NeuroReport 8, 2079–2083 (1997)

# Reduction of excitation by interleukin-1β in rat neocortical slices visualized using infrared-darkfield videomicroscopy

**Giovanna D'Arcangelo, Hans-Ulrich Dodt[1,CA] and Walter Zieglgänsberger[1]**

Department of Experimental Medicine and Biomedical Sciences, II University of Rome Tor Vergata, Via O. Raimondo 1, 00173 Rome, Italy; [1]Max-Planck-Institute of Psychiatry, Clinical Neuropharmacology, Kraepelinstr 2, 80804 Munich, Germany

[CA]Corresponding Author

**THE cytokine interleukin-1 beta (IL-1β) is thought to be critically involved in the neuroendocrine and behavioral changes which occur in response to systemic infection. In the present study, we have employed the novel technique of infrared-darkfield videomicroscopy to examine the effect of IL-1β on the intrinsic optical signal (IOS), an indicator of the spread of neuronal excitation and synaptic transmission in the mammalian central nervous system. Low doses of IL-1β delivered exogenously to rat neocortical slices produced a reduction of the area of the column-like IOS evoked by orthodromic stimulation. The effect of IL-1β was reversible on washout and not mimicked by heat-inactivated IL-1β. These results suggest a possible modulatory role of IL-1β on synaptic transmission in the rat neocortex which is probably mediated through an activation of $GABA_A$ receptors.**

**Key words**: Interleukin; *In vitro*; Neocortex; Synaptic transmission; Videomicroscopy

## Introduction

The cytokine IL-1β has been suggested to be critically involved in both peripheral immune stimulation and in the centrally mediated effects that characterize the response to systemic infection, injury and inflammation.[1] It is considered to be a basic mediator of intercellular communication both within the immune system and between the immune system and other organ systems. This cytokine is produced predominantly by activated macrophages, although other cell types such as monocytes, lymphocytes, vascular cells and brain cells can also secrete it. The activation of the immune system is usually accompanied by a series of symptoms such as fever, neuroendocrine alterations and behavioral changes which can include enhanced sleep duration, reduced locomotory activity and anorexia.[7] In addition, inflammatory diseases can lead to memory impairment and affective disturbances.[3]

Central and peripheral administration of IL-1β has been reported to have a direct influence on brain function, producing changes in neurotransmission in various brain areas, which may underly its behavioural, endocrine and metabolic effects. For example, local injection of IL-1β into hippocampus and hypothalamus increases the release of serotonin, dopamine and noradrenaline in the hypothalamus while the acethylcholine level in the hippocampus is decreased.[4,5] Electrophysiological studies have shown that IL-1β enhances synaptic inhibition in hippocampal CA1 neurons,[6] an effect believed to be mediated via a change in GABAergic transmission. This agrees with the observation that IL-1β augments $GABA_A$-mediated chloride influx into synaptoneurosomes and increases chloride permeability in cortical neurons.[7] In addition IL-1β elevates the threshold for pentylenetetrazol-induced seizures in rats[7] and has anxiolytic effects[8] similar to those of $GABA_A$ergic substances. IL-1β alters sleep structure and duration,[9] which is essentially controlled by $GABA_A$ergic pathways. There is also evidence that IL-1β affects synaptic plasticity,[10] causing a clear inhibition of LTP at low doses, thereby providing a possible explanation for the depressive effects of the cytokine on cognition and behavioural activity. In view of this modulatory action of the cytokine, we investigated the effect of IL-1β on the spread of excitation in rat neocortical slices using infrared-darkfield videomicroscopy.[11,12] This novel technique allowed us to visualize directly the effect of IL-1β on cortical neurons.

## Materials and Methods

Infrared-darkfield videomicroscopy allows the visualization of neuronal excitation in brain slices without

the use of voltage-sensitive dyes. This is achieved by means of a combination of darkfield and infrared videomicroscopy techniques to visualize the intrinsic optical signal (IOS) as an indicator of the spread of neuronal excitation and of synaptic transmission. Rat neocortical slices of somatosensory cortex (400 μm) were prepared from male Sprague–Dawley rats (30–40 days old) using a vibratome. The slices were stored until needed at room temperature oxygenated in Krebs-Ringer solution containing (mM): NaCl 124.25, KCl 5, $NaH_2PO_4$ 1.25, $CaCl_2$ 2.5, $MgSO_4$ 1.3, $NaHCO_3$ 26 and glucose 10, equilibrated with 95% $O_2$ and 5% $CO_2$. to pH 7.4. They were placed in a slice chamber maintained at 32°C and superfused with Krebs-Ringer solution. Slices were illuminated with a condensor modified for darkfield microscopy and examined with an inverted microscope (Axiovert, Zeiss) with a low power 2.5× objective. The darkfield images were projected on the target of an image tube of an infrared sensitive camera (C2400-07, Hamamatsu) and five times analogue contrast enhancement was applied using the camera control unit (C 2400 Hamamatsu). Repetitive orthodromic stimulation (50 Hz trains of pulses of 0.2 ms for 2 s; intensity 1–4 V; stimulation site border between lamina 6 and white matter; concentric bipolar electrode) induced a column-like pattern of increased brightness in the neocortical slice. To visualize this signal, an averaged darkfield image was digitized and stored and subsequently subtracted in real time from the incoming images during tetanic stimulation of the slice. The resulting difference image was smoothed with a digital 3 × 3 filter and the rolling average mode of the system (DVS 1000, Hamamatsu), the contrast was digitally enhanced (64×) and the image was displayed in false colours and recorded on a S-VHS-videorecorder. The experiments were analysed off-line (image analysis software: Optima, Breukmann). Measurements were taken at the peak of the optical signal (3 s after termination of stimulation). The spread of neuronal excitation was quantified by measuring the area of the image for which pixel intensities were higher than half of the maximum signal. For this purpose the pixel intensity was measured in the center of the IOS and the threshold for the area measured was set to half of the maximum intensity value. The area covered by the IOS before substance application was set to 100% and subsequent measurements were expressed as a percentage of this control value. This parameter quantifies the spread of neuronal excitation and represents an even more sensitive indicator for pharmacological effects than changes in light transmittance. Data were analysed for statistical differences using non-parametric Wilcoxon signed rank test. Recombinant human IL-1β (specific activity $1 \times 10^9$ units/mg; Immunex Research, Washington, USA) was made up in aliquots of Ringer, which when diluted gave the final concentrations indicated in the text. Some experiments were performed with heat-inactivated IL-1β, incubated for 30 min at 100°C. After a control period of 20 min, IL-1β was added for 30–60 min to 24 slices, followed by a washout period of 60–90 min.

## Results

In control conditions, tetanic stimulation of white matter or layer VI induced a column-like spread of

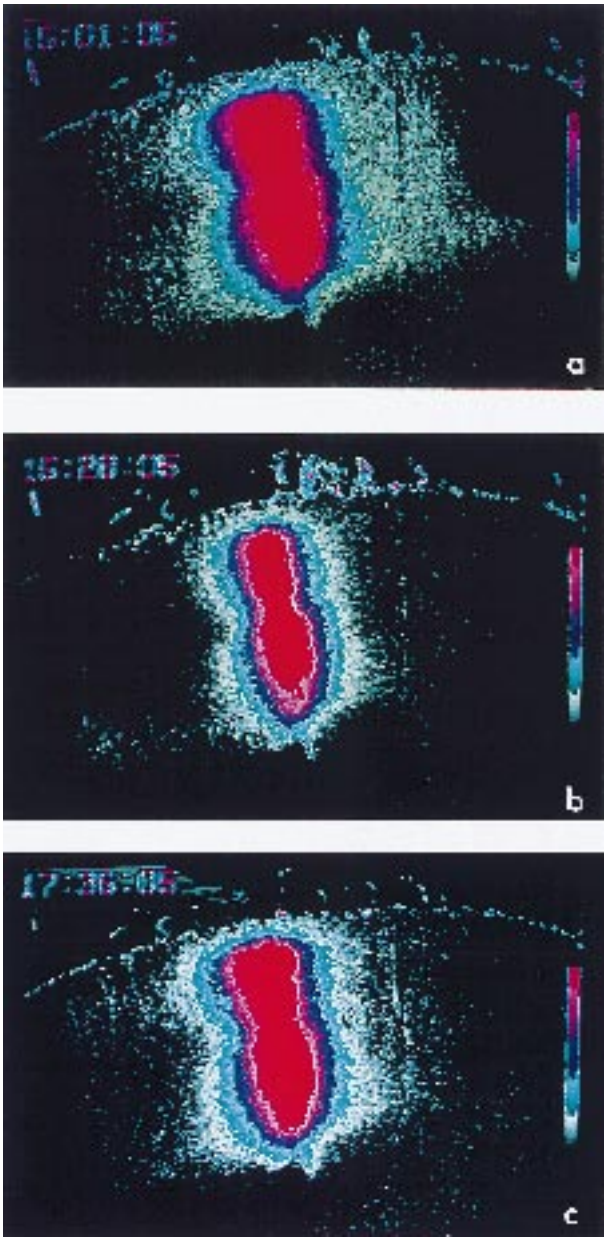


FIG. 1. Effect of IL-1β on the IOS. Tetanic stimulation (2.5 V, 50 Hz, 2 s) induces an area of increased brightness in the neocortical slice, here colour-coded in red. (**A**) control, (**B**) IOS 15 min after beginning of bath application of IL-1β 10 ng/ml, (**C**) recovery after 90 min of washout.

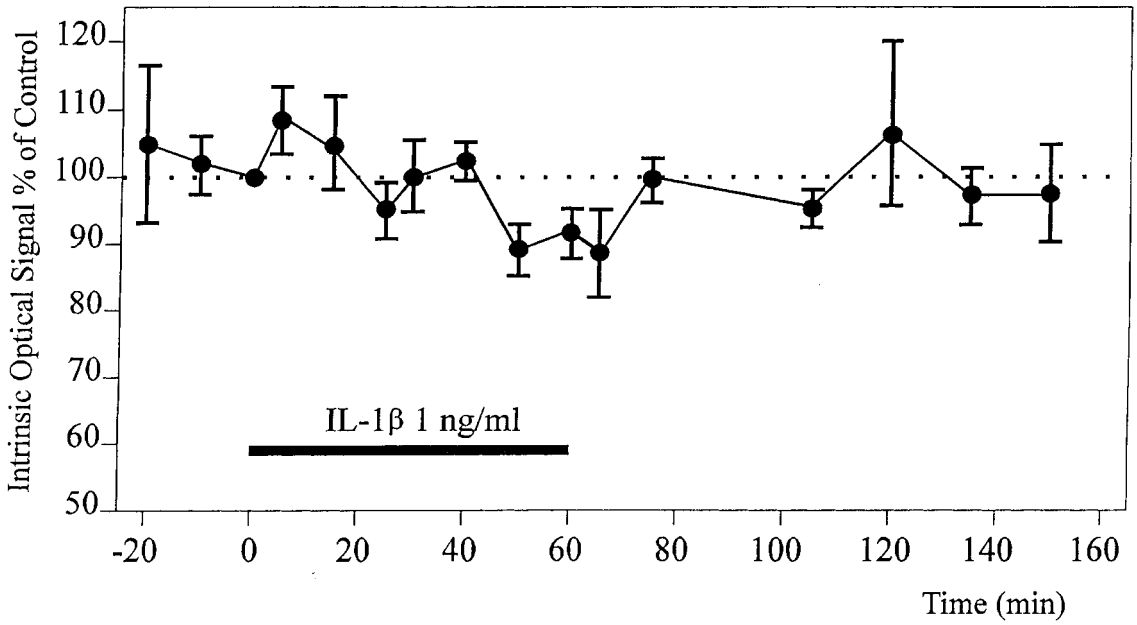


FIG. 2. Time course of the effect of IL-1β on the IOS. The IOS at t = 0 is taken as 100%, points and bars represent mean ± s.e.m. of measurements in eight different slices. Bath application of IL-1β (1 ng/ml) induced a reversible reduction of the IOS to 88% of control after 50 min perfusion ($p < 0.04$, Wilcoxon signed-rank test).

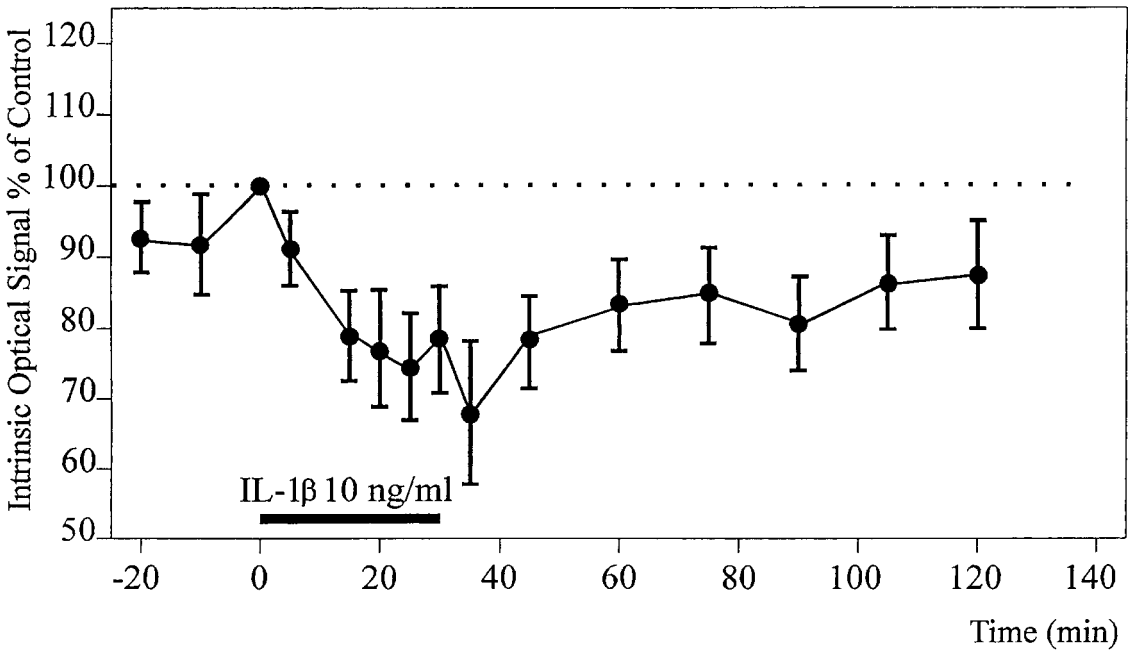


FIG. 3. Time course of the effect of IL-1β on the IOS. The IOS at t = 0 is taken as 100%, points and bars represent mean ± s.e.m. of measurements in eight different slices. Bath application of IL-1β (10 ng/ml) for 30 min decreased the IOS to 67% of control value ($p < 0.03$, Wilcoxon signed-rank test). This reduction was partially reversible after 90 min of washout.

increased brightness (see Fig. 1). The intrinsic optical signal (IOS) reached its maximum amplitude 3 ± 0.3 s after the end of the stimulation and decreased to baseline levels in 30–60 s. The IOS often exhibited a clearly discernable waist in the region of lamina IV. The IOS can be taken as measure of the electrical activity of neurons: a correlation between field potentials and the IOS has been shown previously.[13] The effect of IL-1β on the IOS was investigated by the application of three different concentrations of the substance. First we studied the action of IL-1β at a dose of 10 ng/ml. After recording the control response (Fig. 1a) the cytokine was applied to the bath for 30 min, inducing a reduction of the IOS

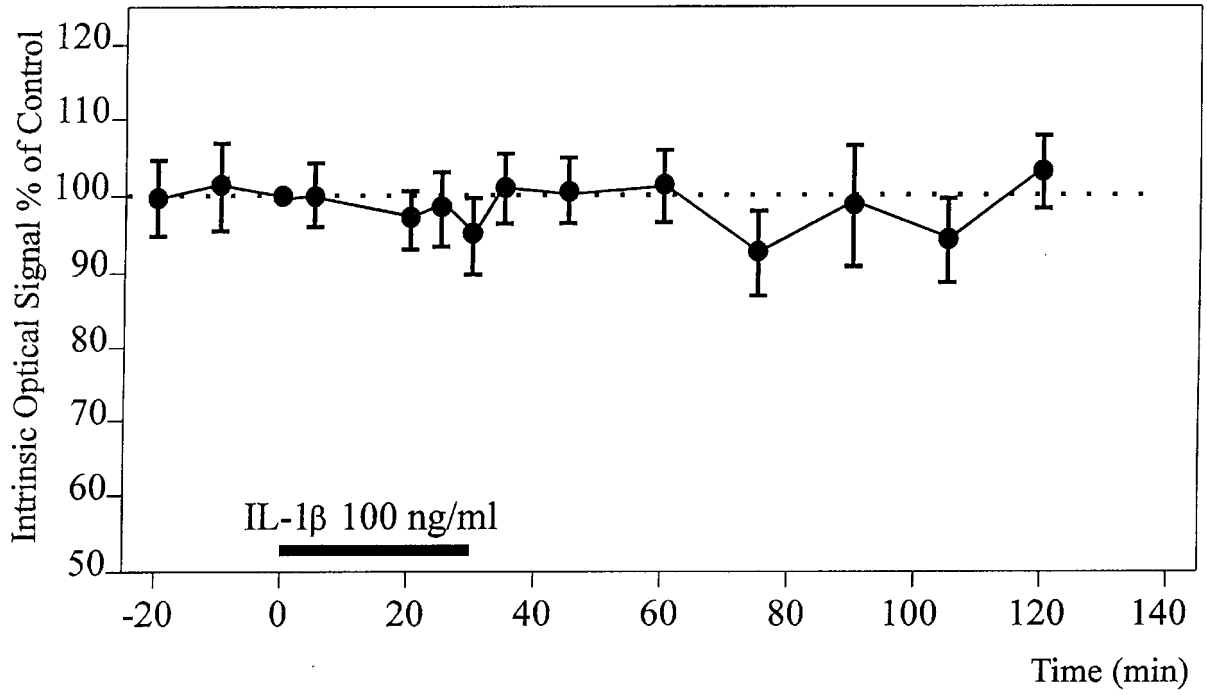


FIG. 4. Time course of the effect of IL-1β on the IOS. The IOS at t = 0 is taken as 100%, points and bars represent mean ± s.e.m. of measurements in eight different slices. Higher concentrations of IL-1β (100 ng/ml) did not apparently affect the IOS.

(Fig. 1b) that was detectable after 15 min of perfusion and reached the maximum after 30 min. This effect was partially reversible after 90 min of washout (Fig. 1c). At this concentration IL-1β exerted an inhibitory effect on the spread of excitation (Wilcoxon signed rank test $p < 0.03$; Fig. 3). Bath application of 1 ng/ml IL-1β for 60 min also significantly reduced the spread of excitation. This effect was observed after 50 min of perfusion and was followed by a recovery after 20 min of washout (Fig. 2). In another series of experiments IL-1β was applied for 30 min at a concentration of 100 ng/ml. At this concentration no significant effect on the IOS was observed (Fig. 4). No change in the spread of excitation was observed when heat-inactivated IL-1β was applied for 30 min under similar conditions ($n = 5$).

## Discussion

If a substance is able to modulate the extent of the spatial spread of neuronal excitation it will profoundly affect the functioning of the brain. As infrared-darkfield videomicroscopy allows one to investigate the spatial spread of excitation, we used this method to investigate the action of IL-1β. Infrared-darkfield videomicroscopy can be regarded as a kind of spatially resolved field potential recording, which allows a rapid acquisition of data. A drawback of the method is that only relatively strong changes in the strength of synaptic inhibition are reflected by changes in the IOS.[13] As IL-1β (2.5 ng/ml) has been shown to increase synaptic inhibition in the hippocampal $CA_1$ region by about 2- to 3-fold, we hoped to see a reduction of the IOS by IL-1β.

Low doses of IL-1β reduced in fact the spread of excitation elicited in response to tetanic stimulation of neocortical slices. It is unlikely that this effect arises from some non-specific action of IL-1β since it was not induced by heat-inactivated IL-1β. The reduction of the IOS observed is probably due to augmentation of GABAergic inhibition, as the effect of IL-1β on the IOS resembles that evoked by the $GABA_A$ agonist muscimol and the neuroactive steroid 5α-THDOC[13] which enhance GABAergic transmission[14] by a direct action on $GABA_A$ receptor-mediated $Cl^-$ currents. Furthermore, the IOS is markedly increased by the application of the $GABA_A$ receptor antagonist bicuculline.[11,13] The finding that the effect of IL-1β was only partially reversible (Fig. 3) could be due to an incomplete wash out of the polypeptide in the slice, as other studies do not suggest genomic effects.[7]

The explanation of the observed effect as an augmentation of GABAergic inhibition is further supported by the results of other studies, using different methods. As mentioned above, synaptic inhibition measured with intracellular recording in the $CA_1$ region of the hippocampus is markedly

enhanced after exposure to IL-1β for 1 h. IL-1β reduces the number of spikes in response to synaptic stimulation via this augmentation of inhibition.[6] Because synaptic inhibition is mainly mediated by $GABA_A$, IL-1β should act through an enhancement of this system. This agrees with the results of Miller *et al.*,[7] who found that IL-1β augments $GABA_A$-dependent chloride uptake in murine synaptoneurosomes. Electrophysiological studies in chick primary cortical neurons showed also that IL-1β enhances $GABA_A$-mediated increase in chloride permeability.

Additional, putatively $GABA_A$ergic effects of IL-1β, are the elevation of the threshold dose for pentylenetetrazol-induced seizures in mice[7] and its anxiolytic properties in rats.[8] Moreover, IL-1β administration suppresses spatial learning in rats in the Morris maze test[15] and reduces schedule-controlled behaviour, social explorative behaviour and spontaneous locomotor activity.[16] In view of the pronounced influence of IL-1β on the spread of cortical excitation it is conceivable that an IL-1β-mediated $GABA_A$ergic potentiation underlies this behavioural suppression.

On the other hand, higher doses (100 ng/ml) of IL-1β do not exert an inhibitory effect on the spread of excitation. This could be explained by a GABAmimetic effect of IL-1β at higher doses: high doses of IL-1β can elicit epileptogenic activity, particularly when injected into the hippocampus.[17] One possible mechanism of such an effect could be an inhibition of inhibitory interneurons.[18]

The present results suggest that IL-1β affects neuronal excitability in neocortical neurons by modulating GABAergic synaptic transmission mediated via the activation of $GABA_A$ receptors.

## References


1. Rothwell NJ and Hopkins SJ. *Trends Neurosci* **18**, 130–136 (1995).
2. Rothwell NJ and Dantzer R. *Interleukin-1 in the* Brain. Oxford: Pergamon Press, 1992.
3. Minden SL and Schiffer RB. *Arch Neurol* **47**, 98–104 (1990).
4. Shintani F, Kanba S, Nakaki T *et al. J Neurosci* **13**, 3574–3581 (1993).
5. Rada P, Mark GP, Yitek MP *et al. Brain Res* **550**, 287–290 (1991).
6. Zeise ML, Madamba S and Siggins GR. *Regulatory Peptides* **39**, 1–7 (1992).
7. Miller L, Galpern WR, Dunlap K *et al. Mol Pharmacol* **39**, 105–108 (1991).
8. Montkowski A, Schöbitz B, Reul JMH and Landgraf R. 3rd International Congress of Neuroendocrinology Budapest Hungary, P4.44, 93 (1994).
9. Krueger JM and Obal F Jr. *FASEB J.* **7**, 645–652 (1993).
10. Patterson PH and Nawa H. *Cell* **72**/*Neuron* **10**(Suppl), 123–137 (1993).
11. Dodt HU, Höllander H and Zieglgänsberger W. *Soc Neurosci Abstr* **19**, 272 (1993).
12. Dodt HU and Zieglgänsberger W. *Trends Neurosci* **17**, 453–458 (1994).
13. Dodt HU, D'Arcangelo G, Pestel E and Zieglgänsberger W. *NeuroReport* **7**, 1553–1558 (1996).
14. Rupprecht R, Reul JM, Trapp T *et al. Neuron* **11**, 523–530 (1993).
15. Oitzl MS, van Oers H, Schöbitz B and de Kloet ER. *Brain Res* **613**, 160–163 (1993).
16. Kent S, Bluthe RM, Dantzer R *et al. Proc Natl Acad Sci USA* **89**, 9117–9120 (1992).
17. Nisticò G and De Sarro G. *Ann NY Acad Sci* **261**, 119–131 (1991).
18. Zieglgänsberger W, French ED, Siggins GR and Bloom FE. *Science* **205**, 415–417 (1979).


**Received 4 March 1997; accepted 18 March 1997**

**General Summary**
Interleukins are involved in the activation of the immune system during infection in mammals, including man. Behavioral changes such as drowsiness which occur during sickness are also mediated by the action of interleukins. To elucidate the effect of interleukins on the brain, we investigated the effect of interleukin IL-1β on neuronal excitation in rat neocortical slices. Using the novel technique of infrared-darkfield videomicroscopy, we measured changes of the spread of neuronal excitation induced by the application of IL-1β. At a concentration of 10 ng/ml, IL-1β reduced the spread of excitation, probably mediated by an increase of synaptic inhibition. The changes in the behavior of the whole animal may directly reflect the effect of IL-1β on neuronal excitation in the brain.