

Neuromodulation by a Cytokine: Interferon- β Differentially Augments Neocortical Neuronal Activity and Excitability

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Hadjilambrea, Gergana, Eilhard Mix, Arndt Rolfs, Jana Müller, and Ulf Strauss. Neuromodulation by a cytokine: interferon- β differentially augments neocortical neuronal activity and excitability. *J Neurophysiol* 93: 843–852, 2005. First published September 22, 2004; doi:10.1152/jn.01224.2003. The immunomodulatory cytokine interferon- β (IFN- β) is used in the treatment of autoimmune diseases such as multiple sclerosis. However, the effect of IFN- β on neuronal functions is currently unknown. Intracellular recordings were conducted on somatosensory neurons of neocortical layers 2/3 and 5 exposed to IFN- β . The excitability of neurons was increased by IFN- β (10–10,000 U/ml) in two kinetically distinct, putatively independent manners. First IFN- β reversibly influenced the subthreshold membrane response by raising the membrane resistance R_M 2.5-fold and the membrane time constant τ 1.7-fold dose-dependently. The effect required permanent exposure to IFN- β and was reduced in magnitude if the extracellular K^+ was lowered. However, the membrane response to IFN- β in the subthreshold range was prevented by ZD7288 (a specific blocker of I_h) but not by Ni^{2+} , carbachol, or bicuculline, pointing to a dependence on an intact I_h . Second, IFN- β enhanced the rate of action potential firing. This effect was observed to develop for >1 h when the cell was exposed to IFN- β for 5 min or >5 min and showed no reversibility (≤ 210 min). Current-discharge ($F-I$) curves revealed a shift (prevented by bicuculline) as well as an increase in slope (prevented by carbachol and Ni^{2+}). Layer specificity was not observed with any of the described effects. In conclusion, IFN- β influences the neuronal excitability in neocortical pyramidal neurons in vitro, especially under conditions of slightly increased extracellular K^+ . Our blocker experiments indicate that changes in various ionic conductances with different voltage dependencies cause different IFN- β influences on sub- and suprathreshold behavior, suggesting a more general intracellular process induced by IFN- β .

INTRODUCTION

Various cytokines are produced within the CNS by different cell classes including lymphocytes (Fabry et al. 1994), resident glial cells (Hopkins and Rothwell 1995), and neurons (Bartfai and Schultzberg 1993; Villarroya et al. 1997). The subgroup of the interferons (IFNs) belongs to a family of endogenous glycoproteins and acts in the defense to virus infections and tumors as well as in the regulation of immune responses of vertebrates (Baron et al. 1991; Bocci 1992).

The latter effect is thought to be responsible for the suppressive activity of IFN- β on autoimmune demyelination in patients with multiple sclerosis (MS), which renders IFN- β an important therapy for these patients (Coyle and Hartung 2002; Durelli et al. 2001; Kinnunen et al. 1993). However, the response to IFN- β is variable, and some patients do not show any improvement, whereas others have to stop the therapy

because of side effects such as fever, muscle rigidity, fatigue, depression, and anorexia (Bramanti et al. 1998; Walther and Hohlfeld 1999). These effects are regulated in CNS structures and depend on specific neuronal activity. Accordingly, IFNs—besides other cytokines (Jiang and Lu 1998; Sternberg 1997)—affect neuronal cells, as reported for IFN- α during cancer treatment. IFN- α and - β are type I IFNs and share several features; this makes a similar activity of both likely. The amino acid sequences of IFN- α and - β indicate they are homologous proteins that bind with high affinity (K_d of 10^{-10} – 10^{-11} M) to a common receptor (IFNAR₁). They induce similar intracellular signaling pathways, but they do not always act synergistically (Ransohoff 1998). Known neuronal effects of IFN- α are divergent. Excitatory as well as inhibitory effects on neurons and the synaptic transmission are reported (for more detailed information, see DISCUSSION) (for review, see Dafny et al. 1997). These effects differed according to the brain region (Dafny et al. 1996); however, as described in further detail in the discussion, antagonistical effects may even occur in one region.

The IFN- α modulation of neuronal activity in the rat neocortex, hippocampus, and hypothalamus (Dafny et al. 1997) could also apply for IFN- β . Therefore we assumed that IFN- β could have a direct effect on the electrical activity of neocortical neurons. To test our hypothesis, we applied intracellular recordings with sharp microelectrodes from neocortical sensorimotor pyramidal neurons in rat brain slices under the influence of IFN- β .

METHODS

Slice preparation

Animals were kept under standard laboratory conditions, and all procedures were performed in agreement with the European Communities Council Directive of November 24 1986 (86/609/EEC). Brain slices were prepared by standard methods (Strauss et al. 2004). After deep anesthesia with ether, adult male Wistar rats (250–400 g) were decapitated. A small block of brain containing the somatosensory cortex was rapidly removed and transferred to cold (4–5°C), oxygenated (95% O₂-5% CO₂, pH 7.34–7.40) artificial cerebrospinal fluid (ACSF). The block was fixed with cyanoacrylate glue ventral side up in the cutting chamber, the neocortex facing the blade. Coronal brain slices of 400- μ m nominal thickness were cut on a vibrating microtome (Vibratome Series 1000, Vibratome, St. Louis, MO) under cold (2–5°C) ACSF. The slices were incubated for ≥ 1 h in ACSF at room temperature (~23°C) before being transferred to a submerged-type chamber for electrophysiological recording. The ACSF contained (in mM) 124 NaCl, 5.0 KCl, 1.25 NaH₂PO₄, 2.0 MgSO₄, 26 NaHCO₃,

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2.0 CaCl₂, and 10 D-glucose with 295–308 mosmol (all compounds were purchased from Sigma-Aldrich, Steinheim, Germany). For six experiments, 2.5 mM KCl had been substituted by 2.5 mM NaCl. When using Ni²⁺, the MgSO₄ was replaced by MgCl₂ and NaH₂PO₄ was omitted. This solution is referred to as divalent cation solution (DCS).

Electrophysiological recordings

Conventional intracellular electrodes were pulled from thin-wall borosilicate glass with 1.2 mm OD and 0.982 mm ID (Hilgenberg, Mansfeld, Germany) using a vertical puller PUL-100 (WPI, Sarasota, FL). When filled with 3 M KCl or 3 M K⁺-acetate, the electrodes had a final DC resistance of 60–100 MΩ. Neurons were impaled in the pyramidal layer 2/3 or layer 5 of the somatosensory neocortex and current clamped. Once a stable intracellular recording was obtained (usually >10 min post impalement), square current pulses (0.3 or 0.5 s) were injected through the micropipette for ≥30 min but ≤240 min. Bridge balance was continuously monitored and adjusted accordingly. When necessary, a tonic current was injected to compensate for changes in membrane potentials induced by carbamylcholin, ZD7288 or Ni²⁺. Voltage signals were amplified using a high-impedance amplifier with active bridge circuitry (npi-amplifier SEC-05LX, npi electronic, Tamm). The superfusion with ACSF was kept at a constant flow (2 ml/min) by a fast gravity system with an onset time of ~1–3 min for 1 μM tetrodotoxin (TTX, Tocris, Bristol, UK). The temperature was held constant (32 ± 0.5°C) by a temperature control unit (TC10, npi electronic, Tamm). All substances were applied by superfusion. Recombinant IFN-β (U-CyTech) was aliquoted after reconstitution and kept at -80°C. For the I_h block, the specific antagonist ZD7288 (Harris and Constanti 1995) purchased by Tocris was prepared as a 100 mM stock solution, aliquoted and stored at -20°C. To reduce M currents, carbamylcholin chloride (Adams et al. 1982) purchased by Sigma-Aldrich (further referred to as carbachol) was prepared as a 100 mM stock solution, aliquoted, and stored at -20°C. Aliquots were diluted in ACSF directly before starting each experiment. Bicuculline methiodide (Sigma-Aldrich; further referred to as bicuculline) was used to inhibit the fast inhibitory synaptic response mediated by GABA_A (Salin and Prince 1996) and was prepared as a stock solution of 10 mM and stored at 4°C. In 22 experiments, 4% Neurobiotin (Vector Laboratories, Burlingame, CA) was included in the pipette solution for staining and injected at the end of the experiment by a DC current (20–30 min, +0.15 to +0.35 nA). Ten neocortical neurons were properly filled with neurobiotin, visualized by diaminobenzidine (DAB) and reconstructed by the use of NeuroLucida imaging system (MicroBrightfield).

Data acquisition and analysis

Data were sampled at 20 kHz by ISO2 (MFK, Niederegge, Germany) after filtering at 2 kHz. To enable a representative comparison

among neurons, all data were normalized on a trial-by-trial basis; that is, each value is expressed in terms of the initial “baseline” value. Evaluation and analysis were performed with ISO2 and Origin7 (Microcal, Northampton, MA). The input resistance was calculated from linear regressions of *I-V* plots including values in the range of ±5 mV from resting membrane potential. Assuming that the surface area of the cell did not change substantially under the conditions of IFN-β superfusion, the input resistance was expected to reflect the membrane resistance (*R_M*). The membrane time constant was obtained as a mean of four to five slowest values of multiexponential least square fits of the initial 200 ms of voltage responses to current steps (Spruston and Johnston 1992) in the vicinity (±5 mV) of the resting potential (*V_M*). The relationship between injected current intensity and the total number of spikes per pulse (normalized by the pulse duration) was calculated, and neurons without a significant correlation between these variables were excluded from analysis. The current-frequency relationship of a given neuron is characterized by the slope (Hz/nA) of the linear regression (see Fig. 6). All values are given as means ± SE. Statistical analysis was performed using one-way ANOVA and paired Student's *t*-test as appropriate. Significance levels were set to *P* = 0.05 if not indicated otherwise.

RESULTS

General neuronal properties

Conventional intracellular recordings were analyzed from a total of 98 somatosensory neocortical neurons. All reported neurons met the criteria of neocortical pyramidal neurons based on the classification of Connors and Gutnick (1990). Neurons recorded after the 15-min pretreatment with ZD7288 (*n* = 6) were not considered for the general characterization of electrophysiological properties. The neurons were segregated by their location (for detailed sub- and suprathreshold membrane properties see Table 1) and their response to suprathreshold current injections (exemplified in Fig. 4A). The neurons were identified by their relatively broad action potentials (action potential width at half height 1.1 ± 0.03 ms, *n* = 80) that exhibited spike frequency adaptation on injection of a depolarizing current pulse. Five neurons in layer 2/3 and 13 neurons in layer 5 generated bursts of action potentials with decreasing amplitudes at the beginning of the depolarizing current pulse through the activation of an afterdepolarization after each action potential. Three of the 6 neurons recorded after pretreatment with ZD7288 responded initially with burst discharges. Irrespective of classification, no decrease in height of single action potentials (86.15 ± 1.1 mV, *n* = 63) was observed during spike trains elicited by depolarizing current

TABLE 1. General sub- and suprathreshold membrane properties of somatosensory pyramidal neurons from the neocortex of Wistar rats

	Layer 2/3	Layer 5
Membrane potential, mV	-75.1 ± 0.9 (45)	-72.6 ± 0.8 (47)
Input resistance, MΩ	34.8 ± 2.5 (45)	33.1 ± 2.5 (47)
Membrane time constant, ms	12.4 ± 0.8 (45)	14.1 ± 1.0 (47)
Action potential amplitude, mV	87.9 ± 1.2 (39)	83.2 ± 3.2 (24)
Action potential width at half height, ms	1.16 ± 0.03 (39)	1.06 ± 0.05 (24)
Action potential rise time (10–90%), ms	0.40 ± 0.01 (39)	0.36 ± 0.01 (24)
Action potential rate of rise, V/s	162.0 ± 7.5 (39)	182.3 ± 13.2 (24)
Rheobase (action potential current threshold), pA	206.8 ± 38.4 (21)	254.0 ± 48.7 (23)
<i>F-I</i> slope, Hz/nA	59.5 ± 6.6 (21)	64.6 ± 9.9 (23)

Data presented as mean ± SE, number of cells in parentheses. Note that the neurons under the pre-treatment with ZD7288 have been excluded from the analysis of the membrane properties. Analysis of single action potential and firing properties were restricted to those neurons which underwent related interferon-β superfusion experiments.

injection, suggesting that the recording location was somatic (Spruston et al. 1995). Examination of the electrophysiological properties and three-dimensional reconstruction of neuron morphology confirmed the identity of the pyramidal neocortical neurons in a random sample ($n = 6$ for layer 2/3, see a typical example in Fig. 1; $n = 4$ for layer 5).

IFN- β augments subthreshold membrane properties

IFN- β concentrations of 500 and 1,000 U/ml were previously reported as biologically active and have been shown to elicit reproducible and significant effects in immune cells (Hallal et al. 2003). Therefore 500 and 1,000 U/ml were chosen for initial test experiments. The IFN- β superfusion was ceased after 30 min because this has been shown to be sufficient for IFN- α to elicit effects on neurons (Mendoza-Fernandez et al. 2000). The result of the superfusion was a substantial change in subthreshold membrane responses to current injections, reflected by an increase of R_M to $242 \pm 34\%$ ($n = 17$) (Fig. 1B). Assuming an unchanged surface area and a subsequent constant membrane capacitance, the reported change in R_M was directly linked to τ , which increased $\leq 163 \pm 10\%$ ($n = 17$; Fig. 1C). The concentrations of 500 and 1,000 U/ml were equally effective doses ($P = 0.37$ and $P = 0.29$ for R_M and τ , respectively), therefore all data were pooled.

The onset of these effects was delayed by 10–15 min and was independent of the depth ($P = 0.51$, $n = 17$ for R_M and $P = 0.08$, $n = 17$ for τ) of the neurons. In detail, normalized values of neurons impaled at 0–100 and 300–400 μm ($n = 5$) were for $R_M = 1.74 \pm 0.39$ and for $\tau = 1.28 \pm 0.03$, compared with normalized values of $R_M = 2.16 \pm 0.36$ and of $\tau = 1.60 \pm 0.11$ for the group between 100 and 300 μm depth ($n = 12$).

The changes could be reversed within 30 min of washout with ACSF. A dose dependence of the changes of R_M and τ was revealed by a series of experiments involving the exposure of a distinct pyramidal neuron to increasing concentrations of IFN- β (Fig. 2A). Each concentration was applied for a period of 30 min and followed by superfusion with a higher one. The investigation of the dose dependence showed that saturation was achieved at doses $>1,000$ U/ml.

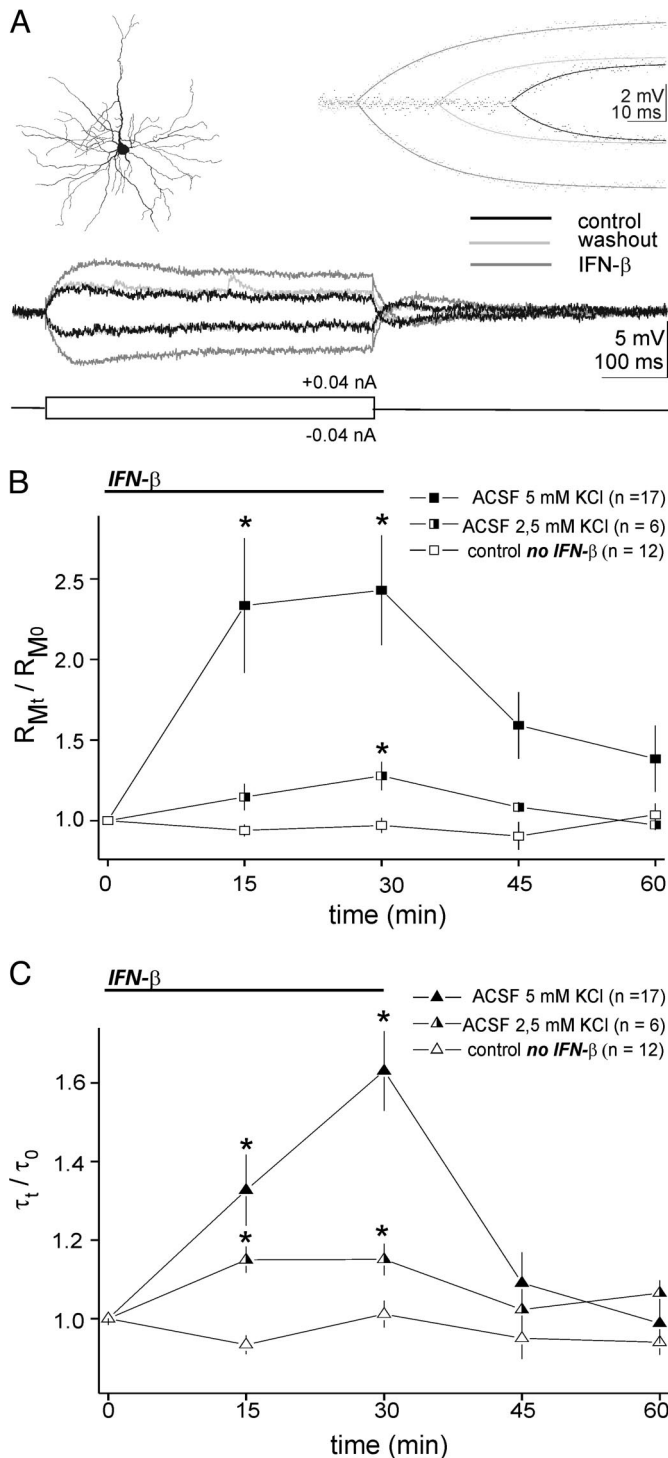
A comparison was performed between the changes after increasing IFN- β concentrations ($R_M = 2.04 \pm 0.32$ and $\tau = 1.65 \pm 0.12$, $n = 6$, respectively) and the changes after immediate application ($R_M = 1.90 \pm 0.39$ and $\tau = 1.44 \pm 0.11$, $n = 11$, respectively) of the submaximal concentration of 500 or 1,000 U/ml. This revealed no difference in kinetics and magnitude of the increase ($P = 0.80$ and $P = 0.29$ for R_M and τ , respectively) which excludes a putative accumulation of the effect during the application of increasing doses. For comparison between neurons, we normalized the data to the initial value (see METHODS). However, we observed a large intercell variability of the subthreshold voltage responses of the neocortical neurons at a distinct concentration. This variability is best reflected in a plot of the individual values (Fig. 2, B and C), considering that the experimental setup did not allow to test for the whole concentration range in one given neuron. A sigmoidal fit revealed a half-maximal dose (ED_{50}) of 29.6 ± 24.9 U/ml ($n = 4$ –10) and 64.1 ± 40 U/ml IFN- β ($n = 4$ –10) and a slope of 0.94 and 0.90 for R_M and τ , respectively. There was no significant change in V_M between the control value and

after 30 min IFN- β application (from -73.3 ± 1.8 to -72.2 ± 1.7 mV, $n = 11$, $P = 0.29$). The effect could be observed in all neurons, and there was neither a difference in the kinetics nor in the magnitude of the effects on R_M and τ between layer 2/3 and layer 5 neurons ($P = 0.77$ for R_M and $P = 0.16$ for τ). Layer 2/3 neurons experienced an increase in R_M to $253 \pm 90\%$ ($n = 7$) and τ to $146 \pm 8\%$ ($n = 7$), compared with an R_M increase to $237 \pm 27\%$ ($n = 10$) and an τ increase to $175 \pm 15\%$ ($n = 10$) in layer 5 neurons. Data for both layers have therefore been pooled. Decreasing the potassium concentration in the ACSF to the lower limit of the physiological range (2.5 mM) did not change the quality of the effect but reduced its intensity (R_M increased to $128 \pm 9\%$, and τ to $115 \pm 4\%$, $n = 6$, for both layers, Fig. 1, B and C). Various substances were preapplied to further determine putative targets of the influence of IFN- β on “resting” properties of neuronal membranes (Fig. 3). We mainly focused on substances known to block potassium conductances that are partially active at rest. IFN- β was added after the effects of the preapplied substances reached a steady state. After the preapplication of 1 mM Ni^{2+} , which blocks Ca^{2+} influx into the neuron and prevents the opening of Ca^{2+} -activated K^+ channels, the superfusion with 1,000 U/ml IFN- β increased R_M to $147.7 \pm 18.0\%$ and τ to $129.4 \pm 06.5\%$ ($n = 7$). Preapplication of 10 μM bicuculline, which blocks the fast inhibitory synaptic response mediated by GABA_A , followed by the superfusion of IFN- β (1,000 U/ml) raised R_M to $161.46 \pm 22.3\%$ and τ to $158.15 \pm 11.2\%$ ($n = 7$). The same extent of increase in R_M to $171.9 \pm 26.7\%$ and τ to $169.5 \pm 28.0\%$ ($n = 4$) was achieved by IFN- β after preapplication of 50 μM carbachol, used to diminish M currents. The amount of relative effect appeared smaller during the preapplication of all three substances compared with the superfusion with IFN- β alone, which might argue for a partial contribution of Ca^{2+} -activated K^+ channels, tonic inhibition mediated by GABA_A and M currents to the effect on R_M and τ . However, these effects were not significant on the population level for any of the substances (for Ni^{2+} $P = 0.27$, for bicuculline $P = 0.46$ and for carbachol $P = 0.73$), possibly due to the large intercell variability (see Fig. 2). However, under the conditions of preapplication of 50 μM ZD7288, a specific blocker of I_h , R_M ($90.1 \pm 13.8\%$, $n = 6$) and τ ($98.5 \pm 7.9\%$, $n = 6$) remained stable despite the superfusion of IFN- β . These results imply that IFN- β causes a dose-dependent and reversible effect on the subthreshold membrane responses of neocortical pyramidal neurons that was mainly mediated by a reduction of I_h .

IFN- β increases the neuronal action potential firing evoked by a 15% suprathreshold current injection

As a second effect of IFN- β , we observed an enhancement of the rate of neuronal action potential firing in a large subset (25/39) of neocortical pyramidal neurons after application of 1–10,000 U/ml IFN- β in ACSF containing 5 mM K^+ . Only neurons with an increase of $>20\%$ in action potential frequency were regarded as IFN- β responsive because control measurements in neocortical pyramidal neurons ($n = 12$) revealed that impalement and measurement itself led to a $\pm 10\%$ variation of the firing rate ($P = 0.56$). Population data of the responding neurons revealed a substantial increase ($\leq 317 \pm 62\%$) in action potential frequency in response to a 15% suprathreshold current injection as calculated from the

first interspike interval (ISI; Fig. 4). The 15% suprathreshold current injection was chosen to mimic the situation of a moderate synaptic input. The observed increase in the frequency developed slowly during the 30-min period of IFN- β superfusion. Most neurons continued to experience an increase in action potential frequency even after the cessation of IFN- β application (Fig. 4, *B* and *C*), i.e., during a washout period of ≤ 60 min the effect was sustained or even increased as long as we held the recording (≤ 210 min after the cessation of the IFN- β superfusion). There was no change in the voltage



threshold for action potential generation in a random sample (control: -52.8 ± 3.2 mV, IFN 1,000 U/ml: -53.0 ± 3.2 mV, $P = 0.44$, $n = 13$).

Some neurons ($n = 8/45$ in layer 2/3 and $n = 6/47$ in layer 5) also experienced an IFN- β induced change in the pattern of action potential firing, i.e., burst discharging at the beginning of the current injection was unleashed, irrespective of the concentrations applied. This resulted in a dose-independent 6.32 ± 1.1 -fold ($n = 14$) increase of the action potential frequency of the first ISI in the group of burst developing neurons. Due to their different response characteristics, these neurons were excluded from the population dose-dependence analysis of the frequency revealed from the first ISI. The effect on the firing rate of the regular spiking neurons was dose-dependent (Fig. 5A). We pooled data for each concentration and calculated means (normalized to initial values, Fig. 5B) for the first and the last ISI as well as for the number of action potentials. The sigmoidal fit for the frequency of the first ISI revealed an ED₅₀ of 24.0 ± 20.6 U/ml ($n = 3-5$) and a slope of 1.04, for the last frequency an ED₅₀ of 29.9 ± 21 U/ml ($n = 2-5$) and a slope of 1.48, and for the number of action potentials an ED₅₀ = 40.6 ± 21.2 U/ml ($n = 3-6$) and a slope of 1.26. A pretreatment with 1 mM Ni²⁺ prevented the IFN- β induced increase in frequency of the first ISI as reflected by the unchanged values as normalized to the first frequency measured in Ni²⁺ alone (1.09 ± 0.2 , $P = 0.68$, $n = 7$), whereas a pretreatment with 50 μ M ZD7288 left the IFN- β induced increase intact (3.40 ± 1.89 , $P < 0.05$, $n = 6$). No correlation was found between the extent of the enhancement and the location of the neuron in either layer 2/3 or 5 or to the depth in the slice. The effect of IFN- β on the action potential frequency varied markedly in strength; the differences in response varied from slight (1.4-fold) to pronounced (7.8-fold) increase in the firing rate on application of 500 U/ml IFN- β . The effect was long-lasting and was established slower as compared with the effect on R_M and τ in neocortical pyramidal neurons. Our experimental protocol itself is unlikely to account for the effects observed in this study, i.e., did not induce long-term potentiation of intrinsic excitability by activation (Cudmore and Turrigiano 2004). The IFN- β -induced effects occurred also with slight activation (15% suprathreshold current injection).

FIG. 1. Interferon- β (IFN- β) reversibly increased subthreshold membrane responses of neocortical sensorimotor pyramidal neurons. *A*: recordings obtained from a layer 2/3 pyramidal neuron (NeuroLucida image of this Neurobiotin-stained neuron in the top left row) on current injection as indicated. The sweeps represent the membrane voltage response at control conditions (black traces) and subsequent to 30 min superfusion of 1,000 IU/ml IFN- β (dark gray traces). Note the increase of the membrane resistance and time constant following interferon application. This increase was reversible (light gray) after a washout period of 30 min with artificial cerebrospinal fluid (ACSF). *B* and *C*: population data of the membrane resistance (squares, *B*) and the normalized membrane time constant (triangles, *C*), both normalized to control values (R_{Mt}/R_{M0} and τ_t/τ_0 , respectively). IFN- β had been applied as indicated by the horizontal bars at submaximal concentrations of 500 and 1,000 U/ml. Measurements were performed before as well as after 15 and 30 min of superfusion with IFN- β and at 15 and 30 min of washout in ACSF containing 5 mM K⁺ (closed symbols) and ACSF containing 2.5 mM K⁺ (half closed symbols). Both membrane resistance and time constant increased compared with control recordings in untreated pyramidal cells (open symbols). Although the effect of IFN- β remained qualitatively the same, it was much more prominent in the higher K⁺ concentration. The reversibility of the effect was confirmed by washout experiments. Asterisks, significant changes compared with initial values calculated with the paired Student's *t*-test ($P = 0.05$), error bars represent SE.

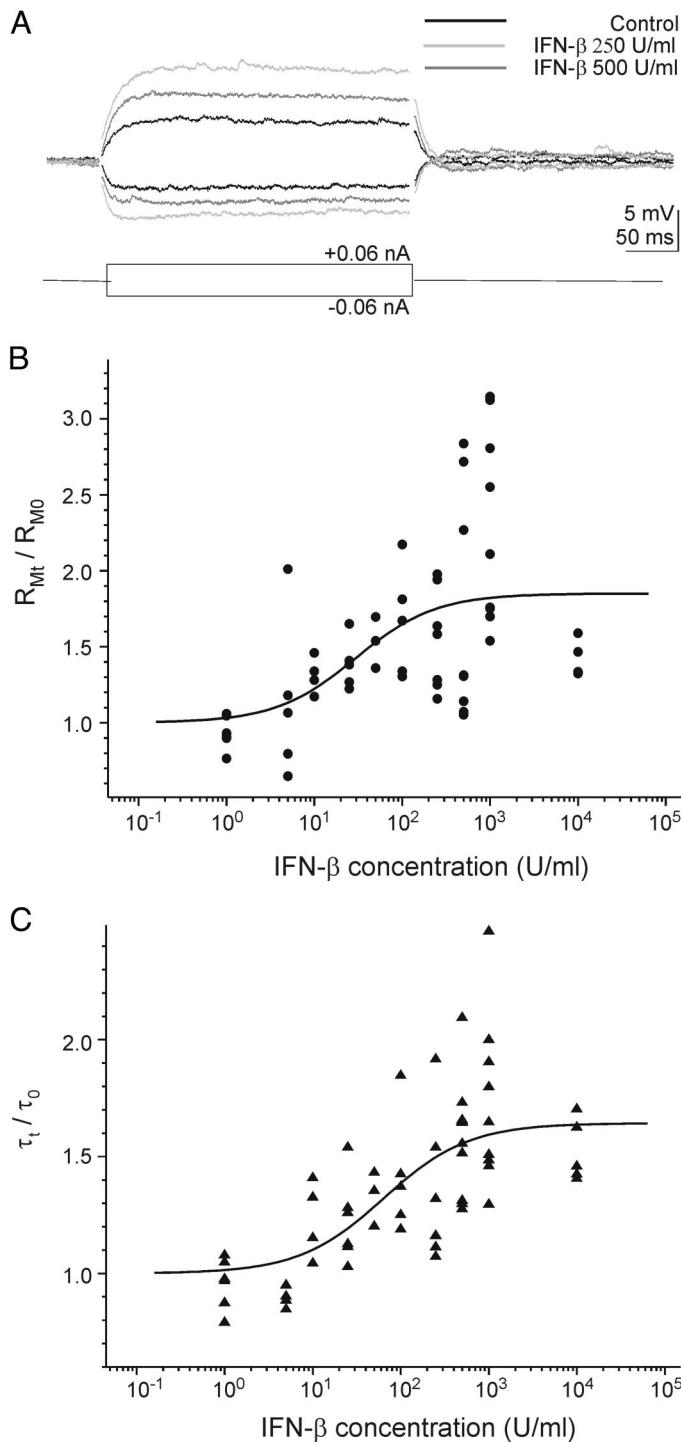


FIG. 2. IFN- β influences the subthreshold membrane characteristics dose-dependently. *A*: traces represent voltage responses from a layer 2/3 pyramidal neuron to current injections as indicated (*bottom*). Increasing concentrations of IFN- β lead to a dose-dependent augmentation of the responses compared with control. The traces were recorded after superfusion with IFN- β of 30 min at the concentrations given in the legend. *B* and *C*: individual values ($n = 4$ – 10 neurons for each concentration) of “resting” membrane resistance (R_M) and membrane time constant (τ) were normalized to the control values estimated before IFN- β application and plotted semilogarithmically against the IFN- β concentration. The plot revealed sigmoidal dose response curves characterizing the dose dependence of both subthreshold membrane characteristics, i.e., R_M (\bullet , *B*) and τ (\blacktriangle , *C*), with an $ED_{50} = 29.62 \pm 24.9$ U/ml and $ED_{50} = 64.1 \pm 40.1$ U/ml, respectively.

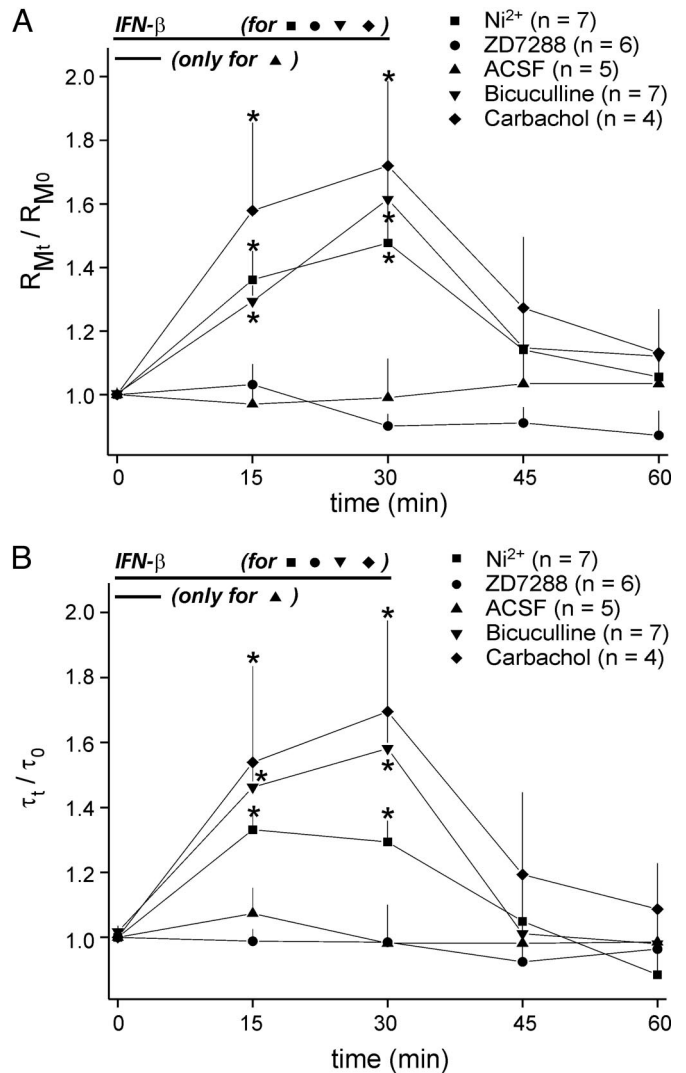
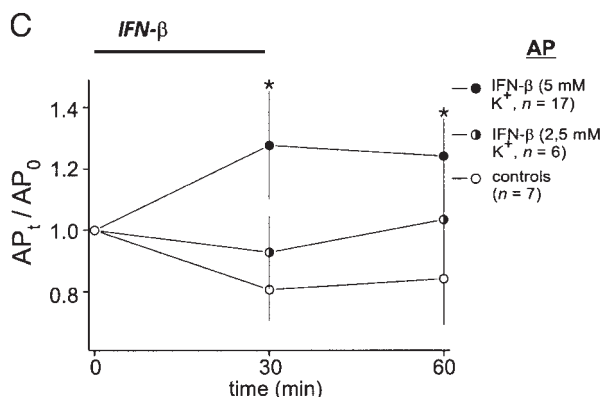
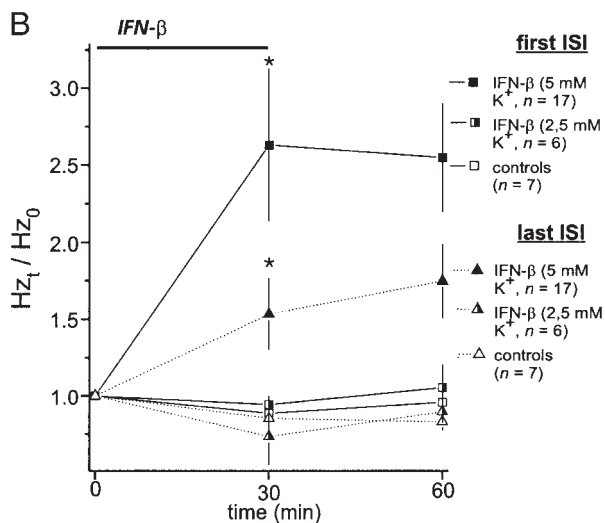
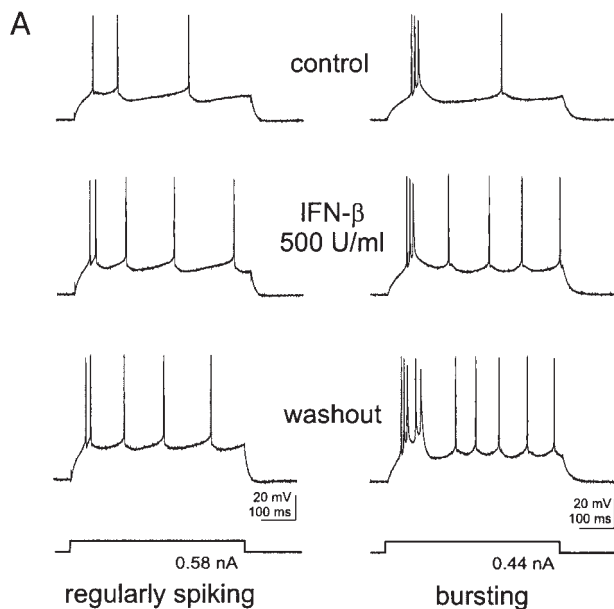


FIG. 3. IFN- β effects on subthreshold membrane responses were differently influenced by varying the IFN- β application time, reduction of various K^+ conductances or decreasing fast inhibitory synaptic transmission. *A* and *B*: population data of the membrane resistance (*A*) and the membrane time constant (*B*), both normalized to control values (R_{Mt}/R_{M0} and τ_t/τ_0 , respectively). IFN- β (1,000 U/ml) had been applied either for 5 min (upward triangle, short horizontal bar) at the beginning of the experiment or for 30 min starting after the effects of the preapplied substances reached a steady state (all other symbols, long horizontal bar). Note that for the latter situation the *time 0* indicates the steady state of the preapplied substance, which was ~ 20 – 25 min after impaling the neurons. Recordings were analyzed for times right before as well as 15, 30, 45, and 60 min after the beginning of the IFN- β superfusion. For all experiments summarized here, the ACSF contained 5 mM K^+ . In case of the preapplication of 1 mM Ni^{2+} , 50 μM carbachol or 10 μM bicuculline additional IFN- β application increased both R_M and τ compared with control recordings in untreated pyramidal neurons (see Fig. 1 and RESULTS). Note that the preapplication of these three substances seemed to prevent partially the effect exerted by IFN- β alone, but this trend did not reach the level of significance (compare with Fig. 1 and see RESULTS). The reversibility of the remaining IFN- β effect was shown in washout experiments. Only the short application of IFN- β and the preincubation with the selective I_h blocker ZD7288 (50 μM) fully prevented the increase of R_M and τ . Asterisks indicate significant changes compared with initial values ($P < 0.05$), error bars represent SE.

tion) at large (10 min) intervals, persisted under the conditions of blocked Ca^{2+} influx (1 mM Ni^{2+}) and increased the slope of the F - I curve, too.

Suprathreshold excitability is increased by IFN- β over a broad range of current injections by a subtractive shift and by an increase in gain of the F-I curves

We additionally determined if IFN- β causes a similar increase in excitability across a range of suprathreshold current injections. Therefore *F-I* curves as a measure of the relation-



ship between firing rate and constant driving current were constructed by injecting a range of current amplitudes before and 30 min after the onset of the IFN- β application. The analysis of these curves (example Fig. 6A, left) revealed that the increase in suprathreshold excitability evolves from a shift of the *F-I* curves, i.e., from a smaller rheobase as calculated from the intersection of the linear regression with the abscissa as well as from a larger gain, i.e., an increase in slope of that linear regression (for values \pm SE, see Fig. 6, B and C, 3rd column). Both effects could be prevented by the reduction of K^+ in the ACSF to low physiological values of 2.5 mM (Fig. 6, B and C, 2nd column). To further address putative mechanisms of action of IFN- β on the neuronal firing rate, we used the application of substances bearing the potency to reduce several candidate currents prior to the superfusion with IFN- β . As depicted in Fig. 6, B and C, pretreatment with bicuculline (10 μ M) prevented the IFN- β induced shift in the rheobase but not the increase in gain. The other two substances used for preapplication (1 mM Ni^{2+} as a blocker of Ca^{2+} conductances with a low selectivity, therewith also reducing Ca^{2+} -dependent K^+ conductances, and 50 μ M carbachol as an inhibitor of M currents) independently prevented the increase in the gain but not the shift of the *F-I* curves.

Short application of IFN- β selectively releases the frequency increase

To describe the development of the IFN- β effects in more detail, experiments with short-term application were performed. This should elucidate whether the IFN- β effect requires continuous presence of the substance or is triggered by an initial exposure to IFN- β . The IFN- β superfusion was limited to 5 min at the beginning of an experiment running for 60 min ($n = 5$), which was considered long enough for diffusion of the IFN- β molecule into the brain tissue. All experiments were performed with the concentration of 500 U/ml, shown to be an effective concentration (see dose-response curves, Figs. 2, B and C, and 5B). Whereas R_M and τ did not change 10 and 25 min after IFN- β application ceased ($P = 0.65$ and $P = 0.29$, respectively, Fig. 3, A and B), the firing rate of four of five neurons in response of a 15% suprathreshold current pulse increased $\leq 155 \pm 5\%$ at 25 min after cessation of the IFN- β application. This effect seems to be mediated more by an increase in the neuronal gain ($P <$

FIG. 4. IFN- β influences the firing behavior of neocortical neurons persistently toward an increase of excitability. A: current injections of 15% above the rheobase were used to evoke a train of action potentials in a regular spiking layer 2/3 pyramidal neuron (left) and a burst discharging layer 5 pyramidal neuron (right). The absolute amount of injected current is given in the inset traces. Regardless the location the firing rate increased when IFN- β at 500 U/ml had been applied. Note that the effect of IFN- β on the frequency achieved by IFN- β application was maintained and even slightly increased throughout the period of washout. B and C: quantification of firing frequencies for the 1st interspike interval (B, squares) and the last interspike interval (B, triangles) in neurons, investigated under 30-min application of 500 or 1,000 U/ml IFN- β ($n = 8$, black squares). Only neurons undergoing a complete experiment with ≥ 30 -min washout after 30-min IFN- β application were included. First and last interspike interval show a significant (*, $P < 0.05$) increase of the frequency values revealed by paired Student's *t*-test. Recordings of untreated neurons (open symbols, $n = 7$ for B and the number of action potentials (AP) (C) for ≥ 60 min did not show time-dependent increase of the firing rate, therewith excluding an effect of penetration and recording procedure itself. For a representative comparison, values are given as ratios to the initial control frequency values (Hz_t/Hz_0). Symbols represent means \pm SE.

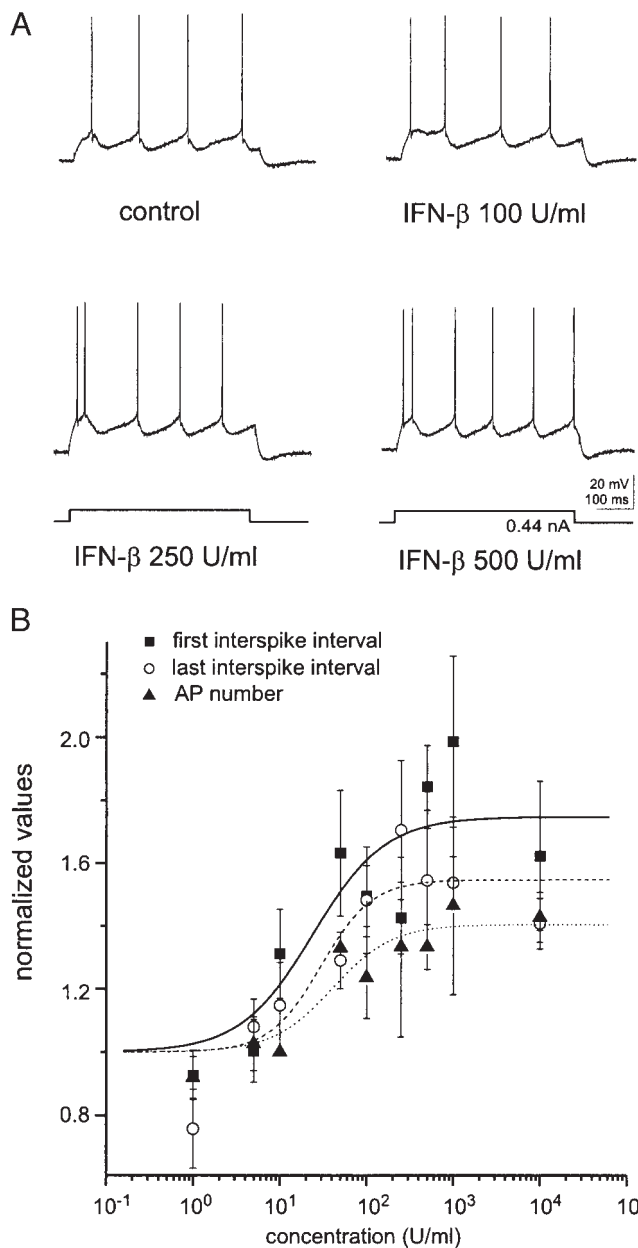


FIG. 5. Dose-dependence of the effect of IFN- β on the action potential frequency. *A*: the membrane voltage response of a layer 2/3 neocortical neuron was recorded during application of progressively increased IFN- β concentrations. Voltage responses to fifteen percent suprathreshold current injections are shown, each trace was recorded after 30-min superfusion with the IFN- β concentration indicated below the respective traces. *B*: the influence of IFN- β on the firing behavior of the neocortical neurons was analyzed separately for changes in the 1st interspike interval (■) and the last interspike interval (○) as well as for changes in the number of the action potentials per pulse (AP number, ▲). All 3 parameters were increased by IFN- β in a dose-dependent manner. The effect was most prominent for the first interspike interval, with an $ED_{50} = 24.03 \pm 20.69$ U/ml ($n = 4-10$). All values were normalized to the control value at the beginning of the experiment, bursting cells are excluded from the analysis of the fast frequency. Mean values are calculated for data at each concentration ($n = 4-10$) and plotted against the IFN- β concentration (log). The error bars represent SE.

0.05) than by a change in $F-I$ shift ($P = 0.13$, Fig. 6*B*, right). A further increase of the firing rate $\leq 257 \pm 14\%$ ($n = 5$) was seen during the 55-min washout period.

In summary, the IFN- β effect on subthreshold membrane properties requires its continuous presence, whereas the action potential frequency is triggered in an "instructive" manner.

DISCUSSION

Our investigations provide evidence for dual excitatory effects of IFN- β on somatosensory neocortical pyramidal neurons: 1) IFN- β augmented the subthreshold membrane responses. Increases in R_M and τ were fast developing, reproducible in every given neuron, reversible, dependent on the continuous presence of IFN- β and determined by an intact I_h . 2) IFN- β caused an enhancement of the neuronal output over a broad range of suprathreshold current injections due to an offset the input-output relationship and an increase in neuronal gain. In contrast to the subthreshold changes, this suprathreshold effect occurs in 64% of the neurons, developed comparatively slower and showed a pronounced variability, appeared to be persistent after cessation of the effect on subthreshold membrane responses, was also released by short-term IFN- β application and was differentially influenced by a set of conductance including I_{Ca} and/or I_{KCa} , I_M and the fast inhibitory GABA $_A$ conductance (see following text).

These observations, although giving first evidence for direct IFN- β actions in neurons, are in accordance with the majority of reports on IFN- α , which shares a common IFNAR $_1$ (type I) receptor with IFN- β . For IFN- α , previous findings include an increase in the spontaneous discharge activity in rat and cat cerebral cultures (Calvert and Gresser 1979), an enhanced single-cell activity in warm-sensitive hypothalamic neurons (Nakashima et al. 1988), short bursts and epileptiform discharges in hippocampal neurons (Müller et al. 1993), an increased activity in cortical and hippocampal neurons in vivo after intravenous IFN- α administration (Dafny et al. 1996), modulated spontaneous activity in glucose-sensitive hypothalamic neurons (Reyes-Vazquez et al. 1997) and cell-type-dependent responses of thermosensitive neurons (Shibata and Blatteis 1991). However, there is also evidence for an unchanged intrinsic and a decreased synaptic excitability in CA1 hippocampal neurons of rats (Mendoza-Fernandez et al. 2000) after application of IFN- α .

We propose a reduction in the hyperpolarization activated nonselective cation current I_h is the key mechanism for the IFN- β effect on subthreshold membrane properties. This is supported by the finding that a reduction in extracellular K^+ , which also reduces I_h , leads to a reduction in the IFN- β effect, and that the selective blockade of I_h by ZD7288 prevented an effect of IFN- β on the subthreshold membrane properties. A reduction of I_h has been shown to be a critical factor affecting membrane and integrative properties of a given neocortical neuron and to increase cortical excitability (Strauss et al. 2004).

The analysis of the nature of the frequency enhancement by IFN- β pointed to an offset as well as an increase in gain of the $F-I$ curves. Both aspects are sufficient to alter neuronal suprathreshold excitability. Offsetting the input-output relationship reduces the possibility of a neuron to distinguish baseline levels of excitation from a signal, whereas increasing the gain alters the sensitivity of the neuron. Pretreatment experiments with ion channel blockers and ion channel modulating substances revealed a number of distinct ion channels putatively

contributing to the frequency enhancement. Whereas the prevention of the increase in gain by carbachol suggests an involvement of the M current, the prevention of the increase in gain by Ni^{2+} points to a contribution of either Ca^{2+} -activated

K^+ currents or T-type calcium currents. The latter could explain the character of the frequency increase (highest influence on the frequency of the first ISI), especially because 1 mM Ni^{2+} prevented the increase in the frequency of the first ISI. However, these changes in single-cell excitability did not translate to an apparent direct increase in network excitability, i.e., there were no signs of spontaneous (epileptiform) discharges like observed for instance under the influence of bicuculline. Nevertheless, the amount of input necessary to evoke an action potential and the number and pattern of action potentials in response to an input will affect network behavior (Cudmore and Turrigiano 2004). Given this our observations might be explained by a parallel increase in the excitability of inhibitory neurons or by a recurrent inhibition.

As shown by our pretreatment experiments with bicuculline, a putatively IFN- β -evoked reduction in the tonic (shunting) inhibition might partially contribute to the enhancement of action potential firing due to a shift in the *F-I* relationship without affecting the gain (see also Ulrich 2003). The prevention of the shift in the *F-I* curve by bicuculline might reflect a contribution of small conductance Ca^{2+} -activated K^+ channels because they have been shown to be affected by bicuculline (Khawaled et al. 1999). However, this mechanism did not appear to have enough impact to exert significant changes in the gain of the subthreshold *I-V* relationship, i.e., the membrane properties R_M and τ . Which conductance is affected and its exact contribution to the effects described as well as whether existing channels are modulated or whether channels are inserted in or removed from the membrane remains to be determined. A putative PKC-mediated shift in I_{NaP} activation (Astman et al. 1998) due to an upregulation of PKC activity by IFN- β (Qiao et al. 2001) is unlikely to account for the enhancement in excitability because there was no change in the spike initiation voltage threshold itself. A change in I_{NaP} , however,

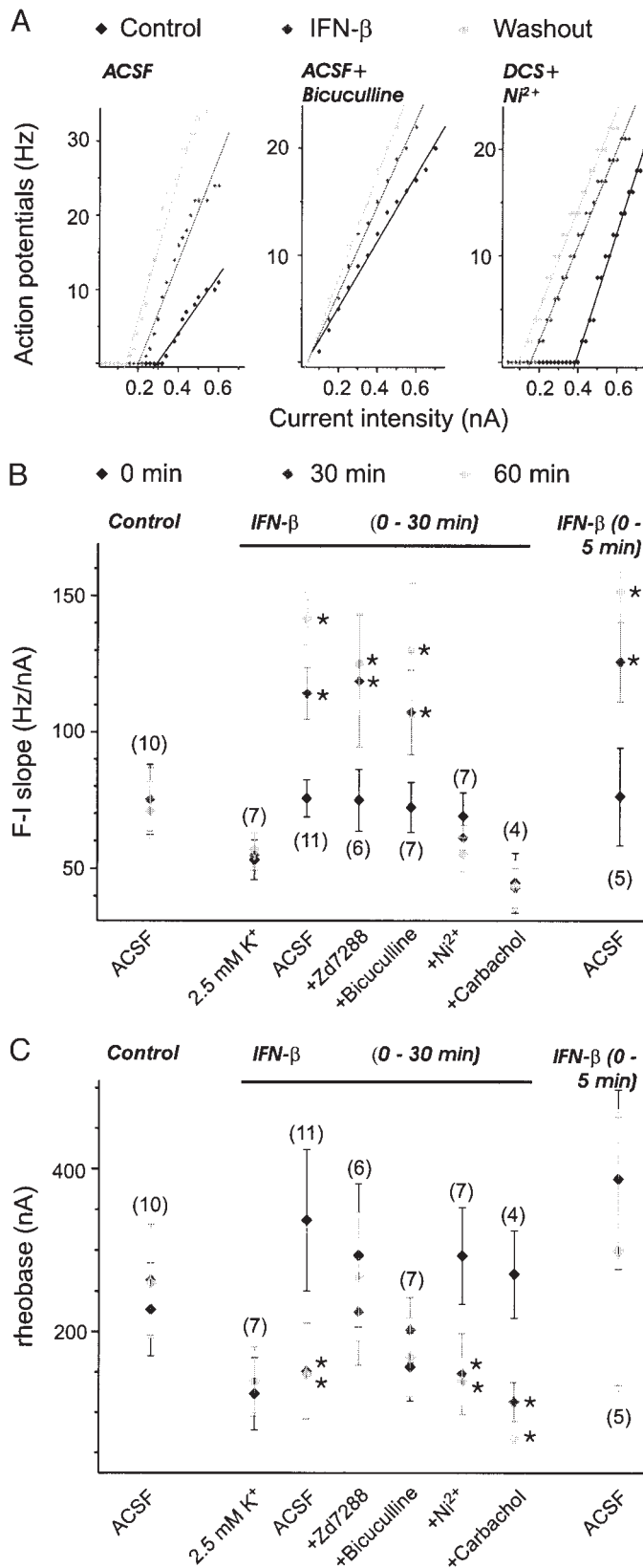


FIG. 6. IFN- β acts additive and multiplicative on the action potential discharge rate. *A*: examples of firing rate vs. constant driving current (*F-I* curves) for 3 different pyramidal neurons that were superfused with 1,000 U/ml IFN- β for 30 min. *F-I* curves of all 3 neurons were recorded before (black diamonds), at 30 min of the IFN- β superfusion (gray diamonds), and 30 min after cessation of the IFN- β superfusion (light gray diamonds). The straight lines represent linear fits under each condition. The slope of this regression (Hz/nA) represents the gain and the intersection with the abscissa has been taken as a measure of the rheobase to determine the amount of shift of the *F-I* curve. Note that the superfusion of IFN- β alone shifted the *F-I* curve and increased the gain (*left*) whereas IFN- β in combination with a pretreatment of 10 μM bicuculline in artificial cerebrospinal fluid containing 5 mM K^+ cause only an increase in gain (*middle*) and IFN- β combined with a pretreatment of 1 mM Ni^{2+} (*right*) in divalent cation solution (DCS, see METHODS) mainly shifted the *F-I* curve. *B* and *C*: population data for the slopes (*B*) and rheobases (*C*) of the *F-I* curves under conditions given at the abscissa. Slopes and rheobases of *F-I* curves were estimated for times before (black diamonds), at 30 min of the IFN- β superfusion (gray diamonds), and at 30 min after cessation of the IFN- β superfusion (light gray diamonds). Except the *leftmost* column (control), which was estimated from neurons without any additional substance recorded in ACSF containing 5 mM K^+ , and the *rightmost* column (IFN- β 0–5 min), which represents data from neurons exposed to IFN- β for 5 min, all other columns consist data of neurons exposed to IFN- β for 30 min. An increase in excitability is either reflected by a decrease in the rheobase (*C*), i.e., a shift in the *F-I* curve as for IFN- β (30 min) alone, in addition to 1 mM Ni^{2+} and to 50 μM carbachol, or by an increase in the gain (*B*), i.e., an increase in slope as for IFN- β alone as well as in addition to 50 μM ZD7288 or 10 μM bicuculline and for short time of IFN application (5 min). Symbols represent means \pm SE (number of neurons), asterisks right of the symbols indicate significance at the level $P < 0.05$.

would be expected to cause a decreased threshold voltage (Crill 1996).

Methodological restrictions prevent to commit the mechanism of the IFN- β action to direct effects on ion channels or activation of signaling pathways. Direct effects were reported for IFN- α in lymphocytes (Schlichter 1992). Other cytokines such as interleukin (IL)-1, IL-2, tumor necrosis factor (TNF)- α , TNF- β , and transforming growth factor (TGF)- β are known to affect ionic currents, Ca²⁺ homeostasis or long-term potentiation, which leads to changed electrophysiological properties of neurons and glial cells (for review see Köller et al. 1997). Additionally, IL-1 β and IL-2 directly inhibit sodium currents (Hamm et al. 1996). Some of the IFN- α effects have been related to both the direct interaction with opioid receptors on neurons (Nakashima et al. 1988) and the release of reactive oxygen species and nitric oxide (Müller et al. 1993).

However, the modulation in the suprathreshold range appeared to be state dependent, i.e., IFN- β had different effects on neuronal activity when applied to the same preparation, even if the starting pattern of activity was similar. Moreover, IFN- β seems to control the intensity and pattern of neuronal activity by regulating sets of conductance rather than by regulating a single conductance. This is in agreement with the observation of Goldman et al. (2001) that fairly large changes in one conductance showed no appreciable change in activity, whereas modestly correlated changes in more than one conductance significantly modify activity. The modulation of multiple conductances by a single neuromodulator has been demonstrated for some cases including 5-HT (Kiehn and Harris-Warrick 1992) and dopamine (Kloppenborg et al. 1999). State dependence and influence on multiple conductances point to a modification of intrinsically programmed regulatory cascades by IFN- β .

Although the sub- and suprathreshold effects seem to be partially independent on each other and putatively based on different mechanisms, we further assume a mediation of the effects via the common IFN- α/β receptor and the subsequent signaling pathway. This could lead to the synthesis of protein(s) that may interfere with membrane molecules. In this case, the relative contribution to sub- or suprathreshold responses would depend mainly on the voltage-dependence of the conductance influenced by intracellular compounds. Previous reports showed that the intracellular signaling pathway of IFN- β involves janus kinases (JAKs) and signal transducer and activator of transcriptions (STATs) for induction of the transcription of target genes (for review, see Ransohoff 1998). Within that scenario, the reversibility and the dependence on permanent presence of IFN- β for the changes in the subthreshold membrane responses would argue for a higher turnover rate of the proteins putatively involved in the suspected reduction of I_h .

We recorded in brain slices to preserve physiological conditions. Therefore we cannot conclusively distinguish between direct IFN- β effects on intrinsic channel properties as they are suspected for IFN- α in lymphocytes (Schlichter 1992), on neuronal gap junctions (Jahromi et al. 2002), on synaptic transmission (D'Arcangelo et al. 1991; Katafuchi et al. 1995) and on glial activity (Blalock 1994; Hu et al. 2000; Szelényi 2001). Regarding the latter possibility some reports described an IFN- β involvement in the activation of multiple signaling cascades in human microglia (Kim et al. 2002) and in the

phosphatidylinositol 3-kinase pathway in astrocytes (Barca et al. 2003). A close communication and interaction between different cell types, i.e., pyramidal cells, interneurons and glia, cannot be excluded to at least contributing to both IFN- β effects. Subsequent investigations will be necessary to dissect these complex interactions.

Our study may give hints for better understanding the pathogenesis and therapeutic manipulations of inflammatory diseases of the CNS because a large number of activated lymphocytes and macrophages infiltrate the brain parenchyma and a variety of proinflammatory and antiinflammatory cytokines are released locally (Sternberg 1997; Szelényi 2001). Furthermore, IFN- β might appear in the brain tissue after systemic administration in MS patients, possibly because of a local increase in the permeability of the blood-brain barrier (Durelli et al. 2001; Smith et al. 1985). Given this fact, the observed changes in neuronal properties and activity may support the therapeutic effects but may also account for CNS-related side effects of IFN- β therapy like stiffness, spasms, and epileptic seizures (Bramanti et al. 1998; Walther and Hohlfeld 1999). However, reports on electrophysiological parameters that could provide evidence for neuromodulatory actions of IFN- β during therapeutic use in humans are limited. Using the latency of the P300 wave of cortical event-related potentials as a sensitive neuroelectric tool to evaluate drugs interacting with the CNS, Gerschlagler et al. (2000) found changes in a subgroup of MS patients treated for 1 yr with IFN- β . If these results can be replicated on a greater number of patients and after a longer treatment period, this might be a link to the experimental data presented here.

Our results give new insights in the action of IFN- β as a neuromodulator in addition to the antiviral, antiproliferative and immunoregulatory effects that have been in the focus of interest for many years (Taniguchi and Takaoka 2002; Teige et al. 2003).

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REFERENCES

- Adams PR, Brown DA, and Constanti A. Pharmacological inhibition of the M-current. *J Physiol* 332: 223–262, 1982.
- Astman N, Gutnick MJ, and Fleidervish IA. Activation of protein kinase C increases neuronal excitability by regulating persistent Na⁺ current in mouse neocortical slices. *J Neurophysiol* 80: 1547–1551, 1998.
- Barca O, Ferre S, Seoane M, Prieto JM, Lema M, Senaris R, and Arce VM. Interferon beta promotes survival in primary astrocytes through phosphatidylinositol 3-kinase. *J Neuroimmunol* 139: 155–159, 2003.
- Baron S, Tyring SK, Fleischmann WR Jr, Coppenhaver DH, Niesel DW, Klimpel GR, Stanton GJ, and Hughes TK. The interferons. Mechanisms of action and clinical applications. *J Am Med Assoc* 266: 1375–1383, 1991.
- Bartfai T and Schultzberg M. Cytokines in neuronal cell types. *Neurochem Int* 22: 435–444, 1993.

- Blalock JE.** Shared ligands and receptors as a molecular mechanism for communication between the immune and neuroendocrine systems. *Ann NY Acad Sci* 74: 1292–1298, 1994.
- Bocci V.** Physicochemical and biologic properties of interferons and their potential uses in drug delivery systems. *Crit Rev Ther Drug Carrier Syst* 9: 91–133, 1992.
- Bramanti P, Sessa E, Rifici C, D'Aleo G, Florida D, Di Bella P, and Lublin F.** Enhanced spasticity in primary progressive MS patients treated with interferon beta-1b. *Neurology* 51: 1720–1723, 1998.
- Calvert MC and Gresser IC.** Interferon enhances the excitability of cultured neurons. *Nature* 278: 558–560, 1979.
- Connors BW and Gutnick MJ.** Intrinsic firing patterns of diverse neocortical neurons. *Trends Neurosci* 13: 99–104, 1990.
- Coyle PK and Hartung HP.** Use of interferon beta in multiple sclerosis: rationale for early treatment and evidence for dose- and frequency-dependent effects on clinical response. *Mult Scler* 8: 2–9, 2002.
- Crill WE.** Persistent sodium current in mammalian central neurons. *Annu Rev Physiol* 58: 349–362, 1996.
- Cudmore RH and Turrigiano GG.** Long-term potentiation of intrinsic excitability in LV visual cortical neurons. *J Neurophysiol* 92: 341–348, 2004.
- Dafny N, Prieto-Gomez B, Dong WQ, and Reyes-Vazquez C.** Interferon modulates neuronal activity recorded from the hypothalamus, thalamus, hippocampus, amygdala and the somatosensory cortex. *Brain Res* 734: 269–274, 1996.
- Dafny N, Prieto-Gomez B, Dong WQ, and Reyes-Vazquez C.** Effects of interferon on the central nervous system. In: *Interferon Therapy of Multiple Sclerosis*, edited by Reder AT. New York: Dekker; 1997, p. 115–137.
- D'Arcangelo G, Grassi F, Ragozzino D, Santoni A, Tancredi V, and Eusebi F.** Interferon inhibits synaptic potentiation in rat hippocampus. *Brain Res* 564: 245–248, 1991.
- Durelli L, Oggero A, Verdun E, Barbero P, Pipieri A, Isoardo G, Ricci A, Clerico M, Bradac G, Bergamasco B, and Bergui M.** Interferon-beta dose and efficacy: the OPTIMS study. *Neurol Sci* 2: 201–203, 2001.
- Fabry Z, Raine CS, and Hart MN.** Nervous tissue as an immune compartment: the dialect of the immune response in the CNS. *Immunol Today* 15: 218–224, 1994.
- Gerschlag W, Beisteiner R, Deecke L, Dirnberger G, Endl W, Kollegger H, Lindinger G, Vass K, and Lang W.** Electrophysiological, neuropsychological and clinical findings in multiple sclerosis patients receiving interferon beta-1b: a 1-year follow-up. *Eur Neurol* 44: 205–209, 2000.
- Goldman MS, Golowasch J, Marder E, and Abbott LF.** Global structure, robustness, and modulation of neuronal models. *J Neurosci* 21: 5229–5238, 2001.
- Hallal DE, Farias AS, Oliveira EC, Diaz-Bardales BM, Brandao CO, Protti GG, Pereira FG, Metzke IL, and Santos LM.** Costimulatory molecule expression on leukocytes from mice with experimental autoimmune encephalomyelitis treated with IFN- β . *J Interferon Cytokine Res* 23: 293–298, 2003.
- Harris NC and Constanti A.** Mechanism of block by ZD 7288 of the hyperpolarization-activated inward rectifying current in guinea pig substantia nigra neurons in vitro. *J Neurophysiol* 74: 2366–2378, 1995.
- Hamm S, Rudel R, and Brinkmeier H.** Excitatory sodium currents of NH15-CA2 neuroblastoma x glioma hybrid cells are differentially affected by interleukin-2 and interleukin-1 β . *Pfluegers* 433: 160–165, 1996.
- Hopkins SJ and Rothwell NJ.** Cytokines and the nervous system. I: Expression and recognition. *Trends Neurosci* 18: 83–88, 1995.
- Hu S, Sheng WS, Ehrlich LC, Peterson PK, and Chao CC.** Cytokine effects on glutamate uptake by human astrocytes. *Neuroimmunomodulation* 7: 153–159, 2000.
- Jahromi SS, Wentlandt K, Piran S, and Carlen PL.** Anticonvulsant actions of gap junctional blockers in an in vitro seizure model. *J Neurophysiol* 88: 1893–1902, 2002.
- Jiang CL and Lu CL.** Interleukin-2 and its effects in the central nervous system. *Biol Signals Recept* 7: 148–156, 1998.
- Katafuchi T, Take S, and Hori T.** Roles of cytokines in the neural-immune interactions: modulation of NMDA responses by IFN-alpha. *Neurobiology* 3: 319–227, 1995.
- Khawald R, Bruening-Wright A, Adelman JP, and Maylie J.** Bicuculline block of small-conductance calcium-activated potassium channels. *Pfluegers* 438: 314–321, 1999.
- Kiehn O and Harris-Warrick RM.** 5-HT modulation of hyperpolarization-activated inward current and calcium-dependent outward current in a crustacean motor neuron. *J Neurophysiol* 68: 496–508, 1992.
- Kim MO, Si Q, Zhou JN, Pestell RG, Brosnan CF, Locker J, and Lee SC.** Interferon-beta activates multiple signaling cascades in primary human microglia. *J Neurochem* 81: 1361–1371, 2002.
- Kinnunen E, Timonen T, Pirttila T, Kalliomaki P, Ketonen L, Matikainen E, Sepponen R, and Juntunen J.** Effects of recombinant alpha-2b-interferon therapy in patients with progressive MS. *Acta Neurol Scand* 87: 457–460, 1993.
- Kloppenborg P, Levini RM, and Harris-Warrick RM.** Dopamine modulates two potassium currents and inhibits the intrinsic firing properties of an identified motor neuron in a central pattern generator network. *J Neurophysiol* 81: 29–38, 1999.
- Köller H, Siebler M, and Hartung HP.** Immunologically induced electrophysiological dysfunction: implications for inflammatory diseases of the CNS and PNS. *Prog Neurobiol* 52: 1–26, 1997.
- Mendoza-Fernandez V, Andrew RD, and Barajas-Lopez C.** Interferon-alpha inhibits long-term potentiation and unmasks a long-term depression in the rat hippocampus. *Brain Res* 885: 14–24, 2000.
- Müller M, Fontana A, Zbinden G, and Gähwiler BH.** Effects of interferons and hydrogen peroxide on CA3 pyramidal cells in rat hippocampal slice cultures. *Brain Res* 619: 157–162, 1993.
- Nakashima T, Hori T, Kuriyama K, and Matsuda T.** Effects of interferon-alpha on the activity of preoptic thermosensitive neurons in tissue slices. *Brain Res* 454: 361–367, 1988.
- Qiao H, Sakamoto T, Hinton DR, Gopalakrishna R, Ishibashi T, Ryan SJ, and Inomata H.** Interferon beta affects retinal pigment epithelial cell proliferation via protein kinase C pathways. *Ophthalmologica* 215: 401–407, 2001.
- Ransohoff RM.** Cellular responses to interferons and other cytokines: the Jak-STAT paradigm. *N Engl J Med* 338: 616–618, 1998.
- Reyes-Vazquez C, Mendoza-Fernandez V, Herrera-Ruiz M, and Dafny N.** Interferon modulates glucose-sensitive neurons in the hypothalamus. *Exp Brain Res* 116: 519–524, 1997.
- Salin PA and Prince DA.** Electrophysiological mapping of GABA_A receptor-mediated inhibition in adult rat somatosensory cortex. *J Neurophysiol* 75: 1589–1600, 1996.
- Schlichter LC.** Acute exposure to human IFN- α affects ion currents in human natural killer cells. *Can J Physiol Pharmacol* 70: 365–376, 1992.
- Shibata M and Blatteis CM.** Differential effects of cytokines on thermosensitive neurons in guinea pig preoptic area slices. *Am J Physiol Regulatory Integrative Comp Physiol* 261: R1096–R1103, 1991.
- Smith RA, Norris F, Palmer D, Bernhardt L, and Willis RJ.** Distribution of alpha interferon in serum and cerebrospinal fluid after systemic administration. *Clin Pharmacol Ther* 37: 85–88, 1985.
- Spruston N and Johnston D.** Perforated patch-clamp analysis of the passive membrane properties of three classes of hippocampal neurons. *J Neurophysiol* 67: 508–529, 1992.
- Spruston N, Schiller Y, Stuart GJ, and Sakmann B.** Activity-dependent action potential invasion into CA1 pyramidal neuron dendrites. *Science* 268: 297–300, 1995.
- Sternberg EM.** Neural-immune interactions in health and disease. *J Clin Invest* 100: 2641–2647, 1997.
- Strauss U, Kole MH, Brauer AU, Pahnke J, Bajorat R, Rolfs A, Nitsch R, and Deisz RA.** An impaired neocortical I_h is associated with enhanced excitability and absence epilepsy. *Eur J Neurosci* 19: 3048–58, 2004.
- Szelényi J.** Cytokines and the central nervous system. *Brain Res Bull* 54: 329–338, 2001.
- Taniguchi T and Takaoka A.** The interferon-alpha/beta system in antiviral responses: a multimodal machinery of gene regulation by the IRF family of transcription factors. *Curr Opin Immunol* 14: 111–116, 2002.
- Teige I, Treschow A, Teige A, Mattson R, Navikas V, Leanderson T, Holmdahl R, and Issazadeh-Navikas S.** IFN-beta gene deletion leads to augmented and chronic demyelinating experimental autoimmune encephalomyelitis. *J Immunol* 170: 4776–4784, 2003.
- Ulrich D.** Differential arithmetic of shunting inhibition for voltage and spike rate in neocortical pyramidal cells. *Eur J Neurosci* 18: 2159–2165, 2003.
- Villarroya H, Marie Y, Ouallet JC, Le Saux F, Tchelingirian JL, and Baumann N.** Expression of TNF alpha in central neurons of Lewis rat spinal cord after EAE induction. *J Neurosci Res* 49: 592–599, 1997.
- Walther EU and Hohlfeld R.** Multiple sclerosis: side effects of interferon beta therapy and their management. *Neurology* 53: 1622–1627, 1999.