

Magnetic stimulation induces neuronal c-fos via tetrodotoxin-sensitive sodium channels in organotypic cortex brain slices of the rat

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Abstract

Repetitive transcranial magnetic stimulation is a novel non-invasive method with antidepressant properties, where electromagnetic fields are applied via an electrode. The aim of the present study was to investigate in an *in vitro* model if magnetic stimulation may activate the transcription factor c-fos. Organotypic brain slices of the parietal cortex were cultured for 2 weeks and then treated with a magnetic stimulator. Immunohistochemistry was used to detect c-fos like immunoreactivity. We show that magnetic stimulation (1 Hz, 10 min, 75% machine output/magstim 200 rapid stimulator) transiently enhanced c-fos 3–6 h after stimulation. Co-localization experiments revealed that c-fos was expressed in neurons but not astroglia. The activation of c-fos by magnetic stimulation was inhibited by the sodium-channel blocker tetrodotoxin (TTX) (10 μ M). It is concluded that magnetic stimulation induces neuronal c-fos via TTX-sensitive sodium channels in organotypic cortex slices. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

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Repetitive transcranial magnetic stimulation (rTMS) is a new non-invasive therapeutic tool in psychiatry in which a pulsed electric current is applied to the scalp via coil. The electric current generates a magnetic field which passes the skull to depolarize the subjacent neuronal tissue [2,13]. rTMS has shown significant antidepressant effects in humans [5].

Among genes, which have been shown to be induced very rapidly are immediate early genes, including those of the fos-jun family [3,11]. C-fos is a rapid indicator for extensive activation of neurons, thus this transcription factor is a suitable and useful marker of fast neuronal activation. Strong central stressors, such as seizures induced by kainic acid [12], kindling [4], electrical stimulation [14] or ischemia [12] activate the c-fos gene. Mild physiological stressors such as changes in the environment [6] or light [1,15] also may induce c-fos expression in different brain areas. We recently demonstrated that chronic rTMS for 14 days markedly enhanced c-fos mRNA and protein expression in the parietal cortex of the rat *in vivo* [7]. The aim of the study was to observe the effects of acute magnetic stimulation on

the expression of the immediate early gene c-fos in an organotypic cortex brain slice model.

Organotypic cultures were established as described by our group [17,18]. Briefly, the parietal cortex of postnatal-day-10 rats was dissected under aseptic conditions, 400 μ m slices were cut with a tissue chopper, and the slices placed on a Millicell-CM 0.4 μ m (Millipore, Vienna, Austria) culture plate (6 slices per membrane). Slices were cultured in petri dishes at 37°C and 5% CO₂ with 1.2 ml/petri dish. After 2 weeks slices ($n = 17$ –32 slices per treatment group) were subjected to magnetic stimulation. The magnetic stimulation was performed with a Magstim 200 super-rapid stimulator (Magstim Company, Whitland, UK) using either 1 Hz (10 min, 75% machine output), 20 Hz (10 s, 75% machine output) or 50 Hz (5 s, 50% machine output). The cathode of the figure-8-coil (70 mm) was placed directly below (0.6 cm) the membrane insert containing the six slices. After 0, 1.5, 3, 6, 14 or 24 h slices were fixed with 4% paraformaldehyde and processed for immunohistochemistry. Incubation with the sodium channel blocker tetrodotoxin (TTX) (10 μ M) was performed 30 min before magnetic stimulation. TTX was present during the magnetic stimulation and for an additional 3 h after the stimulation. Non-stimulated control slices were incubated for 3.5 h with 10 μ M TTX. Immunohistochemistry using the avidin-biotin technique was performed as described previously [7,17],

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Table 1

Quantitative analysis of magnetic stimulation in 2-week-old organotypic brain slices of the parietal cortex, analyzed 3 h after treatment^a

Treatment	c-fos nuclei/0.197 mm ²	n	P
Control	85 ± 23	17	-
MST	432 ± 49	32	***
TTX	56 ± 23	20	n.s.
TTX + MST	89 ± 29	25	n.s.

^a MST: magnetic stimulation with 1 Hz for 10 min and 75% output; TTX: 10 μM TTX. The number of analyzed slices is given (n). Statistical analysis was performed by one way ANOVA with a subsequent Fisher PLSD post-hoc test and compared to the control slices (****P* < 0.001; n.s. not significant).

using a polyclonal anti c-fos antibody (1:1000, Santa Cruz Biotechnology). For the co-localization experiments, slices were first stained for c-fos using the ABC method (the staining was enhanced with nickelchloride) and then incubated with primary antibodies against neuronal microtubuli-associated protein-2 (MAP-2; Chemicon, 1:400) or astroglial fibrillary acidic protein (GFAP; Roche, 1:500) and then with FITC labeled secondary antibodies (Sigma, 1:50).

C-fos expression was analyzed by using a computer-assisted image analysis system (Image pro Plus Software, connected to an Olympus BX60 microscope via a Sony video camera). The number of c-fos positive nuclei was counted in a calibrated 0.197 mm² square under the microscope using a 20× magnification. Multistatistical analysis was obtained by one way ANOVA, followed by a subsequent Fisher post-hoc test by comparing controls against the respective treatments, where *P* < 0.05 represents significance.

Immunohistochemistry of 2-week-old untreated control slices revealed a low number of c-fos positive nuclei per field (Table 1; Fig. 2A). When slices were treated with 1 Hz for 10 min (75% output) the number of c-fos immunopositive nuclei was significantly enhanced 3 h after magnetic treatment (Table 1; Fig. 2B) but transiently declined to control values. (Fig. 1). Magnetic stimulation of 2-week-old slices with 20 Hz for 10 s (75% output) only slightly enhanced the c-fos staining 3 and 6 h after stimulation (Fig. 1). Slices stimulated with 50 Hz for 5 s (50% output) did not show an increase in c-fos like immunoreactivity (Fig. 1). When 2-week-old slices were incubated with 10 μM TTX for 3.5 h the number of c-fos positive cells did not change when compared to controls (Table 1). When slices were incubated with 10 μM TTX and subjected to magnetic stimulation (1 Hz, 10 min) the c-fos activation was totally inhibited (Table 1). The MAP-2 staining in the slices was weak, but clearly neurons and nerve fibers very visible (Fig. 2C). Many MAP-2 positive neurons co-localized with the c-fos positive nuclei (Fig. 2C). A strong GFAP positive staining was found in the slices (Fig. 2D). The c-fos positive nuclei did not co-localize with GFAP-positive astroglia (Fig. 2D).

The organotypic slice model has been thoroughly characterized in our research group and has proved to be an effective in vitro system [8,16–18]. In comparison with dissociated nerve cell cultures, the organotypic slice model resembles more closely the in vivo condition of a 'high density cell system' and allows the survival of neurons, astroglia, oligodendrocytes and microglia. These individual cells are in close contact and do not lose density-dependent regulatory mechanisms and three-dimensional architecture. The present study shows that magnetic stimulation is able to activate neurons in the parietal cortex of brain slices via depolarization and subsequent influx of sodium. This activation was transient and could be blocked by the sodium-channel blocker TTX. Our data are consistent with a previous study using organotypic brain slices [16,18] and show nuclear localization of neuronal c-fos immunoreactivity as well as a transient activation of this transcription factor.

There is strong evidence that rTMS treatment affects cortical brain areas. Kole et al. [10] reported substantial increases in receptor binding in selected layers of the parietal cortex after acute rTMS treatment. A recent study has shown that an acute single rTMS train markedly enhanced c-fos mRNA in the cingulate and frontal cortex, however, the most pronounced increase in c-fos after acute rTMS was found in the paraventricular nucleus of the thalamus [9]. In a previous study we showed, that chronic rTMS treatment (20 Hz, daily for 14 days) activates c-fos mRNA and protein expression in a definite pattern in the parietal cortex in layers I-IV and layer VI of adult rats in vivo [7]. The present study extends our previous one [7] and demonstrates that acute magnetic stimulation is able to activate neuronal c-fos protein in organotypic brain slices of the parietal cortex.

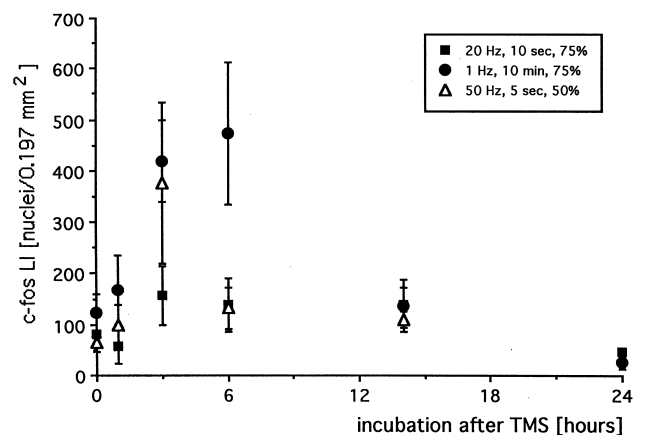


Fig. 1. Time course of c-fos expression after magnetic stimulation. Two-week-old cortex slices were subjected to magnetic stimulation (20 Hz, 10 s, 75% machine output/filled squares; 1 Hz, 10 min, 75% output/filled circles; 50 Hz, 5 s, 50% output/open triangles) and analyzed 0, 1.5, 3, 6, 14 and 24 h after treatment. The number of c-fos positive nuclei were counted under the microscope using an image analysis system. Values are expressed as average ± SEM c-fos like immunoreactive nuclei per 0.197 mm² field. At least 6 slices per time point were analyzed.

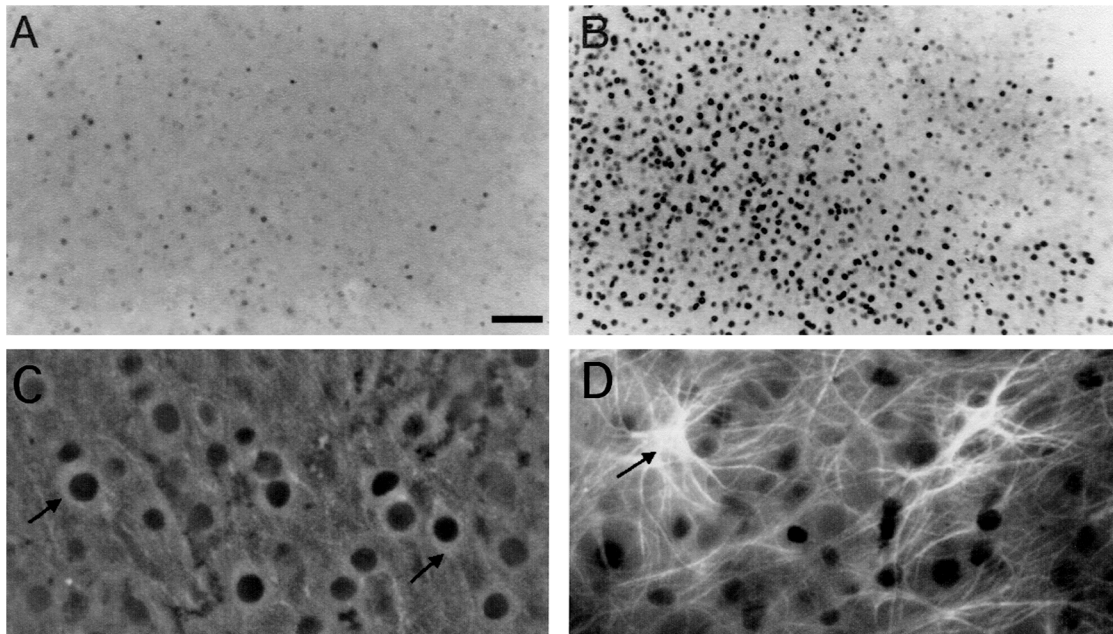


Fig. 2. Immunohistochemical staining of c-fos in slices of the parietal cortex. Two-week-old untreated slices were used as a control (A). When 2-week-old slices were subjected to magnetic stimulation (1 Hz, 10 min, 75% output) a marked increase in c-fos positive nuclei was observed (B). Co-localization experiments show that FITC labeled MAP-2 positive neurons (C, arrows) express c-fos protein. Staining for GFAP-positive astroglia was very prominent in slices (D, arrow), however, did not co-localize with c-fos positive nuclei. Scale bar = 100 μm (A,B), 22 μm (C), 30 μm (D).

Thus, this model is very easy and suitable to optimize the parameters of magnetic stimulation.

In conclusion, our data show that the organotypic brain slice model may offer a convenient method to optimize magnetic stimulation parameters. The activation of neuronal c-fos was induced by magnetic stimulation in a frequency-dependent pattern and may require sodium influx through TTX-sensitive sodium channels.

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