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Research report

# Light-induced Fos expression in phosphate-activated glutaminase- and neurofilament protein-immunoreactive neurons in cat primary visual cortex

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**Abstract**

Previous double-stainings in the cat visual cortex [E. Van der Gucht, S. Clerens, K. Cromphout, F. Vandesande, L. Arckens, Differential expression of c-*fos* in subtypes of GABAergic cells following sensory stimulation in the cat primary visual cortex, Eur. J. Neurosci. 16 (2002) 1620–1626] showed that a minority of Fos-immunoreactive nuclei was located in distinct subclasses of inhibitory neurons following sensory stimulation. This report describes double-stainings between Fos and phosphate-activated glutaminase (PAG) or Fos and neurofilament protein (SMI-32) revealing that, following a short-term visual experience, Fos is also expressed in neurochemically distinct subpopulations of non-GABAergic, pyramidal neurons in supra- and infragranular layers of cat area 17.


*Theme:* Sensory systems
*Topic:* Visual cortex: striate

*Keywords:* Immediate early gene; Neurofilament protein; Glutaminase; Pyramidal neuron; Excitatory; Double-staining

Immunocytochemistry for the immediate early gene (IEG) protein Fos has already been successfully applied to localize activated neurons in response to a variety of cellular signals providing function-related maps with single-cell resolution (for review, see Ref. [17]). Neural stimulation physiologically upregulates the levels of Fos in mammalian sensory systems [20], but only few reports are available about the neurochemical identity of cells showing immunoreactivity for Fos following a stimulation paradigm. Recently, in cat visual cortex, double-stainings revealed that only a confined subgroup of Fos-immunoreactive nuclei was expressed in several subclasses of GABAergic interneurons following a short-term light stimulation [31], indicating that Fos expression is also induced in certain non-GABAergic cell populations. However, several studies suggested that pyramidal neurons are the most expected cellular candidates based on the spatial distribution profile of neurons with induced Fos expression throughout mammalian sensory cortex [5,11–13,22,23]. Therefore, the present study was conducted to assess whether pyramidal neurons in cat visual cortex are also an important neuronal target affected by visual stimulation.

The neurochemical heterogeneity of different phenotypes within the pyramidal cell population is less comprehensive in comparison to the GABAergic population in the cerebral cortex of mammals. In cat primary visual cortex, we have already analyzed the neurochemical features of the pyramidal cell class by single immunocytochemical stainings for phosphate-activated glutaminase (PAG) and for neurofilament protein (SMI-32), and also by double-stainings between both neurochemical markers [30,32]. PAG, the synthesizing enzyme of the excitatory transmitter glutamate

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[4], is considered to be a reliable neurochemical marker to map the glutamatergic cell population [1,10,18,32], whereas SMI-32, a monoclonal antibody recognizing the nonphosphorylated epitopes of the neurofilament protein [24], is only present in a particular subset of pyramidal neurons in rodent, carnivore, and primate visual pathways [3,6,8,14–16,19,30,33]. In this study, we examined these PAG- and SMI-32-immunopositive neurons for Fos expression upon visual stimulation in area 17 of the cat visual cortex. We present qualitative data on light microscopic double-stainings of Fos with PAG or SMI-32 to illustrate that neurochemically distinct pyramidal cell populations exhibit light-induced Fos immunoreactivity.

All animal experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). All efforts were made to minimize the animals' discomfort and to reduce the number of animals used in accordance to the policy established by the Ethical Committee on Animal Research of the Katholieke Universiteit Leuven, based on the disposition of the Belgian Government. These rules were followed strictly in all experiments. Four adult cats were housed in the animal house with a normal 14:10 h light/dark cycle with the normal diurnal light period commencing at 07.00 AM (Katholieke Universiteit Leuven, Belgium). All experimental animals were put overnight in a dark room at 6.00 PM. The next day at 09.00 AM, they were exposed to a normal environment with ambient light for 1 h and were immediately sacrificed thereafter. The IEG c-*fos* is expressed at very low, undetectable basal levels under non-stimulatory conditions, while a light experience for 1 h after a period of darkness results in a maximal increase in the expression levels of the protein Fos in cortical neurons of mammalian visual system (for review, see Refs. [17,20] and references therein, [34]). At our laboratory, control conditions regarding the nature of the stimulus and its specific effect on the induction pattern of Fos in cat visual neurons were already studied and described in detail before [2,26,29,35]. Fos expression was checked (1) in a control, non-visual area like somatosensory area I and II of the adult cat [26], in visual cortex of cats (2) after a long-term light stimulation for 8 h to reveal Fos expression under basal conditions [29,34], (3) following two monocular deprivation conditions [29], (4) where visual input was confined to one hemisphere by sectioning the left optic tract and the visual part of the corpus callosum, this model serves as its own control by comparing the intact with the visually deafferented hemisphere [29,35], (5) following the induction of retinal lesions resulting in visually deprived and surrounding visually non-deprived zones in area 17 and higher order areas [2], and also (6) in the visual cortex of several mammalian species such as rodents and primates upon an overnight dark adaptation in combination with a 1-h visual exposure [28,29].

All animals were deeply anaesthetized (10 mg/kg ketamine hydrochloride im), sacrificed with a large dose of pentothal (60 mg/kg iv) at the same moment of the day (10.00 AM), and perfused transcardially with 0.9% NaCl followed by cold 4% paraformaldehyde (Sigma-Aldrich, St. Louis, MO) in phosphate-buffered saline (PBS, 0.15 M, 0.9% NaCl, pH 7.4). Brains were removed, postfixed, rinsed, and stored at 4 °C in PBS. All primary antibodies have already been successfully used before on cat visual cortex: (1) a rabbit anti-rat brain PAG polyclonal antibody recognizing cat brain PAG specifically (characterized in our laboratory [32]), (2) a rabbit anti-cat brain Fos polyclonal antibody (produced and characterized in our laboratory [29]) [2,26–28,31], and (3) a monoclonal antibody SMI-32 [24,28,30,32,33]. All dilutions were made in Tris-buffered saline (TBS, 0.01 M, 0.9% NaCl, 0.1% Triton X-100, pH 7.6) and all incubations were performed at room temperature under gentle agitation, unless mentioned otherwise. Coronal vibratome sections (50 μm) were pretreated with 0.3% $H_2O_2$ (20 min), preincubated with normal goat serum (NGS, 1:5, 45 min), and subsequently incubated with one of the primary antibodies: PAG antiserum (1:2000) for 7 days at 4 °C or SMI-32 antiserum (1:2000) overnight at room temperature. These antibodies were detected with biotinylated goat anti-rabbit IgGs (1:500, 30 min, Dako, Glostrup, Denmark) or biotinylated goat anti-mouse IgGs (1:200, 30 min, Dako), respectively, and peroxidase-conjugated streptavidin (1:600, 30 min, Dako). PAG was visualized as a dark-purple precipitate using a substrate solution containing 12.5% DAB and 2% Nickel ammonium sulphate (Sigma-Aldrich). The peroxidase labeling of SMI-32 was detected as a dark-gray color reaction using the glucose oxidase-diaminobenzidine-nickel method as described previously [26–33]. After thorough rinsing in TBS, sections were double-labeled by incubating overnight with the second primary antibody against Fos (1:15,000). Fos immunoreactivity was detected with the Envision$^+$ System Peroxidase (DAB) (Dako) according to the manufacturer's recommendations [26–29]. The sections were dehydrated, coverslipped, and viewed through a DM RBE microscope (Leica, Heidelberg, Germany). Possible cross-reactivity between any of the primary and/or secondary antibodies was checked to exclude the presence of any falsely double-labeled cells: (i) sequential omission of one of the various incubation steps completely abolished the immunocytochemical staining of nuclei or cell bodies indicating method specificity; (ii) Fos immunoreactivity was distinguishable from neuronal markers by color and location: the cell cytoplasm was stained dark-gray or dark-purple as an indication for the presence of the neuronal marker, whereas the nucleus was always stained brown locating the Fos protein.

Nissl staining was performed with Cresyl violet (1%, Fluka Chemika, Sigma-Aldrich) according to standard protocols to determine each cortical layer of cat area 17 containing immunoreactive neurons [21].

The protein Fos is expressed at very low, undetectable levels under basal conditions, while visual stimulation