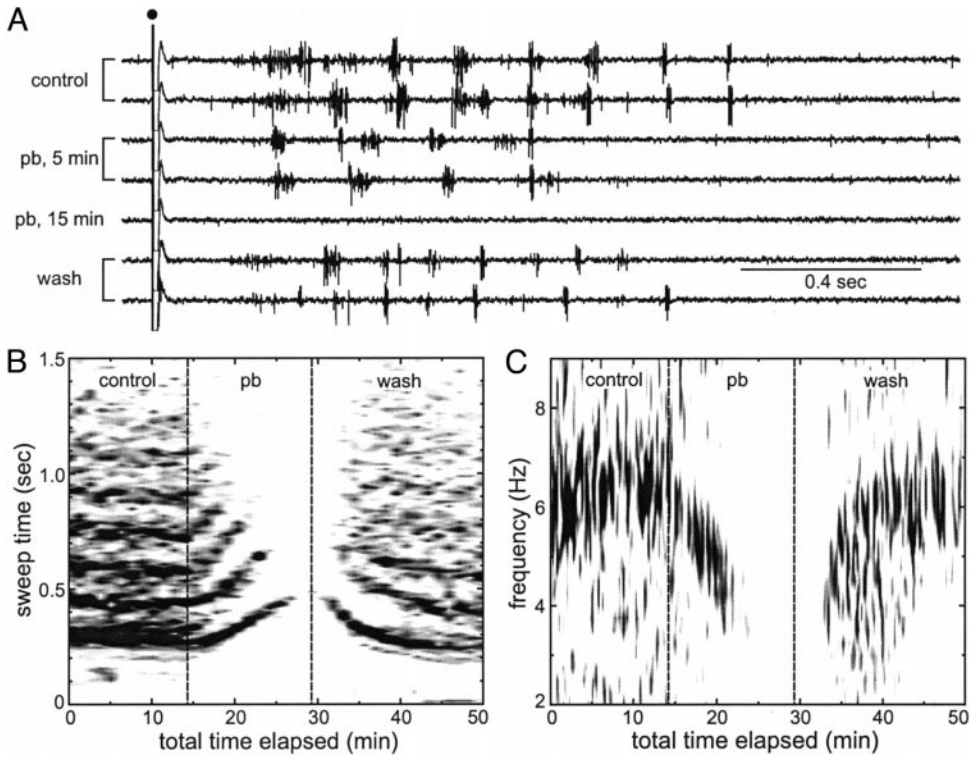

FIG. 5. The effect of the barbiturate, pentobarbital (pb), on spindle-like oscillations. *A*: extracellular, multi-unit recordings of oscillations in VB evoked by electrical stimulation of IC at 15-s intervals. Two consecutive traces each are shown for control conditions and following exposure to 100 μM pb for 5 min. After 15 min exposure to pb, the oscillation was completely abolished (*next trace*), and this effect was reversible after 20 min of wash (*last 2 traces*). *B*: contour plot showing oscillatory activity during a 1.5-s recording period following each stimulus in the experiment above. The contours represent spike rate/10-ms bin, black representing the highest rate. Note that early following application of pb, the oscillation became progressively slower and more organized. *C*: the frequency components of each evoked oscillation in the experiment above were determined by FFT. The raw spectral power of each frequency component of the FFT is indicated by contour intensity, black being most intense.

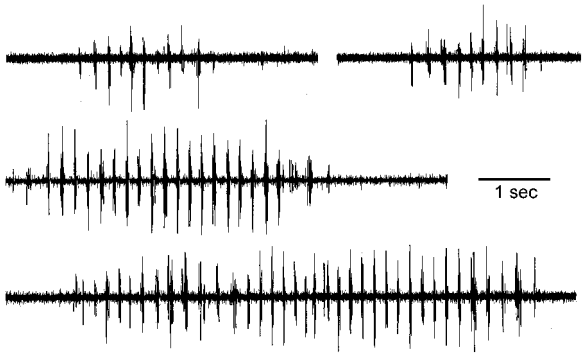

FIG. 6. Spontaneous spindles were occasionally recorded from rat thalamic slices in 2 mM $[Mg^{2+}]_O$. Extracellular, multi-unit recordings of spontaneous spindles from 2 sites in VB of a *P12* rat are shown. The *middle trace* was recorded from a separate location from the *top* and *bottom traces*. The frequencies were 5–6 Hz, and the durations ranged from 1.8 to 6.6 s.

Spontaneous SLOs

Spontaneous spindles were often seen in *P12* slices using 0.65 mM $[Mg^{2+}]_O$ but were observed much more rarely in 2 mM $[Mg^{2+}]_O$. Several examples of spindles recorded in the latter condition from two separate sites in VB are shown in Fig. 6. In these recordings, spontaneous spindles arose every 10–60 s, lasted 1–7 s, and had frequencies of 5–6 Hz.

DISCUSSION

Role of $GABA_B$ receptors in spindle oscillations

We have shown that SLOs with frequencies in the 4- to 9-Hz range can be evoked in thalamic slices from young rats. The oscillations resemble those seen in extracellular recordings from ferret lateral geniculate nucleus (LGN) slices (Bal et al. 1995a; McCormick et al. 1995; von Krosigk et al. 1993), but pharmacological manipulations revealed several differences between the two preparations. In ferret, the blockade of $GABA_B$Rs had no apparent effect on spontaneous (Bal et al. 1995a) or evoked (Sanchez-Vives and McCormick 1997) spindles recorded in 1.2 mM $[Mg^{2+}]_O$. We found that the $GABA_B$R antagonist CGP-35348 had several effects on evoked SLOs when bath applied to rat thalamic slices, and that these effects were sensitive to $[Mg^{2+}]_O$. An increase in oscillation frequency was seen in five of eight experiments where $[Mg^{2+}]_O$ was 2 mM (range, 13–32% increase) and in all six experiments where $[Mg^{2+}]_O$ was 0.65 mM (range, 13–24%; compare Figs. 1*E* and 2*E*). In addition, $GABA_B$R blockade caused an increase in the synchrony of oscillations that was much less consistent in 2 mM than in 0.65 mM $[Mg^{2+}]_O$ (observed in 2/8 and 6/6 experiments, respectively). Finally, based on our measures of oscillation robustness (total spike activity and duration), CGP could have enhancing or suppressing effects in 2 mM $[Mg^{2+}]_O$ but tended to leave unchanged or cause a slight enhancement in 0.65 mM $[Mg^{2+}]$ (compare Figs. 1*D* and 2*D*). These results indicate that in the rat preparation $GABA_B$Rs are activated during evoked SLOs where they may have slowing and/or desynchronizing effects on oscillatory activity.

The actions of CGP could be pre- or postsynaptic, as has been described in several thalamic studies in rat (Ulrich and Huguenard 1996) and ferret (Sanchez-Vives and McCormick 1997; Sanchez-Vives et al. 1997). The increase in frequency seen following CGP application in the present study is likely the result of postsynaptic blockade of $GABA_B$Rs in relay neurons, as relief of presynaptic inhibition would be expected to increase GABA output and, if anything, prolong postsynaptic inhibitory postsynaptic potentials (IPSPs). The loss of slow, $GABA_B$-mediated IPSPs would result in more rapid rebound bursting, since the remaining IPSPs would be shorter duration, $GABA_A$-mediated events. In addition, a homogeneous group of $GABA_A$R-mediated IPSPs in relay cells might synchronize rebound activation and result in more organized oscillations, as was seen in some experiments with CGP (and also transiently during pentobarbital application; see *Effects of pentobarbital on oscillations*). The complex effects of CGP on oscillation robustness (see Fig. 1*D* and 2*D*) could be due to a mixture of pre- and postsynaptic effects at both reticulo-thalamic and intra-nRt synapses. Variations in connectivity and/or membrane properties between preparations might favor the activation of different sets $GABA_B$Rs in different experiments.

A study using paired recordings between synaptically coupled neurons of the perigeniculate nucleus (PGN) and lateral geniculate nucleus (LGN) in ferret slices examined the efficacy of high-frequency IPSCs in promoting $GABA_B$R activation during bursts induced in single PGN neurons (Kim and McCormick 1998; Kim et al. 1997). This study found that only prolonged, high-frequency bursts in a PGN neuron, such as those seen following $GABA_A$R blockade of intra-nRt connections by picrotoxin or bicuculline, were capable of significantly activating postsynaptic $GABA_B$Rs in a monosynaptically coupled LGN neuron. Our results in rat suggest that $GABA_B$R activation can occur under more normal bursting conditions. One possible explanation is convergence of GABAergic input from multiple nRt cells onto single relay neurons. The increase in $GABA_B$R activation in 0.65 mM $[Mg^{2+}]_O$, as indicated by the more pronounced effects of CGP and the maintenance of $GABA_B$-mediated oscillations in the presence of picrotoxin, suggests an increase in the amount of GABA released by nRt neurons under these conditions. The possible contribution of NMDARs to an enhancement in nRt output is discussed in the next section.

While this is the first study to implicate $GABA_B$Rs in normal SLOs in vitro, intracellular recordings in mouse (Warren et al. 1997), rat (Huguenard and Prince 1994a) and ferret (Bal et al. 1995b; Kim et al. 1997; Sanchez-Vives and McCormick 1997; von Krosigk et al. 1993) have demonstrated that $GABA_B$Rs can be activated at synapses between nRt and relay neurons. However, the studies in ferret and mouse found no effect of $GABA_B$R antagonists on in vitro oscillations. Slice preparations of mouse ventroposterior thalamus, for example, were unable to support $GABA_B$R-mediated oscillations following the blockade of $GABA_A$Rs in 1.3 mM (Warren et al. 1994) or 2 mM $[Mg^{2+}]_O$ (Huntsman et al. 1999), despite the functional presence of postsynaptic $GABA_B$Rs (Warren et al. 1997). In contrast, robust $GABA_B$R-mediated oscillations can occur in the rat ventrobasal nucleus (Huguenard and Prince 1994a) and ferret LGN (von Krosigk et al. 1993) under similar conditions. These discrepancies could be explained by species-specific and/or anatomical differences in preparations.

NMDA receptors contribute to SLOs at thalamoreticular synapses

A number of studies in rodent have shown an NMDAR component to excitatory potentials evoked at corticothalamic synapses in both the reticular (de Curtis et al. 1989; Golshani and Jones 1999) and relay neurons (Deschênes and Hu 1989; Golshani et al. 1998; Kao and Coulter 1997; Scharfman et al. 1990; Turner and Salt 1998). An immunocytochemical electron microscopy study identified the NMDAR1 subunit at putative corticothalamic and thalamoreticular type synapses in nRt of cat and rat (Liu 1997). One previous report has attempted to specifically identify an NMDAR component to excitatory postsynaptic potentials (EPSPs) at thalamoreticular synapses during oscillatory activity (Huguenard and Prince 1994a). Consistent with the present results, it was concluded that NMDARs at thalamoreticular synapses contributed to recurrent oscillatory activity.

The finding that the NMDAR antagonist APV reduces the total spike activity and duration of oscillations (see Fig. 3*C*) evoked by electrical stimulation of both corticothalamic and thalamocortical axons in the internal capsule could be explained in two ways (that are not mutually exclusive). NMDAR activation could be reduced at one or both synapses during stimulation, and/or at thalamoreticular synapses during the maintenance of oscillations following initiation. Several observations strongly suggest that NMDARs are functionally present at thalamoreticular synapses and that they contribute to the maintenance of SLOs. The most convincing argument for this is the persistence of oscillations after AMPAR blockade. It is possible that a small fraction of the AMPAR population remained unblocked in 2 μM NBQX, but the complete suppression of the remaining oscillatory activity by APV shows that NMDARs are playing at least a major facilitative role. Support for an NMDAR contribution to SLO maintenance comes from experiments showing that increasing stimulus intensity does not overcome NMDAR blockade, despite the recruitment of additional unit activity during stimulation (Fig. 3, *A–C*). In addition, the frequency of oscillations was reversibly reduced in the presence of APV in several experiments. Since stimulus intensity did not influence the frequency of evoked oscillations (unpublished observations), we conclude that the changes in frequency arise from the influence of NMDAR blockade on the internal structure of oscillations, i.e., at thalamoreticular excitatory synapses, rather than through a reduction in stimulus efficacy.

Effects of $[Mg^{2+}]_O$ on $GABA_BR$ activation: a possible link with NMDAR excitability

When $[Mg^{2+}]_O$ was 2 mM, SLOs were largely blocked by the $GABA_AR$ antagonist picrotoxin, suggesting that postsynaptic $GABA_BR$ activation in relay neurons was insufficient to induce rebound burst generation and sustain the oscillation. In 0.65 mM $[Mg^{2+}]_O$, SLOs were converted into slow, hyper-synchronous oscillations in the presence of picrotoxin. This suggests that $GABA_BR$-mediated IPSPs were sufficiently enhanced under these conditions to drive rebound burst firing in relay neurons, presumably due to an increase in the output of nRt neurons. Also consistent with increased $GABA_BR$ activation in reduced $[Mg^{2+}]_O$ was the more pronounced effect of CGP on SLO frequency under these conditions.

A developmental study in ferret LGN found that bicuculline nearly abolished spindle oscillations in thalamic slices from animals up to *postnatal day 39* but slowed and synchronized them in older animals (McCormick et al. 1995). The authors concluded that the ability of PGN neurons to generate sustained high-frequency bursts of action potentials, which are necessary to activate postsynaptic $GABA_BRs$, was a limiting factor during development. The changes in $[Mg^{2+}]_O$ in our study are unlikely to influence action potential firing in the same way, although there may be other nonspecific effects on excitability, such as changes in ion channel inhibition, charge screening effects, or Mg^{2+} block of NMDARs. Our findings provide some support for the importance of NMDAR excitability in mediating Mg^{2+}-related changes in oscillatory activity. The inhibitory effect of APV and 2 mM $[Mg^{2+}]_O$ on SLOs recorded in 0.65 mM $[Mg^{2+}]_O$ was similar when compared in the same slice. In addition, the inhibitory effect of APV was, on average, more pronounced in 0.65 mM than 2 mM $[Mg^{2+}]_O$, suggesting that the NMDAR contribution to oscillations is enhanced in lower $[Mg^{2+}]_O$. These findings support a link between NMDAR excitability and the sensitivity of SLOs to $[Mg^{2+}]_O$.

A recent study in the amygdala found that NMDAR-mediated EPSPs were more effective than AMPAR currents at activating a low-threshold Ca^{2+} current (Calton et al. 2000). This raises the interesting possibility that NMDAR activation could enhance the bursting capabilities of nRt neurons by influencing the activation of a thalamic low-threshold Ca^{2+} channel. The apparent increase in $GABA_BR$ activation in reduced $[Mg^{2+}]_O$ supports the notion that the output of nRt neurons is enhanced under these conditions and this could be due, at least in part, to the influence of NMDAR activation on the bursting properties of nRT.

Effects of pentobarbital on oscillations

The barbiturate pentobarbital completely blocked SLOs in *P12* slices. A gradual increase in inter-burst duration (slowing of frequency) and a synchronization of the remaining cycles in the oscillation was followed by complete loss of oscillatory activity. These findings are consistent with the slowing of IPSC decay kinetics caused by barbiturates (Macdonald and Olsen 1994), as observed in hippocampal neurons (Otis and Mody 1992; Thompson and Gähwiler 1992). The initial rebound burst, caused by direct activation of nRt cells by the stimulus, was also abolished by 100 μM pentobarbital. This could be explained by an enhancement in the GABAergic inhibitory interactions between nRt cells, which would have a shunting effect on the stimulus and thus reduce the output of nRt cells to a level insufficient to induce rebound burst generation in relay neurons. Another likely possibility is that the GABAergic response of relay neurons was directly prolonged by pentobarbital such that the time course of repolarization during IPSPs was too slow to activate T-type Ca^{2+} channels (Crunelli et al. 1989). An increase in the duration of $GABA_AR$-mediated IPSPs in relay neurons would explain the slowing of oscillations prior to complete blockade. This is consistent with the

idea that it is primarily relay cell IPSPs that determine spindle frequency (Bal et al. 1995b).

In conclusion, the finding that SLOs with frequencies in the 4- to 9-Hz range occur in rat thalamic slices will allow the extension of previous observations in this preparation to oscillations that more closely resemble in vivo spindles. The results presented here outline the contributions of $GABA_A$, $GABA_B$, AMPA, and NMDA receptors to SLOs. Combined with data showing the strong influence of $[Mg^{2+}]_O$ on the pharmacology of SLOs, our findings suggest several new and important factors that may be involved in shaping spindle oscillations and predisposing the thalamic circuit to hyper-synchronous, epileptiform activity (Huguenard and Prince 1994a; Huntsman et al. 1999; von Krosigk et al. 1993).

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