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# Time-dependent distribution and neuronal localization of c-fos protein in the rat hippocampus following 4-aminopyridine seizures

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**Abstract**

The immunohistochemical localization of c-fos protein in the CNS neurons was studied in a model of generalized epilepsy induced by the intraperitoneal injection of 4-aminopyridine to adult Wistar rats. This specific blocker of the voltage-dependent potassium channels proved to be suitable for use in the investigation of epileptogenesis. Following the treatment of adult rats with 5 mg kg of 4-aminopyridine, the animals experienced generalized seizures. At the end of the experiment, the rats were briefly anesthetized and perfused with fixative. Frozen coronal plane sections were cut and processed for immunohistochemistry, using polyclonal c-fos antibody. The number and distribution of immunostained cell nuclei in the hippocampus were analyzed in detail with the help of a digital microscope camera and a morphometry program. The highest level of immunostaining was detected in most of the structures at 3 h, but the level had decreased to the control level by 5 h following 4-aminopyridine injection. In the dentate fascia, immunostaining was highest at 1 h and then decreased slowly until 5 h post-injection. The activated neuronal assemblies were analyzed with the aid of parvalbumin c-fos double immunostaining. These countings revealed the highest inhibitory interneuronal activation in every part of the hippocampus (including the dentate fascia) at 3 h post-injection. The results indicate that systemic 4-aminopyridine induces limbic seizures, which are probably initiated in the entorhinal cortex. 

*Keywords:* Aminopyridine; c-fos; Epilepsy; Hippocampus; Inhibition; Parvalbumin

## 1. Introduction

4-Aminopyridine (4-AP) is a convulsant that blocks some of the voltage-dependent neuronal potassium channels: the $K_A$-channel (or A-channel), which regulates the spike frequency in post-

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synaptic structures, and the $K_V$-channel (or delayed rectifier), which is involved in the repolarization phase of the action potential (Alexander and Peters, 2000), resulting in prolonged action potential duration. Therefore 4-AP increases the inflow of $Ca^{++}$ into the presynaptic axons (Thesleff, 1980). The drug also acts directly through the presynaptic voltage-sensitive $Ca^{++}$ channels, facilitating transmitter release (Rogawski and Barker, 1983). The increased presynaptic activity caused by 4-AP is reflected in the increased synaptic vesicle exocytosis at the ultrastructural level (Tokunaga et al., 1979). Additionally, 4-AP crosses the blood–brain barrier quickly and will be secreted into the cerebrospinal fluid and eliminated by the kidney (Lemeignan et al., 1984). In consequence of its fast action, the latency of the seizure is relatively short, and the convulsive activity probably extends to the whole forebrain (Mihály et al., 1990). 4-AP is used for seizure induction both in vivo (Pasantes-Morales et al., 1987; Szente and Baranyi, 1987; Mihály et al., 1990, 1997, 2000) and in vitro (Kuhnt et al., 1983; Brückner and Heinemann, 2000; Marinelli et al., 2000), and most of its actions are blocked by the standard antiepileptic drugs (Mihály et al., 1990; Brückner and Heinemann, 2000; Mihály et al., 2000). The excitatory properties of 4-AP and its derivatives have been examined in humans (Jones et al., 1983; Andreani et al., 2000). Recent studies from our laboratory indicated significant increases in regional cerebral blood flow (rCBF) in the dentate fascia, neocortex and diencephalon in mice following 4-AP injection (Mihály et al., 2000). However, no detailed systematic studies of the localization and spread of the convulsive activity have yet been performed.

The aim of the present study was to describe the distribution and the rate of appearance of the activated neurons in the hippocampus following 4-AP seizures, using the immunohistochemical detection of c-fos protein as a marker of neuronal activation (Willoughby et al., 1995; Herdegen and Leah, 1998). Several investigations have indicated the occurrence and role of inhibition in chronic epileptic phenomena (for a review, see Engel, 1996), and an increasing number of data point to the participation and importance of inhibitory neurons in the acute seizure process (Watts and Jefferys, 1993; Avoli, 1996; Morris et al., 1996; Mihály et al., 1997). We therefore used double immunolabeling to identify c-fos-protein-containing parvalbumin (PV)-positive interneurons in the hippocampus in order to collect data on the participation of inhibitory cells in the seizure process.

## 2. Methods

### *2.1. Animal handling and immunohistochemistry*

The experiments were performed on 15 male Wistar rats (180–200 g b.wt.). The 4-AP was dissolved in physiological saline (0.67 mg of 4-AP in 1 ml of solvent). Rats were lightly anesthetized with diethylether, and 5 mg/kg of 4-AP were injected intraperitoneally to 12 animals. This dose proved to be epileptogenic in previous pharmacological experiments (Mihály et al., 1990, 2000). The controls (three animals) received the solvent of 4-AP (0.9% NaCl in distilled water). Following the administration of 4-AP, every animal produced generalized tonic-clonic convulsions. The animals were anesthetized with diethylether and perfused through the heart with 500 ml of cold 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) 1, 3 and 5 h after the injection of 4-AP (four animals from each, plus one control). The brain was dissected and postfixed for 1 h at room temperature. Following postfixation, the brains were cryoprotected overnight in 30% sucrose in 0.1 M phosphate buffer. Frozen serial coronal plane sections were cut at a thickness of 24 μm, and every sixth section was processed for immunohistochemistry by one or other of two different methods.

Single primary antibody (polyclonal c-fos antibody raised in rabbit, 1:1000; Santa Cruz Biotechnology, CA), followed by secondary antibody (donkey anti-rabbit IgG, 1:40; Jackson ImmunoResearch, PA), detected by the peroxidase-anti-peroxidase (PAP; Jackson ImmunoResearch, PA) method (PAP dilution 1:1000), and the peroxidase reaction was developed with nickel-intensified 3′3′-diaminobenzidine tetrahydrochloride (Ni-DAB; Sigma, St. Louis, MO) as chromogen

(nine animals treated with 4-AP and three controls were used in these experiments).

Primary antibody cocktails (two primary antibodies: mouse anti-PV, 1:100 000; rabbit anti-c-fos, 1:1000) followed by biotinylated anti-mouse IgG (1:600; Vector Laboratories, CA) and plain donkey anti-rabbit IgG (1:40) detected with streptavidin-peroxidase (1:2000; Vector Laboratories, CA) and PAP (1:1000), respectively. The streptavidin-peroxidase was developed by using plain 3′3′-diaminobenzidine tetrahydrochloride (DAB), while the PAP was developed with Ni-DAB (three animals treated with 4-AP were used in these experiments). The anti-parvalbumin serum was purchased from Sigma (St. Louis, MO).

### 2.2. Image analysis techniques

C-fos immunoreactivity was observed in the cell nuclei. Areas were selected from regions CA 1, CA 2 and CA 3 of the Ammon's horn and from the hilus of the dentate fascia, and the immunoreactive cell nuclei displaying grayish black staining were counted with the aid of a Nikon Eclipse 600 microscope, equipped with a Polaroid DMC digital camera (1600 × 1200 dpi in 8 bits) with 40 × objective magnification, using the Image Pro Plus 4 morphometry program (Media Cybernetics, Silver Spring, MD). Following background subtraction, the threshold was adjusted so that pale- and deep-stained nuclei could be equally recognized by the counting program. The area of interest was the rectangular image-capturing field of the camera. The rectangular area of this field is 0.05 $mm^2$. This value was used when the cell numbers were normalized to 1 $mm^2$. The brains of three animals in each experimental group (1, 3 and 5 h following 4-AP injections: nine animals) were investigated. Counting in a particular brain structure was generally performed on five histological sections from every animal (i.e. 15 samples from each time-interval group). The numbers of samples are indicated in the diagrams. The controls comprised one batch: one animal from each time group (three animals). Counting was not carried out on the control brains, because of the lack of c-fos staining. The double immunostaining was investigated in one animal series: one animal from every time group (three animals). On double-stained sections, the neurons containing *c-fos plus PV* or *PV only* were counted separately, the area of the hippocampus was then measured, and the cell counts were related to the hippocampal area in $mm^2$. The area of interest in this case was determined manually, by labeling the outlines of the hippocampal formation (the fimbria was not included). First, the whole hippocampus was measured, followed by the area of the dentate fascia: the outlines of the dentate fascia were drawn on the image by following the contours of the lower blade and the hippocampal fissure, and connecting the two tips of the granular layers with a straight line. This area is slightly larger than the anatomical area, because it includes not only the hilar region, but also zone 1 of the pyramidal cell layer (Amaral, 1978). However, this segment of the pyramidal layer was not visible on the immunostained sections, and this simplification was therefore chosen in order to have consistent area measurements. The area of the Ammon's horn was calculated from the two measurements. The area measurements were performed with the Image Pro Plus 4 software. Immunoreactive cells were counted by three investigators (R.Sz., B.K-P. and A.M.) in the microscope, manually, at a 20 × objective magnification. The cell counts were normalized to a 1 $mm^2$ tissue area. The numbers of sections measured are indicated on the diagrams. PV staining was not applied to the control brains, and therefore, no control PV counts were made. The PV neurons were counted in the epileptic brains, and the counts obtained in the 1, 3 and 5 h samples were compared.

The cell counts were analyzed by ANOVA (post-hoc test: Bonferroni method). The statistical analysis was performed with the SPSS 9.0 computer program.

## 3. Results

The administration of 4-AP caused characteristic symptoms: increased exploratory activity, followed by tremor of the vibrissal muscles, shivering, and clonus of forelimbs. The frequency of shivering increased so that the animal was

unable to move. At the height of the shivering, generalized tonic-clonic seizure (GS) developed and lasted for 45–60 s. A quiet postictal period followed (1–8 min), and then the animals displayed GS again. The first GS appeared between 15 and 25 min (average: 18 min) after the exposure to 4-AP. The animals in the present experiments experienced two or three GS, and the period of symptoms lasted for 60–90 min. The symptoms were similar to that described in our previous pharmacological experiments, although the latency of the GS was shorter in the present experiments than previously (Mihály et al., 1990).

### *3.1. Localization of c-fos immunoreactivity*

In every animal, c-fos-immunoreactive (c-fosIR) cell nuclei were seen in the entorhinal and piriform cortices, the hippocampus, the lateral septum, the thalamus, the hypothalamus and every area of the neocortex. Some scattered nuclei were visible in the dorsal striatum, the midline nuclei of the thalamus and hypothalamus. The ventral striatum was regularly devoid of immunostaining. C-fos staining was absent from these regions of the control animals, though scattered, pale c-fos staining was detected in the piriform cortex in two control rats. No c-fosIR structures were seen in the hippocampal formation in the controls. In the present study, we analyzed the c-fosIR structures in the hippocampal formation.

The density of c-fosIR nuclei at 1 h post-injection was highest in the granule cell layer of the dentate fascia. The granule cell layer stood out with its very strong immunoreactivity because of the high packing density of the c-fosIR granule cells (Fig. 1A). The intensity of the immunostaining was so strong that counting of the cell nuclei was not possible at 1 h. Accordingly, we did not attempt to count the number of activated granule cells; instead, the changes are illustrated in microphotographs (Fig. 1A–C). The dentate c-fos expression in the granule cell layer decreased gradually between 1 and 3 h and was strongly reduced by 5 h following the injection (Fig. 1B and C). Scattered c-fosIR nuclei were seen in the molecular layer of the dentate fascia. Although we did not count the cell nuclei in the molecular layer, it seemed that the hilus contained many more activated cells. We therefore counted the c-fosIR nuclei in the hilus of the dentate fascia (Fig. 2). Similarly to the c-fos expression in the granule cell layer, the number of c-fosIR cell nuclei in this area was highest at 1 h, and subsequently gradually decreased (Fig. 2). The differences between the cell counts were significant at every time interval (Fig. 2).

One hour after the injection, the Ammon's horn apparently contained few c-fosIR structures (Fig. 1A). Most of the stained nuclei were observed in the pyramidal cell layer of regions CA 1, CA 2 and CA 3: regions CA 2 and CA 3 regularly contained fewer nuclei than CA 1. Apart from the pyramidal cell layer, the stratum oriens, radiatum and lacunosum-moleculare contained scattered (few) c-fosIR cells. The number and staining intensity were increased at 3 h post-injection (Fig. 1B). The cell counts for sectors CA 1, CA 2 and CA 3 of the Ammon's horn reflected these observations: the number of c-fosIR nuclei in these areas increased between 1 and 3 h following 4-AP application and was significantly decreased by 5 h (Fig. 2). However, significant differences could be demonstrated only between the 1- and 5 h and between the 3- and 5 h values.

### *3.2. Parvalbumin localization in the rat hippocampus*

The PV-immunoreactive cell bodies were found mainly in the pyramidal layer of the Ammon's horn (some cells were located in the strata oriens and radiatum, too), and in the hilar region of the dentate fascia. The dendrites of PV neurons were located mainly in the stratum radiatum and lacunosum-moleculare. A dense PV-stained fiber plexus was observed in the stratum pyramidale. In the dentate fascia, most of the cell bodies were located in the hilar region. Some scattered cells were found in the granule cell layer and in the molecular layer. Most of the cells possessed long, thick, strongly stained dendrites. Double-stained neuronal somata were clearly visible; c-fos nuclear staining was displayed in blackish blue, whilst PV immunoreactivity was pale or deep brown (Fig. 3A–D).

The number of c-fosIR PV neurons increased between 1 and 3 h, but then displayed a sharp decrease at 5 h post-injection. This feature of PV neuron activation was the same in the Ammon's horn and the dentate fascia, and the changes were significant for each time interval (Fig. 4). When we investigated the numbers of activated PV cells related to the overall area of the hippocampal formation (Ammon's horn plus dentate fascia), the differences between the 1- and 5 h counts were not significant. However, significant differences were found between the 1- and 3 h counts and

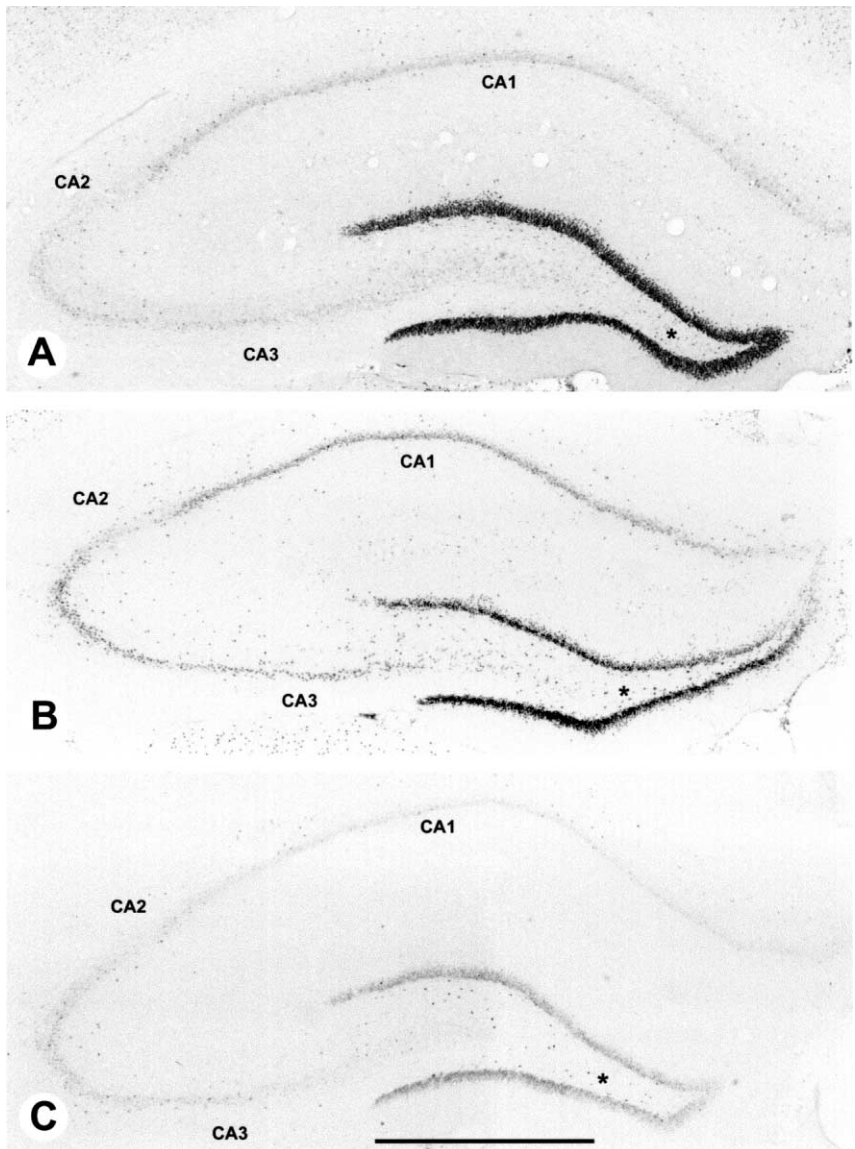


Fig. 1. Low magnification pictures of the distribution of c-fosIR cell nuclei in the hippocampus at 1 h (A), 3 h (B) and 5 h (C) post-injection. The sectors of the Ammon's horn (CA 1, CA 2, CA 3) are indicated. An asterisk denotes the hilus of the dentate fascia. Note the strong immunoreactivity of the granule cell layer at 1 h, and the decrease of the immunostaining at 3 and 5 h. The immunoreactivity in the pyramidal layer of sectors CA 1, CA 2 and CA 3 is strongest at 3 h. Bar: 1 mm.

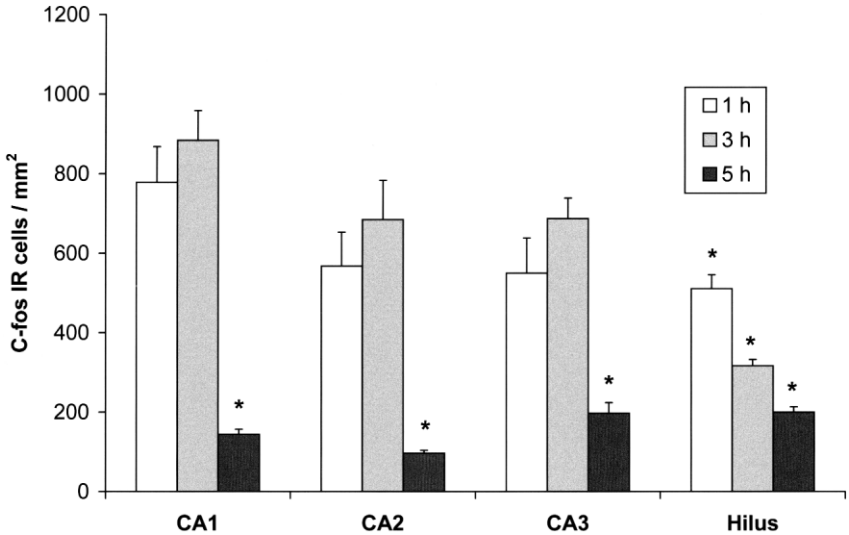


Fig. 2. Results of cell nucleus counts in the Ammon's horn (CA 1, CA 2, CA 3) and in the hilus (HILUS) of the dentate fascia ($n = 15$ in every case). Significant differences are shown by asterisks ($P = 0.001$ in every case, except for the difference between the 3- and 5 h measurements of the hilus, where $P = 0.003$). The standard error of the mean is displayed on the top of the columns.

between the 3- and 5 h counts. The total number of activated PV neurons was highest at 3 h, the 5 h count being very similar to the 1 h count (Fig. 4).

If every PV-stained cell (*c-fos plus PV and PV only*) is taken into consideration, there is a slight increase in their numbers between 1 and 3 h, and a decrease thereafter — this decrease between the 3- and 5 h counts proved to be significant in the hippocampal formation (Fig. 5).

## 4. Discussion

Our results are in accord with literature data as concerns the appearance of c-fos in the convulsing brain (Dragunow et al., 1989; Morgan and Curran, 1991). C-fos belongs to the inducible transcription factors (ITFs; Herdegen and Leah, 1998), the activation of which through second messengers, protein kinases and other transcription factors leads to the accumulation of ITF mRNA and the translocation of the synthesized ITF proteins into the cell nucleus (Morgan and Curran, 1991; Herdegen and Leah, 1998). The ITF c-fos, a 55–62 kDa phosphorylated protein, is normally not expressed in neurons, although there is a low level of c-fos in some structures of the adult forebrain, but not in the hippocampus (Herdegen and Leah, 1998). Seizure activity induced by chemical convulsants (amongst other experimental circumstances) leads to a rapid, massive and transient induction of c-fos mRNA and protein in several brain regions (Gass et al., 1992; Willoughby et al., 1995; Zimmer et al., 1997). The postsynaptic c-fos mRNA expression correlates well with the presynaptic release of excitatory neurotransmitters (Labiner et al., 1993), and the detection of the c-fos protein is therefore suitable for the histological mapping of neuronal hyperactivity (Morgan and Curran, 1991; Labiner et al., 1993; Mihály et al., 1997, 1998). Our present experiments provide evidence that blockade of the 4-AP sensitive neuronal potassium channels leads to activation of the c-fos gene in the hippocampal formation. Although we used immunohistochemistry to detect the c-fos protein, the serum that we applied was characterized on rat-brain homogenates by means of Western blotting in our previous experiments, and we found a single protein band at 62 kDa (Mihály et al., 1997), indicating the high specificity of the antibody.

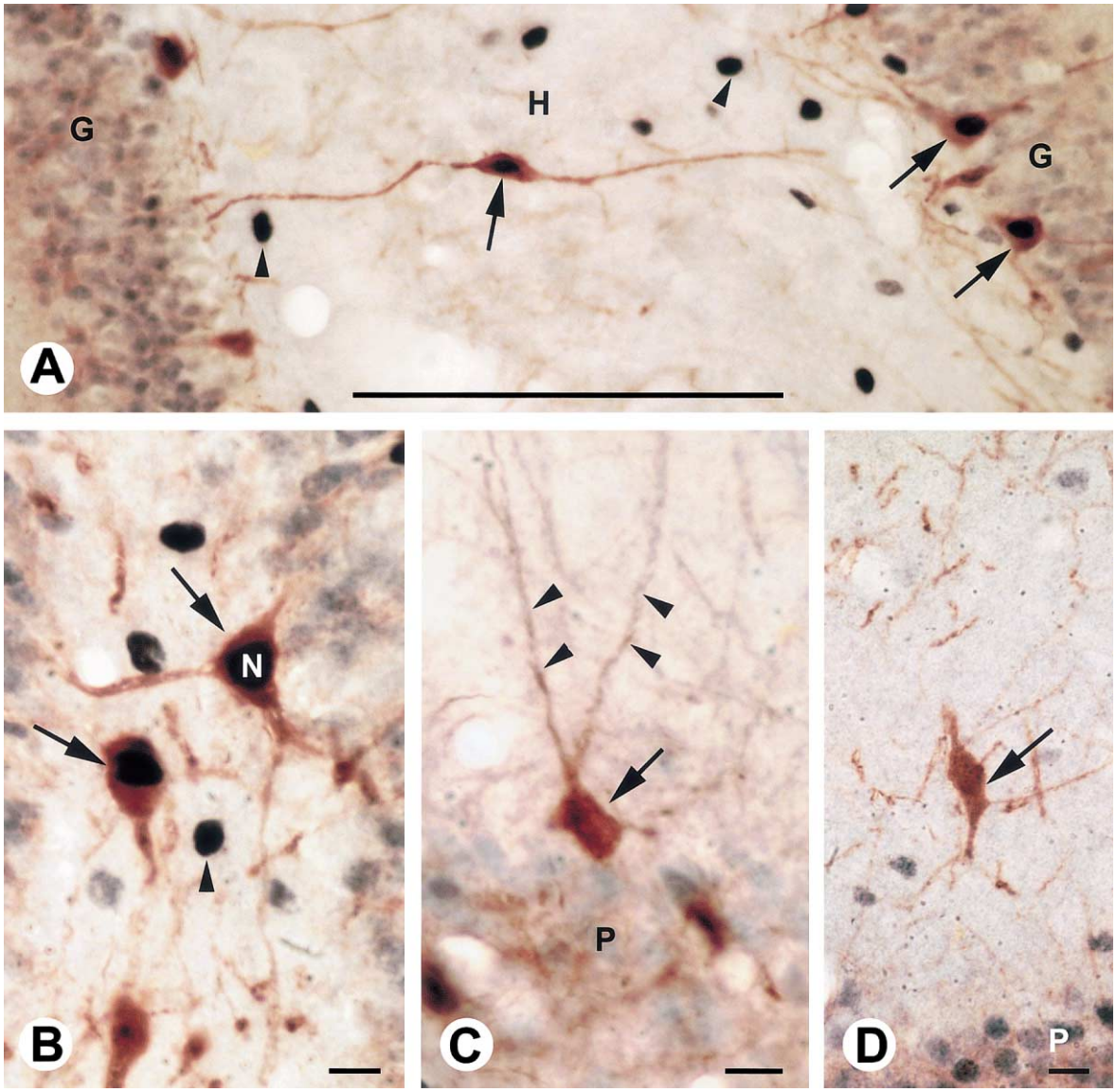


Fig. 3. Appearance of the PV-c-fos double immunostaining in the dentate fascia (A, B), sector CA 2 (C) and sector CA 3 (D). Brown structures are PV-immunoreactive, black, gray and bluish black cell nuclei indicate c-fos immunoreactivity. (A) PV-positive neurons expressing c-fos (arrows) in the dentate fascia at 3 h post-injection. Arrowheads point to c-fos stained neurons of unknown identity. H: hilus; G: granule cell layer containing c-fosIR nuclei. Bar: 100 μm. (B) Double-labelled neurons (arrows) in the hilus at 3 h post-injection. The presence of c-fosIR nuclei (N) in the PV-positive neurons is clearly observable. The arrowhead points to the PV-negative c-fos-containing cell. Bar: 10 μm. (C) PV-positive neuron (arrow) in the stratum radiatum of CA 2 at 5 h post-injection. The neuron does not express c-fos protein. Arrowheads point to the dendrites of the cell. P: pyramidal cell layer. Bar: 10 μm. (D) PV-positive neuron without c-fos immunoreactivity (arrow) in the stratum lacunosum-moleculare of sector CA 3, at 5 h post-injection. P: pyramidal cell layer containing c-fos stained cell nuclei. Bar: 10 μm.

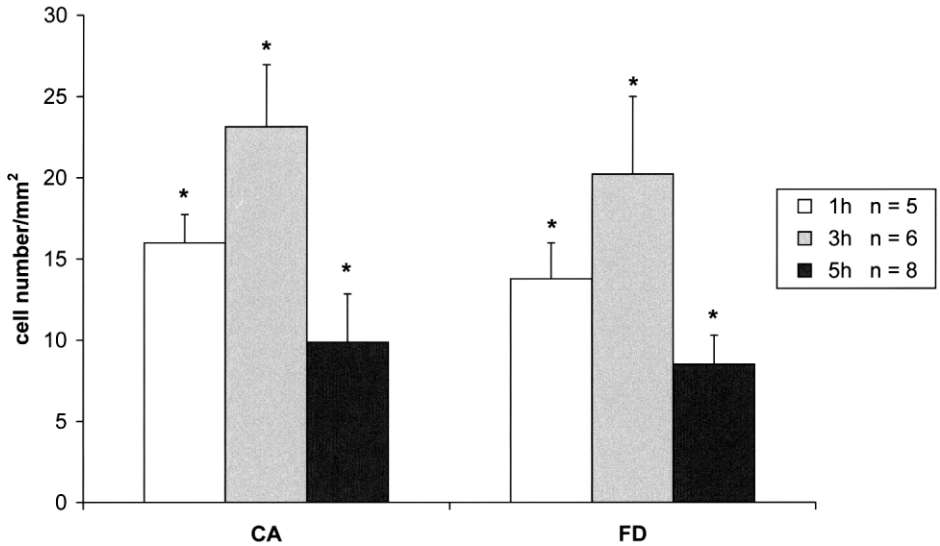


Fig. 4. Changes in the number of PV cells expressing c-fos protein in the Ammon's horn (CA) and in the dentate fascia (FD), at 1, 3 and 5 h post-injection. Asterisks denote significant differences; the standard error of the mean and the number of measurements are indicated (in CA: $P = 0.004$ between 1 and 3 h; $P = 0.008$ between 1 and 5 h; $P = 0.001$ between 3 and 5 h; in FD: $P = 0.011$ between 1 and 3 h; $P = 0.001$ between 3 and 5 h).

### *4.1. Mechanism of c-fos induction by 4-AP*

Literature data (Labiner et al., 1993; Herdegen and Leah, 1998) lead us to assume that the increased transmitter release induced the c-fos expression. We consider that 4-AP acts on several hippocampal pathways and augments the release of excitatory transmitters (e.g. glutamate) from their synapses. This assumption is supported by recent microdialysis experiments, which prove that 4-AP infusion increases the extracellular glutamate concentration in the hippocampus of rats (Peña and Tapia, 2000). Moreover, our recent studies with microdialysis probes in the striatum of rats proved a significant increase in glutamate in the dialysis fluid following intraperitoneal 4-AP injection (Mihály et al., unpublished). However, 4-AP also releases transmitters other than glutamate: the extracellular GABA (Peña and Tapia, 2000), noradrenaline (Versteeg et al., 1995) and dopamine (Bonnano et al., 2000) concentrations increased following 4-AP treatment in vivo and in vitro. It is thought that different glutamate receptors may play a role in c-fos gene expression: the blockade of NMDA receptors inhibited the expression of c-fos mRNA in the dentate fascia (Labiner et al., 1993). In other experiments, ketamine inhibited the expression of c-fos (Huang and Simpson, 1999), again indicating the importance of the NMDA receptor. Interestingly, immunohistochemical studies with the antibody of a subunit of the NMDA receptor revealed strong

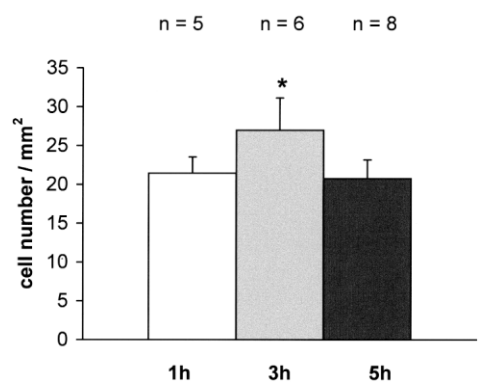


Fig. 5. Time-dependent changes of the number of PV neurons in the hippocampal formation (Ammon's horn plus dentate fascia). The 3 h value differs significantly from the others; the standard error of the mean and the number of measurements are indicated ($P = 0.023$ between 1 and 3 h; $P = 0.004$ between 3 and 5 h).

staining in the molecular layer and hilus of the dentate fascia, and in regions CA 1 and CA 3 of the Ammon's horn (Johnson et al., 1996), which are innervated by glutamate synapses (Amaral, 1978). Accordingly, on the basis of extensive literature evidence (Herdegen and Leah, 1998), we can conclude that mainly NMDA receptors play a role in the c-fos induction in our experiments. However, this issue must be proved by further experiments with NMDA antagonists.

### 4.2. Neuroanatomy of hippocampal seizures

Our present experiments proved that a very strong activation of the dentate fascia occurs at 1 h following the 4-AP injection. This observation is similar to others in the literature relating to other convulsants, though there are some differences in time, depending on the nature of the chemical used (for a review, see Herdegen and Leah, 1998). Recent experiments with 4-AP in our laboratory demonstrated a similar, strong increase of regional cerebral blood flow (rCBF) in the dentate fascia of mice (Mihály et al., 2000). In general, experimental limbic seizures start from the entorhinal cortex both in vivo (Collins et al., 1983) and in vitro (Jones, 1993; Barbarosie et al., 2000). Distinct cell populations of the rodent entorhinal cortex project to the dentate fascia and to the Ammon's horn (regions CA 1 and CA 3) in the perforant pathway (Steward and Scoville, 1976; Jones, 1993), which is thought to be aminoacidergic (Sloviter and Dempster, 1985). This pathway activates the dentate granule cells and the pyramidal neurons of regions CA 1 and CA 3 (Jones, 1993). It also impinges on inhibitory interneurons of regions CA 1 (Gulyás et al., 1999) and CA 3 (Freund and Buzsáki, 1996). This means that entorhinal afferents to these regions are divergent and probably less effective than those ending in the dentate fascia. However, when activated by the perforant pathway, the granule cells of the dentate fascia may prevent the spread of activity towards the Ammon's horn: the mossy fibers innervate not only the CA 3 principal cells, but also inhibitory interneurons of the hilus and region CA 3, which probably exert a feed-forward inhibition on the CA 3 pyramidal cells, delaying activation of the Schaffer collateral system (Acsády et al., 1998). This complex anatomical structure may explain our findings regarding the differences between the dentate fascia and Ammon's horn as concerns the numbers of c-fosIR cells. The fact that the staining intensity in the Ammon's horn increased together with a staining intensity decrease in the dentate granule layer probably reflected the overcoming by excitation of the inhibitory influences in regions CA 1 and CA 3 and the mossy fiber-driven inhibition. The hilus of the dentate fascia followed the granule cell layer as relates to the appearance of c-fos. This can be explained on the basis of the proximity of the hilar neurons and granule cells: the large variety of hilar neurons (Amaral, 1978) receive input from the mossy fibers (Nitsch et al., 1990; Frotscher et al., 1991). This input has been shown to be convergent and probably very effective (Acsády et al., 1998).

### 4.3. C-fos expression in PV-containing neurons

PV-containing neurons comprise a characteristic population of GABAergic interneurons in the hippocampus: in the Ammon's horn, as basket and axo-axonic cells, these cells mediate mainly perisomatic inhibition (Gulyás et al., 1999). A similar perisomatic inhibition occurs in the dentate granule cell layer (Nitsch et al., 1990; Seress et al., 1991). The hilar PV cells could participate in feed-back inhibition to the granule cells, because they are contacted by mossy fibers (Nitsch et al., 1990). Our results indicate the highest number of c-fosIR PV neurons at 3 h not only in the Ammon's horn, but also in the dentate region. This is not surprising in region CA 1, because the afferents of the principal cells and those of the PV cells are similar (Gulyás et al., 1999). The situation is more complex in region CA 3, because part of the input to the PV neurons comes through the mossy fibers (Deller et al., 1994) from the granule cells, which proved to be activated earlier than the cells of region CA 3 in our experiments. It seems that the c-fos expression is not uniform in the hilar neurons: some hilar cells exhibit early c-fos-gene activation, whereas some are activated later. It should be noted, that the number of PV cells in

the hilus is relatively small (Nitsch et al., 1990), and the hilus also contains excitatory mossy cells that are driven by the granule cells (Frotscher et al., 1991). This is probably the explanation of the early peaking of the c-fos counts in the hilus, and the discrepancy between the activation of the PV cells and the activation of the total hilar cell population. However, the sampling differences may also influence the results: the hilar counts were taken strictly from the hilus, whilst the dentate PV counts related to all parts of the dentate fascia, and zone 1 of region CA 3. In any event, the characteristic feature of the dentate PV cell activation was that it outlasted the c-fos expression of the granule cells. A similar c-fos induction has been detected in the somatostatin interneurons in the dentate hilus: the c-fos staining in those cells outlasted that of the granule cells (Dragunow et al., 1992). It is interesting that PV cells in the rat hippocampus express protein subunits of the delayed rectifier potassium channel, and the neurons are sensitive to low 4-AP concentrations: 4-AP increases the amplitude and duration of the action potential (Du et al., 1996). This means that PV cells could have been directly affected by 4-AP, which could contribute to their c-fos expression pattern. The significance of this long-lasting c-fos expression is not clear; it probably indicates some long-lasting cellular alterations, or future cell death. Long-lasting seizures have been shown to induce heat-shock protein expression in hilar neurons, as an indication of cellular injury (Sloviter and Lowenstein, 1992). Further experiments are needed to prove such injury in hilar PV neurons.

Finally, a comment should be made on the changing number of the overall PV cell population in the hippocampus in these experiments. The PV neurons of the thalamic reticular nucleus are known to be susceptible to ischemia (Kawai et al., 1995), but the process of cell loss occurs some days after the insult. However, hippocampal PV neurons are known to be resistant to epilepsy and ischemia (Freund and Buzsáki, 1996). In our case, it seems that limbic seizures first increase the number of PV cells; then, when the seizure activity has disappeared, the number of PV cells decreases again. However, this should be proved by means of in-situ hybridization or Northern blotting of specific mRNA.

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