

Purinergic Inhibition of GABA and Glutamate Release in the Thalamus: Implications for Thalamic Network Activity

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Summary

Adenosine is a CNS depressant with both pre- and postsynaptic actions. Presynaptically, adenosine decreases neurotransmitter release in the hippocampus but only at excitatory terminals. In the thalamus, however, we show that, in addition to its actions at excitatory synapses, adenosine strongly suppresses monosynaptic inhibitory currents both in relay cells of the thalamic ventrobasal complex (VB) and in inhibitory neurons of the nucleus reticularis thalami (nRt). A concomitant increase in transmission failures and results coefficient of variation analysis are both consistent with a presynaptic mechanism. Pharmacological manipulations support an A1 receptor-mediated process. Slow thalamic oscillations induced *in vitro* by extracellular stimulation and recorded with extracellular multiunit electrodes in VB and nRt are dampened by adenosine without affecting their periodicity. We conclude that adenosine can presynaptically down-regulate inhibitory postsynaptic responses in thalamus and exert robust antioscillatory effects, likely by synergistic depression of both excitatory and inhibitory neurotransmitter release.

Introduction

Intrathalamic oscillations that occur in sleep and some forms of epilepsy depend on intrinsic biophysical properties of thalamocortical relay cells and inhibitory neurons of the nucleus reticularis thalami (nRt) and their mutual synaptic connectivity (Steriade and Llinás, 1988; Steriade et al., 1993; von Krosigk et al., 1993; Huguenard and Prince, 1994a; Warren et al., 1994). A variety of neurotransmitters has been shown to modulate directly the firing properties of relay cells and nRt cells, mainly by influencing their resting membrane potentials and/or the activation and inactivation properties of a hyperpolarization-activated cation current (reviewed in McCormick, 1992). However, little is known about presynaptic modulation of neurotransmission in the thalamus. In the hippocampus, an important regulatory role for presynaptic modulation is indicated by the fact that several neurotransmitters differentially inhibit release from excitatory and inhibitory terminals by increasing potassium conductances, reducing calcium currents, and/or interfering directly with the release machinery (reviewed in Thompson et al., 1993). A functional role for this sort of modulation has been demonstrated in the spinal cord, where GABA-mediated presynaptic inhibition is essential for segmental coordination of

the pattern-generating network (Alford and Grillner, 1991; reviewed in Grillner and Matsushima, 1991).

Adenosine is a neuromodulator that can be released under physiological conditions (Mitchell et al., 1993; Manzoni et al., 1994; reviewed in Dunwiddie, 1985; Greene and Haas, 1991) and during pathophysiological states like epilepsy and hypoxia (reviewed in Dragunow, 1988). Adenosine decreases excitatory synaptic currents at central synapses via an A1 receptor-mediated process (e.g., Proctor and Dunwiddie, 1987; Prince and Stevens, 1992; Thompson et al., 1992), likely by reducing calcium currents and/or by direct interference with the release machinery (Scanziani et al., 1992; Wu and Saggau, 1994). On the other hand, adenosine has no effect on inhibitory terminals in the hippocampus (Lambert and Teyler, 1991; Yoon and Rothman, 1991; Thompson et al., 1992).

Here we examine purinergic A1 receptor-mediated presynaptic inhibition in the thalamus. We report that adenosine depresses excitatory as well as inhibitory synaptic terminals. In addition, we show that adenosine exerts antioscillatory effects on thalamic network activity, likely by reducing the excitatory and inhibitory drive on nRt cells and relay cells, respectively. We suggest that endogenous release of adenosine during pathophysiological, and possibly physiological, thalamic and thalamocortical activities would result in activation of a feedback control loop that would limit the duration of these oscillations.

Results

Adenosine Decreases IPSCs in VB and nRt

Thalamic relay cells receive monosynaptic inhibitory input from GABAergic cells of nRt. These inhibitory postsynaptic currents (IPSCs) hyperpolarize relay cells and deactivate a low threshold calcium current, which in turn is responsible for generating rebound calcium spikes and burst firing of action potentials (Steriade and Llinás, 1988; Steriade et al., 1993). The effects of adenosine on IPSCs were studied in relay cells of the thalamic ventrobasal complex (VB). Thalamocortical cells were visually identified in VB and voltage clamped at 0 mV, the reversal potential for excitatory postsynaptic currents (EPSCs). Monosynaptic composite inhibitory postsynaptic currents (IPSCs) were elicited by focal stimulation ($1.5\text{--}2 \times$ threshold, 0.33 Hz) of inhibitory fibers within VB by means of patch electrodes filled with normal saline (Figure 1A). The mean amplitude of the evoked IPSCs was 65 ± 16 pA ($n = 12$), varying in different experiments. The excitatory amino acid blockers amino-phosphono-valeric acid (APV, 50 μ M) and 6,7-dinitroquinoxaline-2,3-dione (DNQX, 10 μ M) were present in all experiments to block excitatory synaptic transmission and putative polysynaptic components of IPSCs. Figure 1A shows an example of the inhibitory effect of bath application of adenosine (50 μ M) on monosynaptic IPSCs in VB. Overall, adenosine decreased IPSC amplitudes in VB to $33\% \pm 8\%$ of control ($n = 7$; Figure 1C; also see Figure

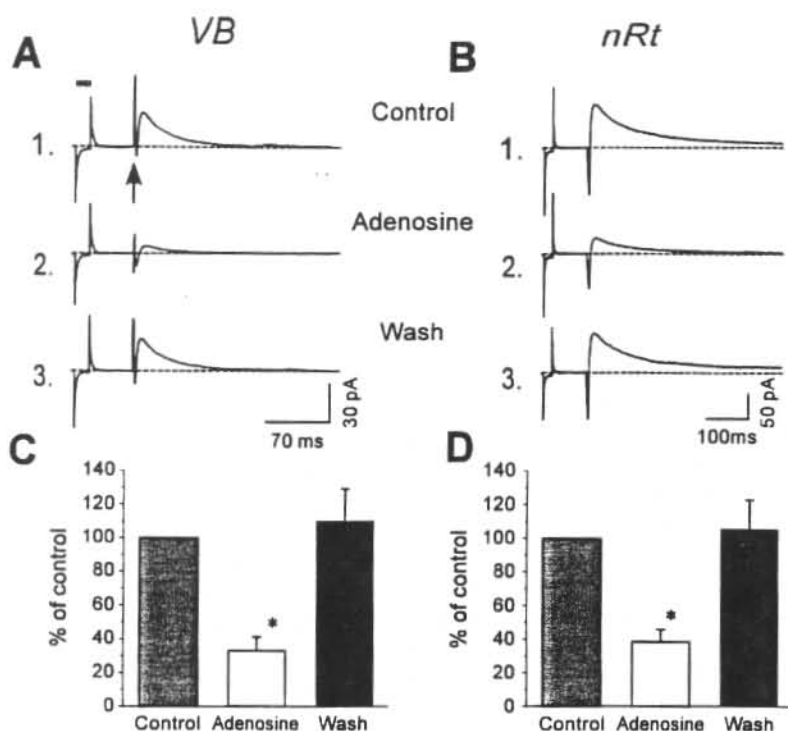


Figure 1. Adenosine Reversibly Depresses IPSCs in VB and nRt

Averages of 100 individual sweeps of evoked composite IPSCs in VB (A) and nRt (B) are shown under control conditions (1), during bath application of 50 μ M adenosine (2), and after washout (3). All recordings were done in the presence of (\pm)APV and DNQX in the bath. The holding potential was 0 mV. Each IPSC is preceded by a current transient generated by a brief voltage step of 1 mV (bar in A1) from the holding potential to assess series resistance. Stimulus artifacts (arrow in A1) have been partially truncated. Note different time scales for VB and nRt. Also shown are mean amplitude and SEM in VB (C) and nRt (D) of the adenosine effect versus control in all cells examined and recovery after washout. The adenosine response was significantly reduced compared with control in VB (*, $p < .0005$; $n = 7$) and in nRt (*, $p < .0001$; $n = 8$).

3A). The mean recovery in nine experiments was $110\% \pm 19\%$ (Figures 1A and 1C).

To confirm the GABAergic nature of the inhibitory synapses, IPSCs were blocked by the specific GABA_A receptor antagonist bicuculline methiodide (BMI, 10 μ M; Figure 2A). In addition, their reversal potential was close to the expected chloride equilibrium potential of -56 mV under

these recording conditions (Figure 2C). The IPSCs were therefore considered to be GABA_A receptor-mediated. Figure 2C shows a typical current-voltage relation for evoked IPSCs in relay cells fitted by the Goldman-Hodgkin-Katz current equation that accounts for the outward rectification seen here and in other preparations (e.g., Barker and Harrison, 1988).

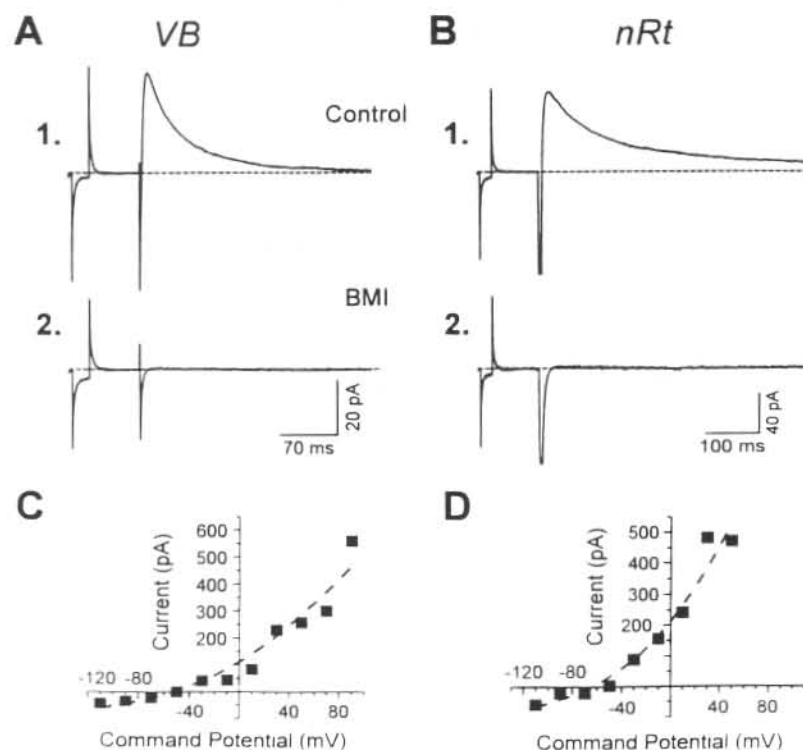


Figure 2. IPSCs Are Mediated by GABA_A Receptors

Average IPSCs in VB (A) and nRt (B) under control conditions in presence of (\pm)APV and DNQX (1) and after application of 10 μ M BMI (2). Current-voltage relation of IPSCs in VB (C) and nRt (D). Individual data points are shown by closed squares, and the dotted line shows the best fit to the Goldman-Hodgkin-Katz equation. In both cases, the reversal potential of the IPSCs is close to the chloride equilibrium potential of -56 mV calculated from the Nernst equation.

The nRt forms a small band between VB and the internal capsule. GABAergic cells within nRt are reciprocally connected by axon collaterals resulting in mutual inhibition (Huguenard and Prince, 1994b; Cox et al., 1995). Therefore, the effect of adenosine on inhibitory terminals within nRt was studied to see whether they were similarly affected compared to their counterparts on the projection fibers within VB. GABAergic cells within nRt were identified by their localization and fusiform shape. Composite monosynaptic IPSCs with a mean amplitude of 95 ± 28 pA ($n = 8$) were elicited by focal stimulation within nRt in the presence of APV and DNQX (see Figure 1B). Adenosine ($50 \mu\text{M}$) reversibly reduced monosynaptic IPSCs in nRt to $39\% \pm 7\%$ ($n = 8$) of control (Figures 1B, 1D, and

3B) recovering to $106\% \pm 17\%$ ($n = 8$) after washout. As was the case in VB neurons, the evoked IPSCs were abolished by BMI in nRt (Figures 2B and 3B) and their reversal potential was close to the chloride equilibrium potential calculated from the Nernst equation (see Figure 2B), implicating GABA_A receptor-mediated processes. These findings indicate that inhibitory terminals on projection fibers and local collaterals of nRt cells are similarly affected by adenosine.

Pharmacological Characterization

Competitive purinerergic agonists and antagonists were used to characterize pharmacologically the receptor subtypes involved in the reduction of IPSCs by adenosine in the somatosensory thalamus. The inhibition of IPSCs in VB and nRt by adenosine was mimicked by the A1 receptor agonist N6-cyclopentyladenosine (CPA; Figure 3B; Figures 4C and 4D); $0.5 \mu\text{M}$ CPA decreased IPSCs in VB to $47\% \pm 8\%$ of control ($n = 5$; Figure 4C) and in nRt to $40\% \pm 13\%$ ($n = 3$; Figure 3B and Figure 4D). The decreases of IPSCs by adenosine and CPA were both reversed by the A1 receptor antagonist 8-cyclopentyltheophylline (CPT, $1 \mu\text{M}$) in VB and nRt cells (Figure 3). CPT reversed the adenosine effect in VB to $81\% \pm 3\%$ ($n = 3$; Figure 4A) of control and in nRt to $90\% \pm 5\%$ ($n = 3$; Figure 4B). Analogously, the depression of IPSCs by CPA was partially reversed by CPT in VB to $74\% \pm 17\%$ of control ($n = 4$; Figure 4C) and was completely reversed in nRt to $97\% \pm 20\%$ ($n = 3$; Figure 4D). To examine the possibility that endogenous adenosine had a tonic inhibitory effect on IPSCs, the effect of CPT alone was assessed on evoked IPSCs in VB and nRt. However, CPT ($1 \mu\text{M}$) application did not affect IPSC amplitude in VB ($105\% \pm 14\%$ of control; $n = 4$) or in nRt ($95\% \pm 13\%$; $n = 3$), suggesting that the endogenous concentration of

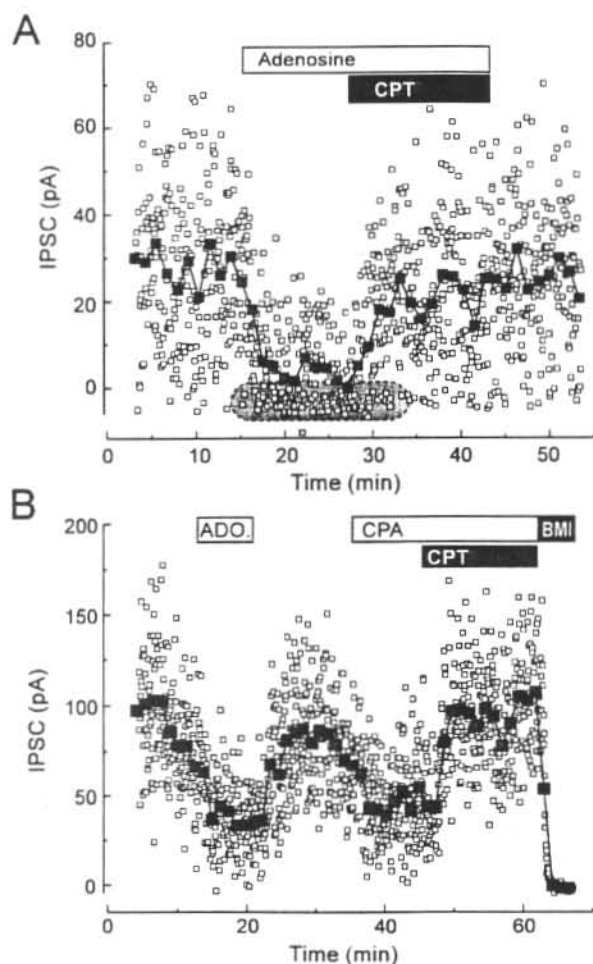


Figure 3. Time Course of Drug Responses

Individual amplitudes of IPSCs (open squares) and local averages of 24 consecutive points (closed squares) are shown for a VB neuron (A) and an nRt neuron (B) versus time. Throughout the experiments, (\pm)APV and DNQX were present. Drugs were applied during time intervals indicated by horizontal bars.

(A) Bath application of $50 \mu\text{M}$ adenosine resulted in a decreased IPSC amplitude with a concomitant 10-fold increase of failures in synaptic transmission (shaded area). The competitive A1 receptor antagonist CPT ($1 \mu\text{M}$) reversed the adenosine effect.

(B) Adenosine reduced evoked IPSCs and a partial recovery was obtained after washout. The A1 receptor agonist CPA ($0.5 \mu\text{M}$) mimicked the adenosine effect, which was reversed by the addition of $1 \mu\text{M}$ CPT. Finally, BMI ($10 \mu\text{M}$) abolished the evoked IPSCs.

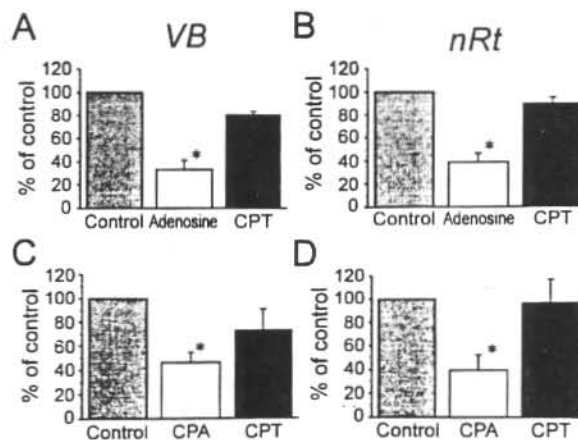


Figure 4. Pharmacology of the Adenosine Response

Mean and SEM of the average adenosine effect in VB (A and C) and nRt (B and D). In VB neurons (A), $50 \mu\text{M}$ adenosine depressed IPSCs to 33% of control ($^*, p < .001$; $n = 3$) with a partial reversal by $1 \mu\text{M}$ CPT. In nRt (B), IPSCs were reduced to 39% of control, which was again statistically significant ($^*, p < .0001$; $n = 3$). These effects were mimicked by the A1 receptor agonist CPA ($0.5 \mu\text{M}$), which reduced the mean IPSC to 47% of control ($^*, p < .005$; $n = 4$) in VB (C) and to 40% of control ($^*, p < .05$; $n = 3$) in nRt (D).

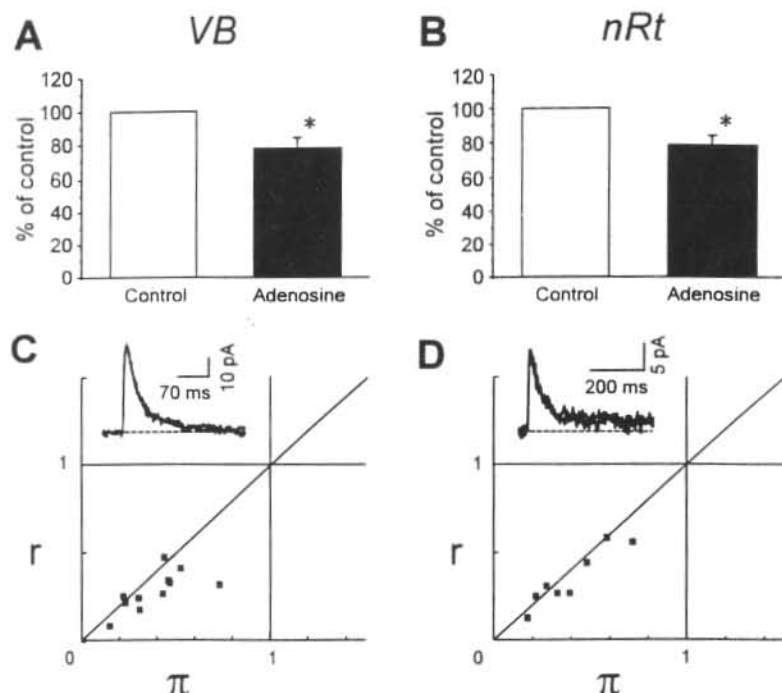


Figure 5. Presynaptic Site of Action
Mean \pm SEM of successful synaptic responses in VB (A, *, $p < .005$; $n = 12$) and in nRt (B, *, $p < .01$; $n = 8$) were significantly reduced with 50 μ M adenosine compared with control. Scatter plots show r versus the modification factor, π (C, $n = 12$; D, $n = 8$). Insets, average IPSCs after adenosine application rescaled to peak amplitude of control and superimposed on the average control IPSCs. Average IPSCs were subtracted by the average of failures to eliminate stimulus artifacts. Note that in both cases the signals are well superimposed.

adenosine in the slice was too low to exert a tonic effect on inhibitory terminals (see Discussion).

Presynaptic Site of Action

The decrease in IPSC amplitude in VB and nRt by adenosine was accompanied by an increase in the number of transmission failures at these synapses (Figures 5 and 6; also see Figure 3A). Adenosine decreased the percentage of successful synaptic transmissions in VB to $78\% \pm 6\%$ of control ($n = 12$; Figure 5A) and in nRt to $79\% \pm 5\%$ ($n = 8$; Figure 5B). The coefficient of variation (CV) of the IPSCs was assessed as outlined in the Experimental Procedures. Figures 5C and 5D show scatter plots of the ratio of the squared CVs (r) before and after adenosine application versus the modification factor (π ; Faber and Korn, 1991). In VB, the mean r and π were 0.26 ± 0.04 and 0.34 ± 0.06 ($n = 12$), respectively, and in nRt, the mean r and π were 0.35 ± 0.05 and 0.40 ± 0.07 ($n = 8$), respectively. In both VB and nRt, the mean r was smaller than the mean π . This implicates a presynaptic modification site (Faber and Korn, 1991). However, whereas most of the data points in Figure 5 lay below the diagonal (slope = 1), occasionally points were observed on the other side of the line as well (see Discussion).

Amplitude histograms of IPSCs in VB and nRt were multimodal, comprising a broad range of values (Figure 6). No attempt was made in the present study to resolve the underlying quantal components. However, adenosine increased the probability of smaller events, including failures, with a concomitant reduction of larger events (Figures 6C and 6D). Both findings are consistent with a presynaptic mechanism (Blum and Idiart, 1994). The insets in Figure 5 show average IPSCs after adenosine application that have been rescaled to and superimposed on the average control IPSCs. In these signals, stimulus artifacts were

eliminated by subtracting the average failure traces from the average IPSCs. The superposition of the IPSCs suggests that the same synaptic boutons were activated in control conditions and after adenosine application (see Discussion).

Adenosine Decreases EPSCs in nRt

Burst firing of GABAergic cells in nRt can be triggered by monosynaptic excitatory input from relay cells (Bal and

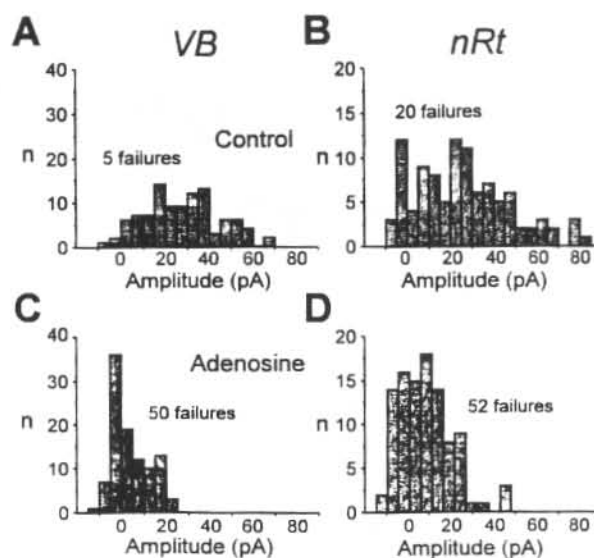


Figure 6. Amplitude Distributions

Examples of amplitude histograms are shown for 100 evoked IPSCs in VB (A and C) and nRt (B and D) under control conditions (A and B) and after bath application of 50 μ M adenosine (C and D). Note that after adenosine application the probability of smaller events including failures is higher (C and D versus A and B). Bin width, 5 pA.

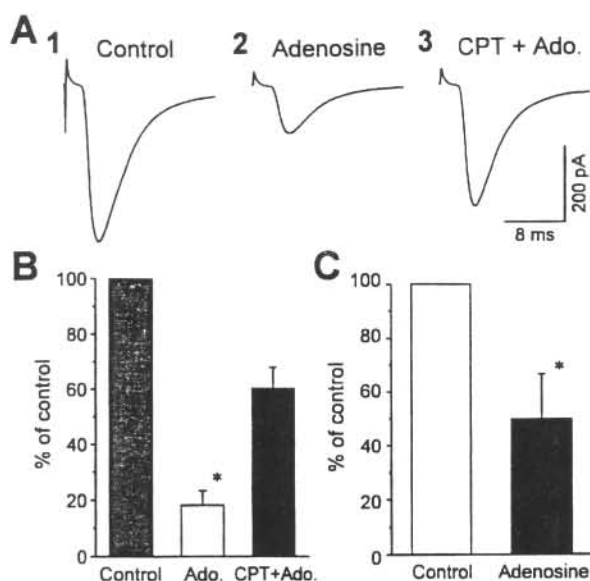


Figure 7. Adenosine Reduces EPSCs in nRt

(A) Evoked EPSCs in nRt in control (1), during application of 50 μ M adenosine (2), and after addition of 1 μ M CPT (3). Sweeps are averages of 100 individual traces. Throughout the experiment, (\pm)APV and BMI were present. Holding potential, -70 mV.

(B) Effect of adenosine (Ado.) on EPSCs in nRt in all experiments (mean \pm SEM). Overall adenosine decreased EPSCs in nRt to $18\% \pm 4\%$ of control (*, $p < .0001$; $n = 5$). A partial recovery is obtained by addition of CPT (1 μ M).

(C) Successful excitatory synaptic transmissions in nRt were reduced after adenosine application to $52\% \pm 13\%$ of control (*, $p < .05$).

McCormick, 1993). In addition, intrathalamic oscillations depend critically on excitatory input from relay cells onto nRt cells (von Krosigk et al., 1993; Huguenard and Prince, 1994a). Therefore, the effects of adenosine on EPSCs were studied within nRt. When nRt cells were voltage clamped at -70 mV in the presence of BMI (10 μ M) and APV (50 μ M), composite EPSCs could be elicited by extracellular stimulation of thalamocortical and corticothalamic fibers in VB (Figure 7A1). These EPSCs were likely to be monosynaptic since very few, if any, recurrent excitatory collaterals exist in dorsal thalamus (Jones, 1985). The EPSCs had a mean amplitude of -162 ± 65 pA ($n = 5$) and were blocked with DNQX (10 μ M, data not shown). They were therefore considered to be non-NMDA receptor mediated, in agreement with previous reports (von Krosigk et al., 1993; Huguenard and Prince, 1994a). Adenosine (50 μ M) decreased the EPSCs to $18\% \pm 4\%$ of control ($n = 5$; Figures 7A and 7B). This blockade was partially reversed to $64\% \pm 7\%$ of control by addition of 1 μ M CPT (Figures 7A and 7B). The reduction of EPSCs by adenosine was accompanied by an increase of failures of synaptic transmission. Adenosine reduced the relative percentage of successful synaptic transmissions to $52\% \pm 13\%$ of control ($n = 5$; Figure 7C). In four out of five experiments analyzed, the ratio of the squared CVs (r) was smaller than the π , and in one experiment, r was slightly bigger than π . Overall, the mean r was 0.1 ± 0.02 and the mean π was 0.18 ± 0.04 ($n = 5$). Together, these

findings indicate that adenosine presynaptically decreases EPSCs in nRt, likely by activating an A1 receptor. The amount of inhibition by adenosine and the sensitivity to reversal by CPT were similar for EPSCs and IPSCs, as well as the concomitant increase in transmission failures. Therefore, EPSCs and IPSCs are similarly affected by adenosine in the somatosensory thalamus.

Adenosine Decreases Thalamic Oscillations

Since excitatory drive and inhibitory drive on nRt cells and relay cells, respectively, are essential in maintaining thalamic oscillations (von Krosigk et al., 1993; Huguenard and Prince, 1994a), it is likely that presynaptic inhibitory effects demonstrated here would decrease thalamic network activity as well. Thalamic oscillations were studied in acute slices by extracellular multiunit recordings in VB and nRt (Huguenard and Prince, 1994a). Phasic oscillations (~ 3 Hz) in VB and nRt were evoked by extracellular stimulation of corticothalamic fibers. Figure 8A shows individual sweeps in control, adenosine (50 μ M), and wash. Figure 8B shows a contour plot of the whole experiment. Note the decrease in number of cycles after perfusion with adenosine and the recovery after washout. Overall, adenosine decreased the number of spikes in these oscillations to $52\% \pm 6\%$ of control in VB ($p < .001$; $n = 8$) and to $50\% \pm 9\%$ of control in nRt ($p < .001$). To assess quantitatively the influence of adenosine on the synchronicity and rhythmicity of thalamic oscillations, autocorrelograms of unit activities were constructed and fitted by a modified Gabor function (König, 1994; see Experimental Procedures; Figure 9A). The amplitude of the Gabor function is a measure of synchronous cell firing, whereas the frequency and duration of the oscillations are described as the period (T) and decay time constant (τ_D), respectively (König, 1994). The mean τ_D in VB and nRt were 574 ± 41 ms and 886 ± 246 ms, respectively. Adenosine significantly reduced τ_D in VB to $63\% \pm 9.5\%$ of control ($p < .01$; $n = 8$) and in nRt to $65\% \pm 13\%$ of control ($p < .05$; $n = 8$; Figures 9A and 9B1). The period of the oscillations in VB and nRt in control was 366 ± 27 ms and 348 ± 26 ms, respectively, reflecting a mean oscillation frequency of 2.8 Hz. The period was not significantly changed by adenosine in VB or nRt (Figures 9A and 9B3). However, cells fired less synchronously after adenosine application. This is reflected by a reduction in amplitude of the corresponding Gabor function (Figure 9A). In VB and nRt the synchronicity was reduced to $46\% \pm 11\%$ and $63\% \pm 12\%$ of control, respectively ($p < .02$; $n = 8$; Figure 9B2). The effects of adenosine on thalamic oscillations were occluded by 1 μ M CPT ($n = 2$; data not shown). Together, these findings indicate that adenosine acting at A1 receptors can dampen thalamic oscillations without disrupting their periodicity.

Discussion

Presynaptic inhibition is likely to be an important factor in regulating synaptic transmission and the behavior of neural network activity (e.g., Grillner and Matsushima,

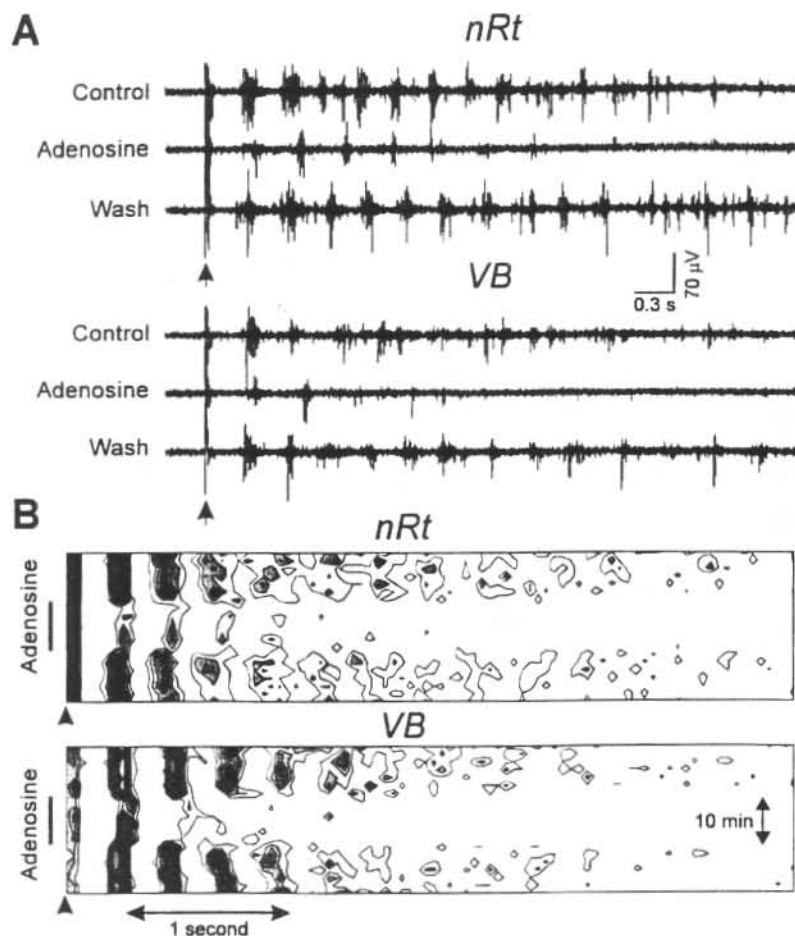


Figure 8. Antioscillatory Effects of Adenosine. (A) Simultaneous extracellular multiunit recordings in nRt and VB under control conditions, during application of 50 μ M adenosine, and after washout. Phasic oscillatory activity lasting several seconds was evoked in nRt and VB by extracellular stimulation of corticothalamic and thalamocortical fibers (arrows). (B) Contour plot of the same experiment. The top of each plot is the control period, the middle portion is the period during which adenosine was applied, and the bottom is the washout. Phasic activity evoked by the stimulus (arrowheads) was especially synchronous in control for the first 3–5 cycles and then continued for 8–12 cycles in a less synchronous fashion. During the period of adenosine application (vertical bars), the activity was much briefer with only 2–4 total cycles of activity. These effects were completely reversible after washout of adenosine. Contour intervals for nRt and VB were 6 and 4 spikes, respectively.

1991; Thompson et al., 1992). Adenosine has been shown to be a selective presynaptic modulator in the hippocampus affecting excitatory synaptic terminals (Proctor and Dunwiddie, 1987; Prince and Stevens, 1992; Thompson et al., 1992) with no effect on inhibitory synaptic transmission (Lambert and Teyler, 1991; Yoon and Rothman, 1991; Thompson et al., 1992). Here we report that, unlike in the hippocampus, in somatosensory thalamus, 50 μ M adenosine depresses IPSCs to about one third of control. This is the first direct demonstration of a reduction in GABAergic synaptic conductance by adenosine although a reduction of inhibitory potentials by adenosine has been described by conventional intracellular recordings in the amygdala (Nose et al., 1991) and nucleus accumbens (Uchimura and North, 1991). The concentration of adenosine used in the present study has been shown to be saturating for the depression of EPSCs in the hippocampus (Prince and Stevens, 1992), where EPSCs were reduced to about half of control. The concentration of adenosine in the slice may be altered by uptake and ectonucleotidases (Dunwiddie, 1985), leaving some uncertainty about the exact concentration of the modulator at the synaptic site. Our pharmacological manipulations indicate that the depression of the IPSCs in the thalamus is mediated by a purinergic A1 receptor, as is the case for EPSCs in the hippocampus (e.g., Thompson et al., 1992). The classification of purinergic receptors is still incomplete (Collis and Hourani,

1993). However, a contribution of A2 receptors on the depression of IPSCs is unlikely, since they have been shown to potentiate synaptic signals at central synapses (Umemiya and Berger, 1994). No attempt was made to characterize the intracellular effector mechanisms involved in the reduction of PSCs. Endogenous adenosine concentrations in vivo and in vitro (Dunwiddie, 1985; Prince and Stevens, 1992) have been shown to be in the low micromolar range, thus exerting a tonic inhibition on excitatory synaptic transmission. In our experiments, the endogenous concentration of adenosine in the slice was too low to affect IPSCs significantly. Our recordings were, however, restricted to cells that were located near the surface of the slice, where the endogenous adenosine concentration might have been altered by the superfusion solution. In agreement with this idea, tonic inhibition of EPSCs was absent in hippocampal slice cultures that are only a few cell layers thick (Thompson et al., 1992).

It is interesting to note that IPSCs in both VB and nRt neurons may arise from activity in nRt neurons. Anatomical evidence exists for recurrent axon collaterals within nRt (Yen et al., 1985; Mullen et al., 1986; Spreafico et al., 1988; Cox et al., 1995), and there is physiological support for the functioning of the recurrent collaterals during thalamic oscillatory activity (von Krosigk et al., 1993; Huguenard and Prince, 1994b). As IPSC in VB and nRt cells were similarly modulated by adenosine, these results sug-

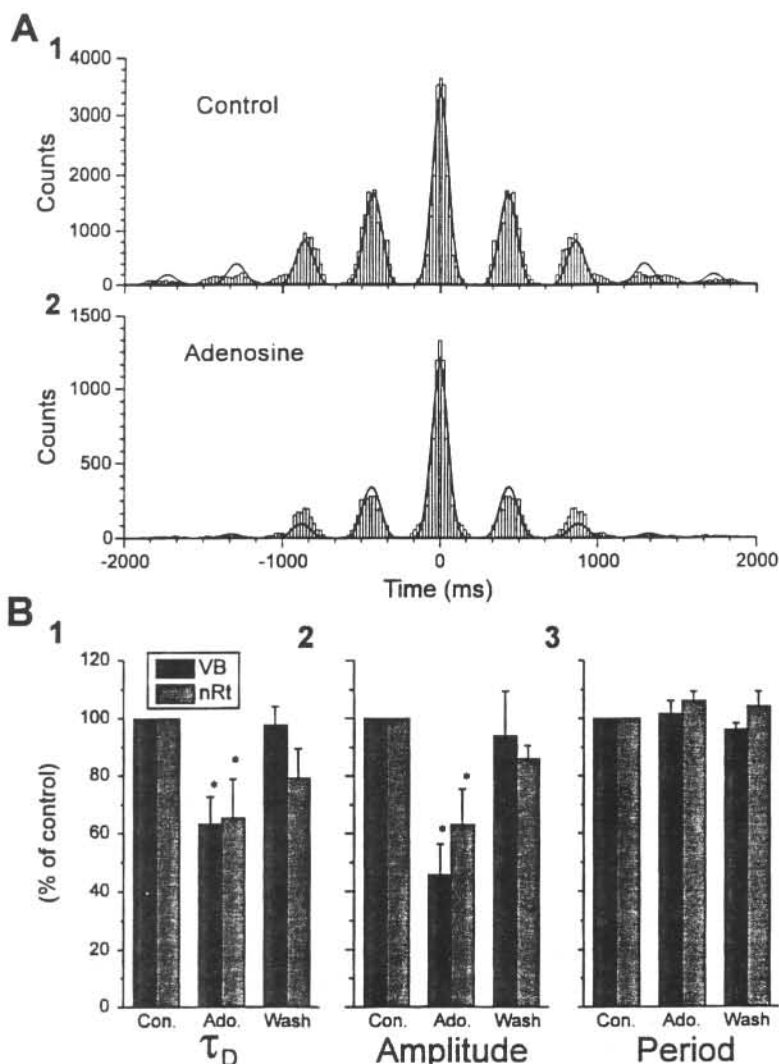


Figure 9. Summary of Adenosine Effects on Intrathalamic Oscillations

(A) Autocorrelograms of interevent intervals for a relay cell under control conditions (1) and after 50 μ M adenosine application (2). The continuous line shows the best fit of a Gabor function to the histogram. Control: T , 433 ms; A , 3450; τ_D , 600 ms. Adenosine: T , 444 ms; A , 1210; τ_D , 352 ms. In this experiment, τ_D was decreased to 59% of control. With washout, τ_D recovered to 607 ms and amplitude returned to 2930 (data not shown).

(B) Summarized effects of 50 μ M adenosine (Ado.) on τ_D , amplitude, and T of the fitted Gabor functions are shown as mean \pm SEM for VB and nRt (*, $p < .05$, see text for details; Con. = control).

gest the following corollary to Dale's principle: not only do different terminals of the same neuron release the same neurotransmitter (Whittaker, 1983), but they may also be identical in terms of their presynaptic modulation.

Pre- Versus Post-

A presynaptic site of the adenosine effect is indicated by the increased failure rate (Del Castillo and Katz, 1954; Kuno, 1964), which reflects probabilistic transmitter release (Allen and Stevens, 1994), and the change in the CV (Harrison, 1990; Faber and Korn, 1991). Theoretically, a postsynaptic decrease of active synapses will result in an increased rate of transmission failures as well. This could happen if whole clusters of receptors would be switched off by some as yet unidentified mechanism. The opposite, i.e., a switching on of receptor clusters, has been proposed to account for the increase of synaptic strength after induction of long-term potentiation (Kullmann, 1994; Liao et al., 1995). However, since the recorded postsynaptic signal is the sum of all quantal events originating from different electrotonic sites, a permanent knockout of synapses will likely affect the overall shape of the signal at

the soma (Henneman et al., 1984). This possibility has been tested by rescaling the depressed IPSCs to the size of control responses (see Figures 5C and 5D [insets]). However, no changes in the overall shape of the control and rescaled IPSCs have been observed in the present study, thus arguing against a permanent knockout of entire synapses. The CV analysis can be ambiguous if release parameters change nonlinearly (Faber and Korn, 1991). Whereas most of our data points were within a region that has been classically related to a presynaptic site, some data points were located above the diagonal (Figures 5C and 5D). This may still be attributable to a presynaptic mechanism if nonlinear changes in release probabilities occur (Faber and Korn, 1991). This is possible, since release probabilities within clusters of boutons have been shown to be nonuniform (Hessler et al., 1993; Rosenmund et al., 1993), changing to different degrees after appropriate conditioning (Larkman et al., 1992).

As in other parts of the brain, adenosine postsynaptically hyperpolarizes thalamic relay cells in the dorsal lateral geniculate nucleus (dLGN) by increasing a potassium conductance (Pape, 1992). This may result in a shunting of

synaptic currents as well. However, we have used cesium, which is a nonspecific potassium channel blocker, as the major intracellular cation. In addition, it has been shown that the postsynaptic effect of adenosine on potassium currents is rapidly washed out during whole-cell recordings (Trussell and Jackson, 1985). Furthermore, simulation studies have shown that even a 10-fold change in membrane conductance only moderately influences the amplitude and time course of synaptic currents (Spruston et al., 1993). Therefore, a postsynaptic effect of adenosine on potassium currents is unlikely to account for the reduction of the IPSCs observed in this study.

Adenosine Effects on EPSCs

Although EPSCs were less comprehensively studied than IPSCs, there were obvious similarities between the adenosine effect on both types of signals. Both EPSCs and IPSCs were depressed by adenosine to a similar degree. In each case the depression is accompanied by an increased rate of synaptic transmission failures, and the adenosine effect is reversed by the A1 receptor antagonist CPT. Furthermore, with both EPSCs and IPSCs, the CV analysis suggests a presynaptic site of action. The present finding that adenosine reduces EPSCs in nRt is in line with reports from other areas of the mammalian brain, where presynaptic inhibition of glutamatergic synapses by adenosine is well documented (Dunwiddie, 1985; Greene and Haas, 1991).

Implications for the Regulation of Thalamic Oscillations

Intrathalamic oscillations depend on reciprocal connectivity between relay cells and nRt cells and on the intrinsic ability of both types of neurons to generate burst discharges (von Krosigk et al., 1993; Huguenard and Prince, 1994a). Adenosine hyperpolarizes relay cells in lateral geniculate nucleus of the guinea pig by increasing a potassium conductance and by an apparent shift in the voltage dependence of a hyperpolarization-activated cation current (I_h ; Pape, 1992). However, the postsynaptic effects of adenosine on nRt cells are unknown. Blockade of intra-nRt inhibition leads to an increased inhibitory output to relay cells, likely via disinhibition of reciprocally connected GABAergic nRt cells (Huguenard and Prince, 1994b). These opposing pre- and postsynaptic effects make a prediction of the overall effect of adenosine on thalamic oscillations difficult. In the present study, we show that moderate concentrations of adenosine can effectively reduce the duration of thalamic oscillations without disrupting the rhythmicity of the associated spiking. It has been reported that small concentrations of adenosine may be more effective in depressing synaptic transmission than hyperpolarizing cells (Siggins and Schubert, 1981). Concentrations of adenosine in the millimolar range have been used to generate postsynaptic effects in relay cells (Pape, 1992). However, since in this study adenosine was focally applied, a direct comparison of the concentration dependence of pre- and postsynaptic adenosine effects in the thalamus is not possible at present. Pape (1992) con-

cluded that the postsynaptic effects of adenosine on relay cells (increase in I_K and decrease in I_h) will favor burst firing behavior in these cells and that this would lead to an increase in rhythmicity. Furthermore, because local application of adenosine counteracted the increase in I_h produced by β -adrenergic or serotonergic agonists, purinergic receptor activation was postulated to mediate an antagonism of brainstem activation of thalamus. Thus, the *in vivo* actions of adenosine in thalamus will likely result in powerful hyperpolarization of relay neurons. Our data suggest, however, that low concentrations of adenosine can reduce the synchronicity and duration of thalamic oscillations *in vitro*, likely via presynaptic inhibition of IPSCs and EPSCs. Thus, any pro-oscillatory postsynaptic actions of adenosine appear to be overcome by the robust and synergistic, presynaptic antioscillatory effects.

Adenosine has sometimes been termed an endogenous antiepileptic drug, as it can be released under conditions of increased cellular activity (e.g., Dragunow, 1988). Our findings support the idea that adenosine might act as an endogenous antiepileptic by dampening epileptiform thalamic oscillations. In addition, since IPSCs and EPSCs are important in triggering burst firing in relay neurons and nRt cells (von Krosigk et al., 1993; Huguenard and Prince, 1994a), it can be expected that adenosine may also play a regulatory role for normal thalamic oscillations, such as spindle waves.

Experimental Procedures

Tissue Slice Preparation

Thalamic slices were prepared as described previously (Huguenard and Prince, 1994a). In brief, rat pups of either sex, 9–14 days old, were anesthetized (50 mg/kg pentobarbital) and decapitated. The brain was removed and transferred into an ice-cold slicing solution containing 234 mM sucrose, 11 mM glucose, 24 mM NaHCO_3 , 2.5 mM KCl, 1.25 mM NaH_2PO_4 , 10 mM MgSO_4 , and 0.5 mM CaCl_2 , aerated with 95% O_2 and 5% CO_2 . The whole brain was glued onto a coverslip, and 200 μm horizontal slices were obtained with a vibratome (TPI, St. Louis, MO). The thalamus and parts of the adjacent striatum were dissected out and kept in an incubator containing standard artificial cerebrospinal fluid (ACSF; see below) at 32°C for at least 1 h prior to recording.

Electrophysiology

Whole-cell patch-clamp recordings (Hamill et al., 1981) were performed under visualized control with Hoffman optics and infrared video microscopy (Edwards et al., 1989; Stuart et al., 1993). Brain slices were transferred into a recording chamber and superfused (2 ml/min at room temperature) with standard ACSF containing 126 mM NaCl, 26 mM NaHCO_3 , 2.5 mM KCl, 1.25 mM NaH_2PO_4 , 2 mM MgCl_2 , 2 mM CaCl_2 , and 10 mM glucose, equilibrated with 95% O_2 , 5% CO_2 . Patch pipettes were pulled from borosilicate glass (Garner Glass, Claremont, CA) and filled with a solution containing 120 mM Cs-gluconate, 11 mM CsCl, 1 mM MgCl_2 , 1 mM CaCl_2 , 10 mM HEPES, and 11 mM EGTA (adjusted to a pH of 7.3 with CsOH, osmolarity 290 mOsm). Cs^+ was replaced by K^+ in the tip filling solution to increase successful seal formations. Positive pressure was applied to the pipette to clean the cell surface prior to gigaseal formation. Whole-cell configuration was established by application of short pulses of suction generated by a vacuum valve controlled by a pulse generator. A liquid junction potential of 10 mV was subtracted on line. Continuous voltage-clamp recordings were performed with a List EPC 7 amplifier (List, Darmstadt, FRG). Access resistance (5–20 M Ω) was monitored throughout the experiment and unstable recordings were rejected. Individual sweeps were low pass filtered at 10 kHz (3-pole Bessel filter) and digitized on line at 1–2 kHz by a Labmaster TM-100 A/D converter at a 12 bit

resolution (Scientific Solutions, Solon, OH) controlled by the software package Pclamp (Axon Instruments, Foster City, CA). Drugs were obtained from RBI (Natick, MA) and applied through the perfusion system. Recordings with stable seal resistances ($\pm 20\%$) were selected out of 36 VB cells and 26 nRT cells.

Data Analysis

Amplitude measurements of IPSCs and EPSCs were obtained by averaging consecutive data points of individual sweeps within a time window of 2–5 ms before the stimulus artifact and around the peak (Sayer et al., 1989). Estimates of noise were obtained by subtracting two periods of averaged baseline recordings, which preceded the stimulus artifact, from each other. The CV was calculated from 100 consecutive sweeps:

$$CV = \sqrt{\text{var}_{\text{signal}}}/\text{mean}.$$

The variance of the noise was subtracted from the variance of the recorded signal:

$$\text{var}_{\text{signal}} = \text{var}_{\text{recorded signal}} - \text{var}_{\text{noise}}.$$

r is defined as (Faber and Korn, 1991):

$$r = CV^2_{\text{control}} / CV^2_{\text{adenosine}},$$

and the modification factor π as:

$$\pi = \text{mean}_{\text{adenosine}} / \text{mean}_{\text{control}}.$$

The number of failures of synaptic transmission was assessed within a series of 100 consecutive recordings by visually inspecting individual sweeps for significant deflections from the baseline noise within a time window of 15 ms after the stimulus artifact. Population data are given as the mean \pm SEM. Statistical comparisons were made by Student's t test.

Thalamic Oscillations

Horizontal 400 μm thalamic slices were put in an interface type recording chamber and perfused with normal saline containing low Mg^{2+} (0.2 mM) and BMI (2–20 μM) at 34°C (Huguenard and Prince, 1994a). Extracellular stimuli (20–60 V, 30–50 μs) were delivered through bipolar tungsten electrodes. Extracellular multiunit activity was recorded with monopolar tungsten electrodes (1–5 M Ω). Signals were band-pass filtered (10 Hz–3 kHz) and digitized at 1.7 kHz (Axotape, Axon Instruments, Foster City, CA). A software Schmitt trigger was used to detect spikes. Autocorrelograms were calculated for time shifts of ± 2 s with a bin size of 200 ms. A modified Gabor function was fitted to the autocorrelograms by means of a simplex algorithm:

$$CF(t) = A \times e^{-\frac{t}{T_0}} \times \cos\left(\frac{1 \times \pi}{T} t\right) + A_0 \times e^{-\frac{t}{T_0}},$$

where T is the period, T_0 is the decay time constant, and A is the amplitude of the Gabor function (König, 1994). The second term of the equation allows for independent fitting of the central peak. The cosine term was raised to the sixth power, because autocorrelograms only displayed sharp positive peaks.

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