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NEUROMODULATORY ROLE OF ACETYLCHOLINE IN VISUALLY-INDUCED CORTICAL ACTIVATION: BEHAVIORAL AND NEUROANATOMICAL CORRELATES

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Abstract—Acetylcholine is released in the primary visual cortex during visual stimulation and may have a neuromodulatory role in visual processing. The present study uses both behavioral and functional neuroanatomy investigations to examine this role in the rat. In the first set of experiments the cholinergic system was lesioned with 192 immunoglobulin G (IgG) saporin and the visual acuity and performance in a visual water maze task were assessed. The cholinergic lesion did not affect the visual acuity measured pre- and post-lesion but it did reduce the efficiency to learn a novel orientation discrimination task measured post-lesion. In order to better understand the involvement of the cholinergic system in the neuronal activity in the visual cortex c-Fos expression induced by patterned visual stimulation was further investigated. Results obtained following lesion of the cholinergic fibers (192 IgG-saporin or quisqualic acid), muscarinic inhibition (scopolamine), or NMDA receptor inhibition (CPP) were compared with control conditions. Double and triple immunolabeling was used in order to determine the neurochemical nature of the activated cortical cells. The results demonstrated that patterned stimulation elicited a significant increase in c-Fos immunolabeled neurons in layer IV of the contralateral primary visual cortex to the stimulated eye which was completely abolished by cholinergic fibers lesion as well as scopolamine administration. This effect was independent of NMDA receptor transmission. The c-Fos activation was predominantly observed in the glutamatergic spiny stellate cells and less frequently in GABAergic interneurons. Altogether, these results demonstrate a strong involvement of the basal forebrain cholinergic system in the modulation of post-synaptic visual processing, which could be related to cognitive enhancement or attention during visual learning.

Key words: c-Fos immunoreactivity, cholinergic system, muscarinic receptor, use-dependent activity, visual discrimination, visual acuity.

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Abbreviations: ACh, acetylcholine; ChAT, choline acetyltransferase; CPP, 3-(2-carboxypiperazin-4-yl)-propyl-propyl-L-phosphonic-phosphonic acid; GAD, glutamic acid decarboxylase; GFAP, glial fibrillary acidic protein; GlutT, glutamate reuptake transporter; HDB, horizontal limb of the diagonal band of Broca; IgG Sap, immunoglobulin G saporin; LM, lateromedial part of the visual extrastriate cortex; PBS, phosphate buffer; PV, parvalbumin; RBPC, rat brain pyramidal cells; S1HL, somatosensory cortex; V1, primary and visual cortex.

The cholinergic system is a key system in cognitive functions such as learning, memory and attention (Sarter et al., 2005). Its involvement in these higher functions could be exerted through its neuromodulatory action on the cerebral cortex as early as in the primary sensory areas, in which cholinergic pathway activation is likely stimulus-driven or controlled by top-down mechanisms including attention (Gilbert et al., 2000; Laplante et al., 2005; Sarter et al., 2005). In sensory areas, electrically-induced release of acetylcholine (ACh) concomitant with auditory or tactile stimulation has been demonstrated to generate the reorganization of the functional cortical map (Kilgard and Merzenich, 1998; Thiel et al., 2002) or to induce long-term changes in evoked somatosensory field potentials (Verdier and Dykes, 2001; Gu, 2002).

In the visual cortex, ACh has been shown to influence the function or the connectivity of visual neurons (Murphy and Sillito, 1991; Brocher et al., 1992; Kimura et al., 1999; Kirkwood et al., 1999; Kuczewski et al., 2005; Roberts et al., 2005; Levy et al., 2006; Zinke et al., 2006). We have recently shown that ACh release was specifically increased in the primary visual cortex (V1) during finely-tuned patterned visual stimulation (Laplante et al., 2005). These results corroborated previous data showing ACh release in the visual cortex during visual field illumination (Inglis and Fibiger, 1995; Jimenez-Capdeville et al., 1997; Arnold et al., 2002) and suggest that ACh release during visual stimulation could influence visual processing in V1. This neuromodulator system therefore could contribute to change the cortical representation of visual stimuli and consequently their efficacy to create perceptual sensations or behavioral responses.

The aim of the present study is to determine the involvement of the cholinergic system in visual processing. It is hypothesized that ACh is involved in the learning process of visually guided behaviors and that this is achieved in part by enhancing the use-dependent neuronal activity of V1. More specifically, the effect of selective cholinergic fiber lesion is hypothesized to affect the capacity to learn a visual orientation discrimination task but not affect the memory of the water maze task itself verified through repeated measure of the visual acuity task. To determine experience-dependent neuronal activity, immunostaining for the immediate early gene *c-fos* product (c-Fos) was used as a cellular marker of neuronal activity in the visual cortex (Kaczmarek and Chaudhuri, 1997). The neurochemical specificity of the cells labeled by c-Fos, i.e. GABAergic and/or glutamatergic, was determined using double immunostaining. c-Fos activation was examined

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following 1) lesions of cholinergic projections to the visual cortex using the cholinergic selective immunotoxin 192 immunoglobulin G saporin (IgG Sap) or the quisqualic acid, and 2) blockade of the muscarinic receptors with scopolamine. In order to further identify the interaction of the cholinergic and glutamatergic transmission, the effects of NMDA receptor blockade by 3-(2-carboxypiperazin-4-yl)-propyl-L-phosphonic acid (CPP) on the visually-induced neuronal activity were also investigated.

EXPERIMENTAL PROCEDURES

Animals

Male Long-Evans rats (275–325 g) were obtained from Charles River Canada (St-Constant, Québec, Canada) and housed individually in a 12-h light/dark cycle with free food access. All experiments conformed to international guidelines and to the Canadian Council for Animal Care guidelines on the ethical use of animals and were approved by le Comité de déontologie de l'expérimentation sur les animaux de l'Université de Montréal. All efforts were made to minimize the number of animals used and the suffering.

Two groups of rats were used for behavioral testing (control sham-lesion, $n=7$ and 192 IgG Sap lesion, $n=8$) in order to test the functional involvement of the cholinergic system in the visual discrimination. Six groups of anesthetized rats were used in parallel for c-Fos immunocytochemistry to further analyze the influence of the cholinergic transmission in the cortical microcircuitry functioning during visual stimulation. These six groups of rats were kept in the dark for 24 h prior to experimentation and divided into control sham-stimulation ($n=4$, kept in the dark), control stimulation ($n=5$), scopolamine treatment+stimulation ($n=5$), quisqualic acid lesion of basal forebrain projections+stimulation ($n=4$), 192 IgG Sap lesion of cholinergic projections+stimulation ($n=5$) and CPP treatment+stimulation ($n=5$).

Lesion of the basal forebrain cholinergic neurons

Rats were anesthetized with a mixture of ketamine (85 mg/kg), acepromazine (2.5 mg/kg) and xylazine (15 mg/kg) and placed in a stereotaxic apparatus for intracerebral injection of either 192 IgG Sap or quisqualic acid. 192 IgG Sap (Advanced Targeting Systems, San Diego, CA, USA; 2.4 μg/μl in PBS, as provided by the manufacturer) was injected within the lateral ventricle (2 μl/ventricle, mm from Bregma, AP −0.3; L ±1.5; V −3.6; (Paxinos and Watson, 1995)) either bilaterally for the behavioral study to avoid interhemispheric compensation or unilaterally for the c-Fos study to allow interhemispheric comparison of activity-induced c-Fos neurons number in the same animal. Moreover, in the case of the behavioral study, sham animals received an i.c.v. injection with a mouse IgG Sap (non-targeted saporin control molecule, Advanced Targeting Systems, San Diego, CA, USA). Quisqualic acid dissolved in 10 mM phosphate buffer (PBS) was injected unilaterally within the horizontal limb of the diagonal band of Broca (HDB) at four different sites (0.4 μl/site, during 4 min, mm from Bregma, AP +0.3; L +0.7; V −8.6/AP 0; L +1.4; V −8.8/AP −0.3; L +2; V −9.0/AP −0.8; L +2; V −9.0; (Paxinos and Watson, 1995)). These sites correspond to the location of the cholinergic neurons that project to the visual cortex as shown previously by track tracing methods (Gaykema et al., 1990; Laplante et al., 2005). The rats were allowed to survive for 21 or 7 days after the 192 IgG Sap or quisqualic acid injections, respectively, and were used for behavioral testing or neuronal activity examination. Location of the lesion sites was confirmed by Cresyl Violet histochemistry. Visualization of the cholinergic and GABAergic neuronal loss was performed on cryostat sections using choline acetyltransferase (ChAT, 1:500, Chemicon, Temecula, CA, USA) and parvalbumin (PV, 1:10,000, made in mouse, Sigma, St. Louis, MO, USA) double immunocytochemistry. Extent of the cholinergic fiber loss was quantified on two consecutive vibratome sections at the level of the V1 (using the same coordinates than c-Fos analysis, see below). Briefly, microphotographs were taken (magnification ×40, area of the region studied: 0.04 mm^2) and fibers were reproduced (one pixel thick line) on a distinct optical layer using Adobe Photoshop. The total length of the replicates was quantified using Scion Image (Scion Corporation, Frederick, MD, USA) and expressed as total length of the fibers (mm) under 1 mm^2 (see Mechawar et al., 2000).

Behavioral tests

The rats were trained and tested in a visual water maze, a two-alternative, forced choice, water-based visual discrimination task (Prusky et al., 2000) in order to test visual acuity or orientation discrimination ability. Learning of the task consisted in the association of a submerged platform with the positive stimulus, a sinusoidal grating (0.12 cpd, 100% contrast, 90° orientation) versus the negative stimulus (a gray screen with equal luminescence to the positive stimulus) according to previously described methods (Prusky et al., 2000). The task exploits the natural water aversion of the rat to motivate animals to perform (escape from water). The two arms of the trapezoidal-shaped pool were separated by a 45 cm divider with Sony Trinitron monitors (18 inches, 36 cd/m^2) at the end of each arm which displayed sinusoidal grating or gray screen generated by VPixx software (v 8.5, Sentinel Medical Research Corporation, Québec, Canada). The entire water maze was made of Plexiglas with the interior painted in black and the water was made opaque with approximately 200 g of powdered milk (final concentration 2 g/L) and kept at 27 °C. A platform (15 cm depth) was placed at the end of the trapezoid arms which corresponded to the positive stimulus. After each swimming session rats were dried and kept in a warm environment to avoid discomfort.

Behavioral analysis consisted of three phases: pretraining, training, and test (Prusky et al., 2000). During the pretraining phase, animals were placed on the platform directly in front of the positive stimulus and were allowed to remain on the platform for 10–30 s. This was repeated for 10 trials for 1 day in order to condition the animals to the escape platform. The training phase was designed to have the animals associate the escape platform and positive stimulus. During this phase, rats were placed in front of the positive stimulus and allowed to swim to the escape platform submerged in front of it and to remain on the platform for 10–30 s. The distance between a low grating stimulus (0.12 cpd) and the release site was increased gradually until the rat swam in a straight line from the beginning of the pool (a centered release chute) to the platform, a total distance of 130 cm. A successful trial consisted of the rat being released into the chute, swimming to the level of the divider (45 cm from the end of the maze), then swimming to positive stimulus to reach the platform. If the animal swam toward the negative stimulus past the divider the trial was recorded as an error and the animal was left in water, as a punishment, until it found the platform for a maximum of 60 s. The task was considered as acquired when the rats were able to reach a rate of 80% of success over 20 trials. For the visual acuity determination, the test phase consisted of determining the threshold value for detection of a pattern stimulus versus a gray screen. The positive stimulus was displayed pseudorandomly in the left or right arm. Small incremental increases in the spatial frequency of the stimulus (from 0.12–0.9 cpd) were made between blocks of trials until the ability to distinguish the grating from gray screen fell to chance (<70%). To minimize the testing time, a full cycle on the screen was added for each correct choice for low spatial frequencies (0.12–0.5 cpd). As the animal approached threshold frequencies (0.5–0.9 cpd) subjects needed four consecutive correct trials

or 7 correct out of 10 trials. Using this method of limits (Prusky et al., 2000) the testing phase can be completed in 2–3 days (40 trials/days). The pretraining and training phases were performed prior to the lesion. All of the rats were first tested for visual acuity prior to any treatment. They were then randomly divided into two groups, whereby one group received intra-ventricular injections of 192 IgG Sap in order to lesion the cholinergic fibers and a sham lesion group which received non-targeted saporin in the left lateral ventricle. After 21 days post-surgery, rats were tested for their ability to recall the visual acuity task.

Following the post-lesion testing phase for visual acuity, the rats were required to learn a novel discrimination task, i.e. the orientation discrimination, for which rats did not receive any training before the lesion. Similar to the visual acuity task, the method of limits was used where subjects had to discriminate between a negative stimulus (90° horizontal bars, 0.20 cpd, 90% contrast) and a positive stimulus that varied between 45° and 90° (positive stimulus, 0.20 cpd, 90% contrast, 15° increments), again using a pseudorandom alternation. An 80% success rate over 20 trials was used to define successful performance which was reached within 3–5 days.

Drug administration

Thirty minutes prior to the visual stimulation, anesthetized rats received an i.p. injection of either the muscarinic receptor antagonist scopolamine (1 mg/kg, Sigma) (Dauphin et al., 1991) or the NMDA receptor antagonist CPP (10 mg/kg, Sigma) (Clapp et al., 2006) dissolved in sterile saline (sodium chloride injection USP 0.9%, Abbott Laboratories, Montréal, QC, Canada).

Visual stimulation for neuronal activity mapping

The visual stimulation was adapted from a previous study (Laplante et al., 2005). The animals were maintained in total darkness 24 h before the visual stimulation in order to drive constitutive expression of c-Fos to its lowest level. To prevent head movements and to ensure constant exposure to the stimulus in the desired eye, rats were anesthetized with urethane (1.3 g/kg) and positioned in a stereotaxic frame placed into a closed black chamber. The eyelid of the non-stimulated eye was kept closed with opaque black tape. Body temperature was maintained at 37 °C with a thermostatically controlled heating pad (FHC, Bowdoinham, ME, USA). Pupil dilation and accommodation paralysis with atropine was not used. Drying of the eye was prevented with a natural eye lubricant (Refresh Tears, Allergan Inc., Markham, ON, Canada). A computer monitor (30×25 cm, Titanium, Apple Computer Inc., Cupertino, CA, USA, luminance 21 cd/m^2) was placed 32 cm parallel to the long axis of the rat and centered on the eye. In these conditions, 80% of the visual field of the animal was stimulated. Sinusoidal and square grating (90% contrast) of variable orientation (30°, 60°, 90°, 120°, 180°), spatial and temporal frequencies (1–5 Hz and 0.02–1.5 cpd, respectively) were generated at random by VPixx software and were alternatively displayed on the monitor for 30 min by contrast reversal. At the end of the visual stimulation period, rats were removed from the stereotaxic apparatus and kept in the dark for 60 min. They were then killed and brains were preserved with an intracardiac perfusion with 4% paraformaldehyde for subsequent immunostaining. Control sham-stimulated animals were processed exactly the same way except that both eyes were kept closed with an opaque tape and an opaque screen covered the computer monitor during the presentation of the stimulus to prevent any luminance or patterned stimulation.

Immunocytochemistry

Section selection and preparation. Brains were sliced into 35 μm sections using a vibratome (Leica Microsystems) at the level of (1) the visual cortex/superior colliculus (mm from Bregma, AP −7.3±0.5; Paxinos and Watson, 1995) to visualize the V1 and the lateromedial part of the extrastriate cortex (LM, we prefer to use this nomenclature than V2L as used in the cited atlas, because it is not clear whether this area has a second order function), and (2) the somatosensory cortex (S1HL)/visual nuclei of the thalamus, i.e. optic tectum, lateral geniculate body (mm from Bregma, AP −5.3±0.5 (Paxinos and Watson, 1995). Brain sections were collected serially in 24 wells plates, so the AP level of the sections could be easily identified according to their position in the plate and anatomical references. In order to determine the extent of the visual activation and the possible regional disparity the entire visual cortex (from AP −5.8 to −8.0) of three control stimulated animals was immunostained for c-Fos.

Immunohistochemistry and cell counting. Five consecutive sections for each rat were selected according to anatomical features (for the visual cortex: at the level of the subiculum) and sections were pre-incubated for 20 min at room temperature in PBS (0.1 M, pH 7.4) containing 0.3% hydrogen peroxide, followed by 30 min in PBS containing 0.25% Triton X-100 and 0.2% gelatin. They were then incubated overnight at room temperature with rabbit–anti-c-Fos primary antibody (1:10,000, Oncogene Research Products, San Diego, CA, USA) in PBS–triton–0.2% gelatin. This was followed by a 2 h incubation in donkey–anti-rabbit secondary antibody (1:500, Jackson ImmunoResearch, Westgrove, PA, USA) and then for 1 h in the avidin–biotin complex (ABC Elite kit, Vector Laboratories, Burlingame, CA, USA). After each incubation step, rinses were carried out in PBS containing 0.25% triton. A peroxidase-substrate-kit Vector SG (Vector Laboratories) was used to develop the reaction product during a standardized period of 5 min. Sections were then mounted onto slides, dehydrated and coverslipped with Permount. Twenty photographs per cortical hemisphere were taken with a Leica DC 500 digital camera in the selected regions. Full-image reconstruction of the entire region was carried out using Adobe Photoshop. The number of c-Fos labeled cells was counted in a region of interest (a rectangle tangent to the external capsule) in layer IV or VI of V1 (mm from Bregma, AP −7.3, L ±3–5, area of the region of interest: 0.38 mm^2 adjusted to every section at the tip of the internal capsule as a constant anatomical reference) or in S1HL taken as a control cortical area (AP −0.3±0.1, L ±2–3 mm, area 0.19 mm^2) and in layer II/III/IV of LM (AP −7.3, L ±5–6.5, area 0.36 mm^2) of two consecutive sections for each rat. This quantity of c-Fos immunoreactive cells was compared between the contralateral and the ipsilateral hemisphere to the stimulated eye.

To verify specificity of c-Fos activation, Zif268 another reported marker of neuronal activity for the visual cortex was performed on a subset of subjects (Kaczmarek and Chaudhuri, 1997). Immunolabeling with Zif268 (1:1000, antibody kindly provided by Dr. Chaudhuri, McGill University, Montreal, Canada) was performed on the three most reactive rats for the visual stimulation as described for the c-Fos procedure. The pattern and quantity of Zif268 and c-Fos immunolabeling were comparable in these sections. c-Fos immunolabeling produced a lower background staining and was subsequently used for all analysis.

Double and triple immunostaining. In order to determine the cell-specificity of the c-Fos immunoreactive cortical cells, we performed a double immunocytochemical study to examine whether c-Fos was expressed within (1) GABA cells labeled for PV, a marker of the basket cells, the most abundant GABA interneurons of the rat layer IV (Gonchar and Burkhalter, 2003) or glutamic acid decarboxylase, GAD67, which label all types of GABAergic neurons, (2) glutamatergic neurons using rat brain pyramidal cell marker (RBPC), a marker of the pyramidal cells and glutamate reuptake transporter (GlutT) which is strictly located on the glutamatergic neurons including the spiny stellate cells or (3) cortical astrocytes using glial fibrillary acidic protein (GFAP) antibody. The

antibodies were used at the following dilution: PV (1:10,000, made in mouse, Sigma), GAD67 (1:5000, made in mouse, Chemicon), RBPC (1:1000, made in mouse, Swant, Bellinzona, Switzerland), GlutT (EEAC1, 1:500, made in mouse, Chemicon) or GFAP (1:300, made in rabbit, DAKO, Denmark). Vibratome sections from the visual cortex were processed with the same protocol as that used for c-Fos immunostaining. Sections were first incubated in the anti-c-Fos antibody and developed with Vector SG then consecutively incubated overnight in the other primary antibody and developed with DAB (Vector Laboratories). The proportion of the double-labeled cells compared with total c-Fos cells was evaluated in both hemispheres total number of cells counted over five animals in the control stimulated group are shown in parentheses GAD67 (481), PV (425), RBPC (553), GlutT (902). The same procedure was used for the double immunostaining ChAT/PV in the HDB cryostat sections.

Additional sections were processed with fluorescent secondary antibodies for triple immunofluorescent labeling for (1) confirming the observation made on double immunolabeling in photonic microscopy and (2) examining the relationship between ChAT fibers and the c-Fos neurons under confocal microscopy. Multiple combinations of markers were analyzed: c-Fos/ChAT/GlutT, c-Fos/ChAT/RBPC, c-Fos/ChAT/PV and c-Fos/ChAT/GAD67. The secondary antibodies were conjugated with Cy5, FITC or Cy3 (Jackson ImmunoResearch) and observed with a confocal microscope (Leica Microsystems) with appropriate filters.

Statistical analysis

For the visual learning task, an unpaired Student's *t*-test was used to analyze the significant differences between the performances of the lesioned rats compared with the sham-lesioned rats. For the analysis of the visually-activated neurons, a Kruskal-Wallis test was performed on c-Fos neurons number for each visual cortical area examined (V1 layer IV, V1 layer VI, LM layer II/III/IV) to determine the effect of treatments (control sham-stimulation; control stimulation; 192 IgG Sap lesion of cholinergic projections+stimulation; quisqualic acid lesion of HDB projections+stimulation; scopolamine treatment+stimulation and CPP treatment+stimulation). This test was followed by a Mann-Whitney *U* test in each region showing an effect of treatment for determining the regions showing statistical changes when compared with their sham-stimulated counterparts. A Wilcoxon signed ranks test was used to determine the statistical difference of c-Fos neurons number in contralateral versus ipsilateral side to the stimulated eye in V1 and LM regions for each cortical layer examined and for each group. A Mann-Whitney *U* test was used to determine the statistical difference between the different layers of V1 compared with S1HL. For the analysis of cholinergic fibers loss a Kruskal-Wallis test followed by a Mann-Whitney *U* test was used to compared fibers density of the "non-lesioned" side in the quisqualic injection (taken as intact cortex) to the "non-lesioned" side of IgG Sap or lesion side after quisqualic acid or IgG Sap injection. A significance level of $P \leq 0.05$ was chosen for each test.

RESULTS

Effect of the basal forebrain lesion on the cholinergic innervation of V1

IgG Sap i.c.v. injection destroyed the cholinergic neurons of the HDB and their projections in V1 (Fig. 1). As expected, the GABAergic cell bodies located within the HDB were not lesioned, indicating that the GABAergic projections to the visual cortex (Freund and Gulyas, 1991) were spared. Cortical cholinergic interneurons as well as pedunculopontine cholinergic neurons were also spared, which testifies the specificity of the IgG Sap for the basal forebrain neurons expressing $p75^{NTR}$. Quisqualic acid infusion in the HDB destroyed the cholinergic as well as GABAergic neurons and projections of the HDB. No change in behavior or locomotor activity was noted. In case of unilateral injection of IgG Sap or quisqualic acid, the fiber density in

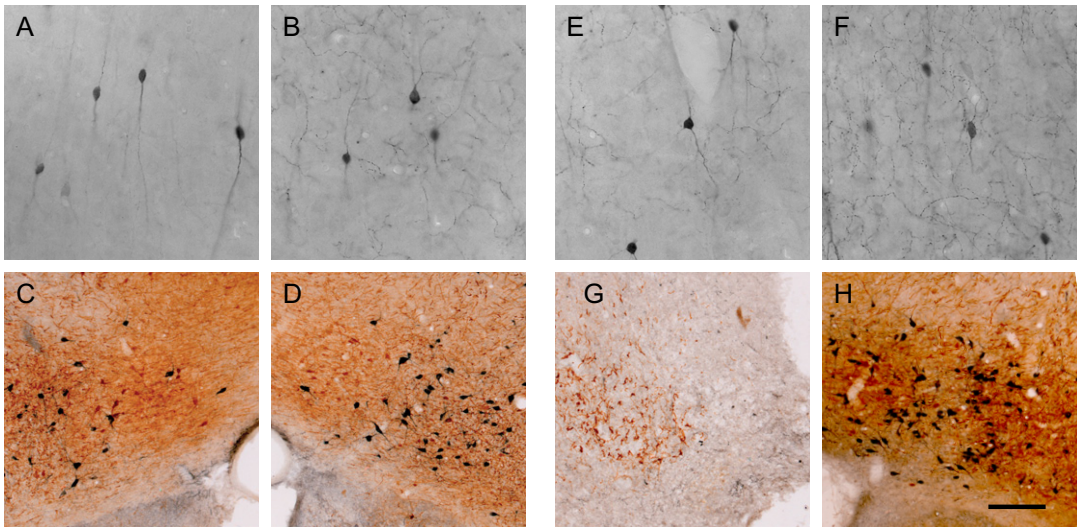

Fig. 1. Microphotographs showing the effects of unilateral 192 IgG Sap or quisqualic acid lesion on visual cortex cholinergic denervation. (A, B, E, F) ChAT immunoreactive fibers (bluish–gray) in the V1 (mm from Bregma, AP −7.3) and (C, D, G, H) ChAT (bluish–gray) and PV (brown) immunoreactive neuronal elements in the HDB (mm from Bregma, AP −0.3). (A–D) Lesions 21 days after intra-ventricular injection of 192 IgG Sap; (A) the loss of the cholinergic innervation of the V1 from the hemibrain which received the i.c.v. injection compared with the contralateral V1 of the injection (B); (C) the loss of the neuronal cell bodies within the HDB from the hemibrain which received the i.c.v. injection compared with the contralateral side of the injection (D). Note that only cholinergic but not PV cells are damaged by the immunotoxin. (E–H) Lesions 7 days after quisqualic acid injection within the HDB; (E) the loss of the cholinergic innervation of the V1 in the hemibrain which received the quisqualic acid infusion compared with the contralateral side from the lesion site (F); (G) the loss of the neuronal cell bodies within the lesion sites in HDB compared with non-injected HDB (H). Note that both cholinergic and PV (GABAergic) cells are destroyed. Scale bar=50 μm (A, B, E, F); 200 μm (C, D, G, H).

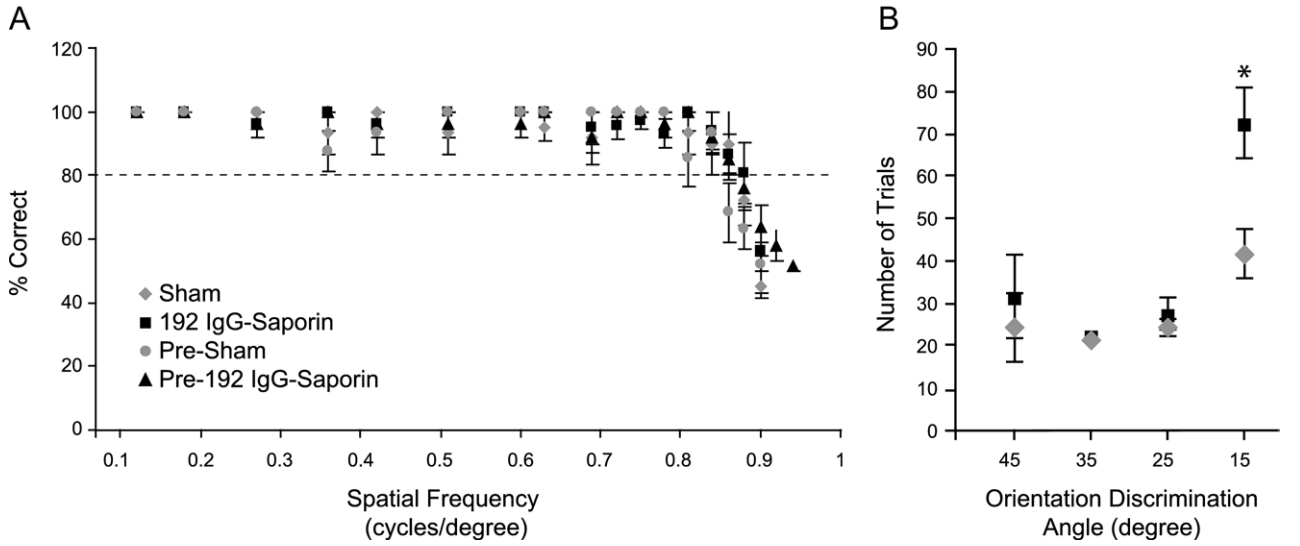

Fig. 2. Schematic representation of the effect of the cholinergic deficit on the (A) visual acuity and (B) the orientation discrimination performance of the rats in the visual water maze. (A) The visual acuity of the rats (spatial frequency) was not affected by intra-ventricular injection of non-targeted saporin (sham group) or 192 IgG Sap (192 IgG Sap group) compared with the acuity measured before injection (pre-sham and pre-192 IgG Sap groups). Thus, the cholinergic deficit did not affect the visual acuity of the rats. (B) The specific lesion of cholinergic fibers (192 IgG Sap group) increased the number of trials needed to perform close to the threshold (angle to discriminate: 15°). The cholinergic system is thus necessary for a proficient acquisition of the orientation discrimination task.

the ipsilateral side of the injection was highly reduced compared with the contralateral side which do not received the toxin (53% of reduction for quisqualic acid injection and 41% for 192 IgG Sap injection). Compared with an intact cortical cholinergic innervation (contralateral side of quisqualic injection, 314.3±40.3 mm/mm^2) the loss of cholinergic fibers was significantly reduced in the ipsilateral side of quisqualic acid (144.6±24.9 mm/mm^2, P=0.04) as well as IgG Sap (55.6±3.3 mm/mm^2, P=0.02) injection but also in the contralateral part of IgG Sap (94.0±12.6 mm/mm^2, P=0.04), which denotes a bilateral action of the IgG Sap although the severity of cholinergic fiber denervation was higher in the ipsilateral side.

Effects of cholinergic fibers lesion on the visual water task performance of the rats

The effect of the cholinergic deficit on visual capacities of the rats was examined using the visual water task (discrimination of pattern stimuli of increasing spatial frequency from a gray screen). The cholinergic deficit did not affect the retention of the task or the visual acuity of the lesioned rats compared with (1) its value before the lesion (0.88±0.01 vs. 0.88±0.02 cpd, P=0.349) or to (2) sham-lesioned animals (0.87±0.01 vs. 0.88±0.02 cpd, P=0.349) (Fig. 2). The percentage of errors made by the animals to reach the threshold was also not affected by the lesion (1.12±0.06 vs. 1.16±0.06, P=0.69), indicating a similar performance in the task. To test their learning abilities, rats were then exposed to patterns of different orientation and had to discriminate a diagonal pattern of increasing orientation from a horizontal one (learning of a new task). Pilot experiments suggested that the orientation discrimination threshold in rats was 15° over 10 trials (data not shown). In the present study, both groups were able to learn the task and to discriminate orientation until 15° resolution, however, performance of the lesioned rats was inferior to that of sham-operated animals (Fig. 2, Table 1). This difference in performance is expressed in terms of number of errors made to reach threshold. The lesioned rats had a greater number of errors than the sham animals in discrimination of a 45 degree orientation (45° vs. 90° grating, P=0.05; Table 1). Similarly, for finer orientation discrimination, i.e. for discrimination of orientation close to the threshold (15°, discrimination of a 75° vs. 90°grating), lesioned rats made more errors (P=0.03; Table 1) and they required more trials to perform the task than sham animals (75.7±8.7 vs. 41.7±6.0, P=0.01).

Effects of visual stimulation on c-Fos immunoreactivity in control-stimulated group

Subcortical structures were systematically observed to verify the validity of the stimulation although they were not the purpose of this study. As previously reported, the results showed c-Fos neuronal responses to the unilateral

Table 1. Number of errors performed by the rat to succeed in discriminating a diagonal pattern from a horizontal one

Angle to discriminate	Control n=7	IgG Sap lesion n=8
45°	1.8±0.5	3.9±0.7*
35°	0.5±0.3	1.4±0.5
25°	2.2±0.6	2.5±0.7
15°	3.7±0.3	5.6±0.6*

Values are expressed in mean±S.E.M. of number of errors performed by the rat in an orientation discrimination task in the visual water maze; n, number of animals per group.

* P<0.05, 192 IgG-saporin lesion+stimulation vs. control stimulated animals, unpaired Student t-test.

Table 2. Number of c-Fos immunoreactive neurons in selected regions of the ipsilateral and contralateral visual cortex to the visually-stimulated eye in control and cholinergic deficient rats

Cortical area	Control/sham-stim. $n=4$	Control/stimulation $n=5$	IgG Sap/stimulation $n=5$	Quisqualic/stimulation $n=4$	Scopo./stimulation $n=4$	CPP/stimulation $n=5$
V1 layer IV						
Ipsi	129±27	237±35	262±56	472±80†	105±45	164±60
Contra	165±27	423±45*†	237±72	321±85	51±15†	341±99*
V1 layer VI						
Ipsi	188±35	258±77	294±42	441±107	226±53	266±73
Contra	202±44	409±40	260±56	347±88	181±42	306±66
LM layers II/III/IV						
Ipsi	81±20	270±59†	328±77†	606±76†	183±54	151±44
Contra	134±35	336±48*†	231±17	485±62†	132±52	190±55

Values are expressed in mean±S.E.M. of c-Fos immunoreactive neurons/mm^2; n, number of animals per group.
* $P<0.05$, stimulated hemisphere vs. non-stimulated hemisphere, Wilcoxon signed rank test.
† $P<0.05$, compared to the sham-stimulated group counterparts, Mann-Whitney U test.

patterned stimulation in retinal projection regions, i.e. the contralateral stratum griseum superficiale of the superior colliculus, the optic tectum, the intermediate and ventral part of the lateral geniculate body but not the dorsolateral part—no c-Fos labeling in this last region is a known puzzling feature of the c-Fos labeling pattern induced by visual stimulation—in agreement with previous studies (Beaver et al., 1993; Kaczmarek and Chaudhuri, 1997; Correa-Lacarcel et al., 2000; but not Montero and Jian, 1995). The number of c-Fos immunoreactive neurons was significantly increased only in the contralateral hemisphere to the stimulated eye compared with sham animals, and only in layer IV of V1 (50–150% compared with ipsilateral cortex) and layer II/III/IV of LM, which are the thalamo-cortical receiving and feedforward cortico-cortical input receiving layers, respectively (Table 2). This is consistent with the anatomy of the visual pathways in the rat which are virtually totally crossed. This functional reactive area extended up to 2.4 mm (mm from Bregma, −5.6 to −8.0, data not shown) within the cortical area representing all parts of the visual field according to retinotopic cortical maps (Espinoza and Thomas, 1983; Paxinos and Watson, 1995). A 20% increase of number of immunoreactive cells in layer IV of the contralateral V1 compared with ipsilateral (675±46 vs. 786±40, $P<0.01$; data not shown) was also detected using Zif268 labeling. Enhanced c-Fos expressing neuron density was also observed in layer VI bilaterally with no detectable interhemispheric differences ($P=0.5$). No significant immunostaining was observed in the other cortical layers of V1. These cortical effects of the visual stimulation were modality specific since no change of c-Fos immunoreactivity in the contralateral S1HL was detected ($P=0.55$, Table 3).

Effect of the lesion of the cholinergic projections on the visual stimulation-evoked c-Fos immunoreactivity in the visual cortex

There was a significant effect of treatment on the number of c-Fos cells in layer IV of V1 (contralateral, $P=0.036$; ipsilateral $P=0.031$) as well as in LM (contralateral, $P=0.013$; ipsilateral $P=0.023$) among the different groups (Fig. 3; Table 2). However, comparison with the sham-stimulated counterparts showed that this change took place only in the control-stimulated rats with no substantial changes in the other groups (see Table 2), except for two regions described below. Accordingly, the number of c-Fos neurons in layer IV of V1 was not increased by the visual stimulation in IgG Sap–lesioned animals ($P=0.5$, Fig. 3c, Table 2). This indicates that the destruction of cholinergic projections in the activated cortex prevented the visually-driven upregulation of c-Fos production. However, the basal level of c-Fos neurons number was not affected, compared with sham-stimulated animals, by the partial destruction of the cholinergic fibers in the “non-injected” side in the 192 IgG Sap group (Table 2, first line).To verify that cholinergic fibers exclusively issued from the HDB were involved in this response, HDB pathway lesion was performed using quisqualic acid. Similar to IgG Sap lesion, cortical c-Fos staining induced by the patterned stimulation was abolished in the V1 and LM deprived of cholinergic basalo-cortical afferents ($P=0.14$, Fig. 3d, Table 2). It has to be noted that the global density of c-Fos immunoreactive neurons was slightly increased throughout the cortex in this condition ($P=0.03$ compared with sham-stimulated animals for V1 and LM, see Table 2), probably due to the concomitant destruction of the basal forebrain GABAergic projections resulting in cortical disinhibition (Freund and Gulyas, 1991).

Table 3. Number of c-Fos immunoreactive neurons in the V1 or the S1HL cortices ipsilateral or contralateral to the visually-stimulated eye

	V1 $n=5$	S1HL $n=5$
Layer IV		
Ipsi	237±35	163±25
Contra	423±45*	137±24
Layer VI		
Ipsi	258±77	166±35
Contra	409±40	140±18

Values are expressed in mean±S.E.M. of c-Fos immunoreactive neurons/mm^2; n, number of animals per group.
* $P<0.05$, visual cortex vs. somatosensory cortex, Mann-Whitney U test.

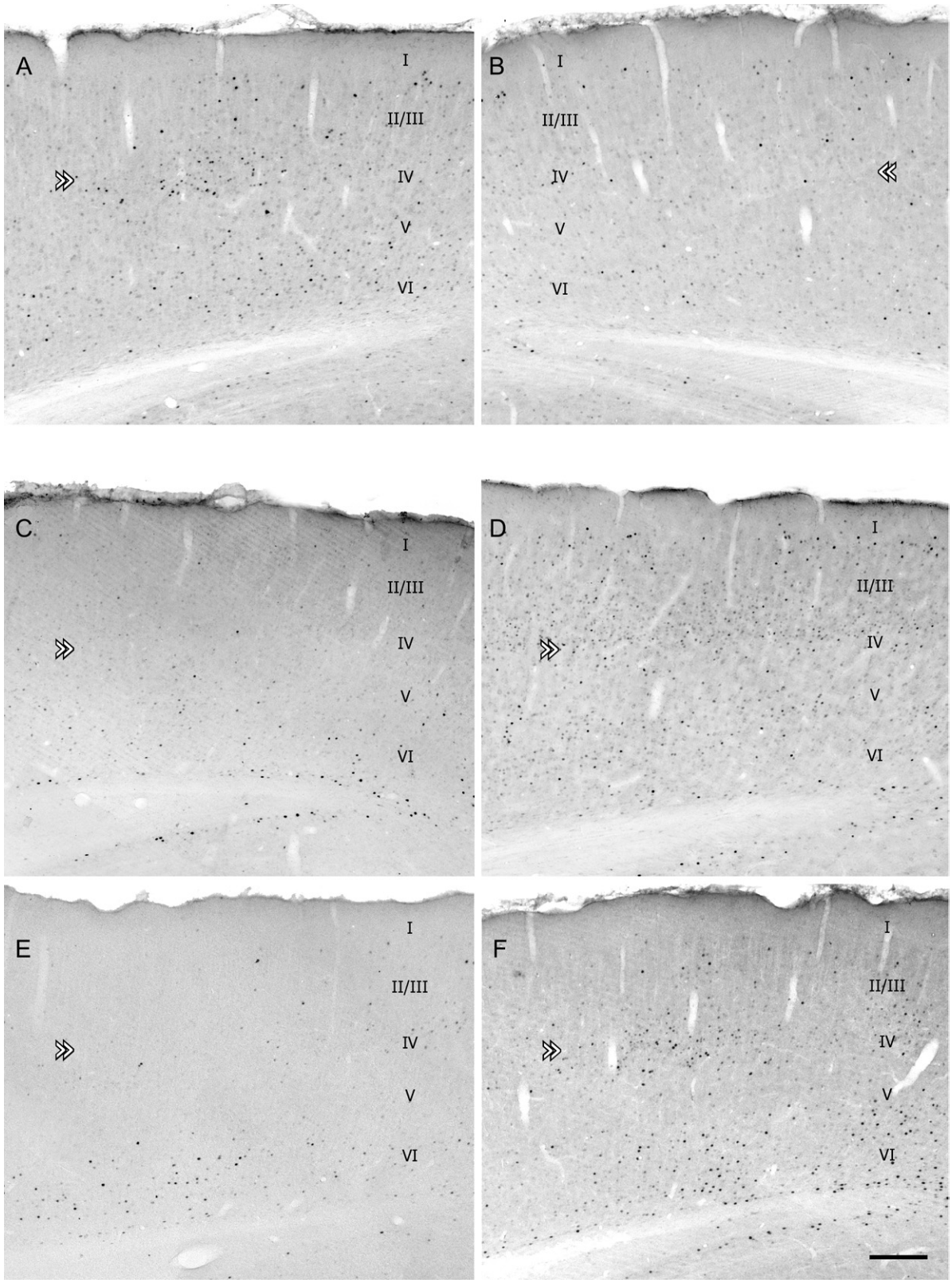

Fig. 3. Details of coronal brain sections at the level of V1 (mm from Bregma, AP −7.3, L 3–5) immunostained for c-Fos following patterned visual stimulation. (A, B) c-Fos immunoreactivity evoked during visual stimulation in the contralateral V1 (A) to the stimulated eye compared with the ipsilateral V1 (B) in one representative control-stimulated animal. Note that c-Fos positive neuronal density is mostly increased in layer IV of the contralateral (receiving the input from the stimulated eye) but not the ipsilateral cortex. (C–F) c-Fos immunoreactivity observed in the contralateral V1 to the stimulated eye in the 192 IgG Sap lesion+stimulation (C), quisqualic acid lesion+stimulation (D), scopolamine treatment+stimulation (E) and CPP treatment+stimulation (F) groups. Note that in all experiments except CPP treatment, the activity-dependent increase in c-Fos positive neuron number (shown in A) is abolished by the inactivation of the cholinergic transmission. Scale bar=200 μm.

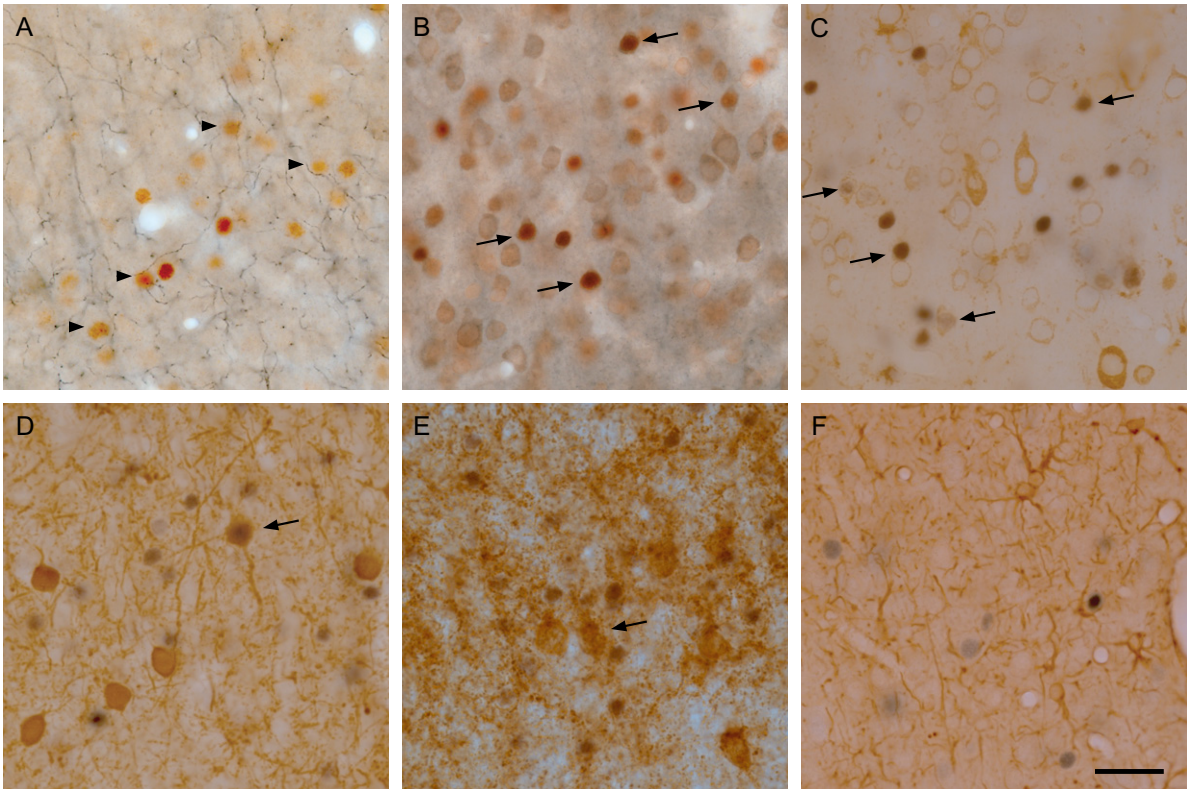

Fig. 4. High magnification of coronal brain sections (mm from Bregma, AP −7.3) double immunostained for c-Fos (brown in A, B and bluish–gray in C–F) and ChAT (bluish gray, A), GlutT (bluish gray, B), RBPC (brown, C), PV (brown, D), GAD67 (brown, E) or GFAP (brown, F). The photographs are taken within the cortical layer IV of V1. c-Fos immunoreactive cells (whose staining is located within the nucleus) are often embedded by ChAT immunoreactive neural processes (A) (arrowheads). The larger population of activated neurons was glutamatergic as shown by co-localization (arrows) of c-Fos with GlutT and less frequently GABAergic interneurons co-localizing GAD67 (see Results). c-Fos was never localized in astrocytes (F). Scale bar=50 μm.

Effects of blockers of cholinergic and glutamatergic receptors

c-Fos immunoreactivity in V1 and LM was virtually abolished bilaterally after the peripheral administration of the muscarinic antagonist scopolamine (Fig. 3e, Table 2), showing statistical difference compared with the sham-stimulated animals. There was thus no difference between this group and the dark-restrained animals. No apparent effects of scopolamine treatment were observed in the subcortical structures. In contrast, peripheral administration of the NMDA receptor antagonist CPP showed the same pattern of c-Fos immunolabeling in layer IV of V1 (contralateral-ipsilateral changes: $P=0.043$, Fig. 3f, Table 2) as in the sham/stimulated animals. This suggests a specific role of ACh in the activation of c-Fos by visual stimulation.

Cell type specificity of the cortical c-Fos immunoreactive cells

In the control-stimulated animals, the double immunocytochemistry study showed no co-localization of c-Fos with GFAP, confirming that the immunoreactive cortical cells were neuronal elements. Rare co-localization of c-Fos with either GAD67 (7.4%), PV (1.9%) or RBPC (7.4%) was detected (Fig. 4), whereas the most abundant c-Fos immunoreactive neurons were stained for GlutT (14.4%, Fig. 5). This indicates that visually-induced c-Fos expression mostly occurs in the spiny stellate cells of layer IV rather than the inhibitory interneurons or star pyramidal cells. It should however be mentioned that the RBPC labels the majority of pyramidal cells but not all of them (Staiger et al., 2002), suggesting that some non-immunoreactive star pyramidal cells of layer IV could also be activated. Triple immunolabeling with confocal microscopy showed close proximity between cholinergic fibers and c-Fos neurons co-localizing GlutT (Fig. 5), PV or GAD67 markers (data not shown) suggesting a cholinergic modulation by, at least, volume transmission.

DISCUSSION

The primary goal of this study was to elucidate the neuromodulatory role of the cholinergic system on visual cortex activation. This study shows that the deficit of ACh transmission results in a diminished efficiency of visual processing without affecting visual acuity or memory of the task. Furthermore this deficit abolishes the visually-elicited expression of c-Fos in glutamatergic neurons of the thalamo-recipient layer in V1 independent of NMDA receptor activation. These results suggest that the cholinergic input to the V1 improves the efficiency of the visual processing, particularly when a high level of attention is required.

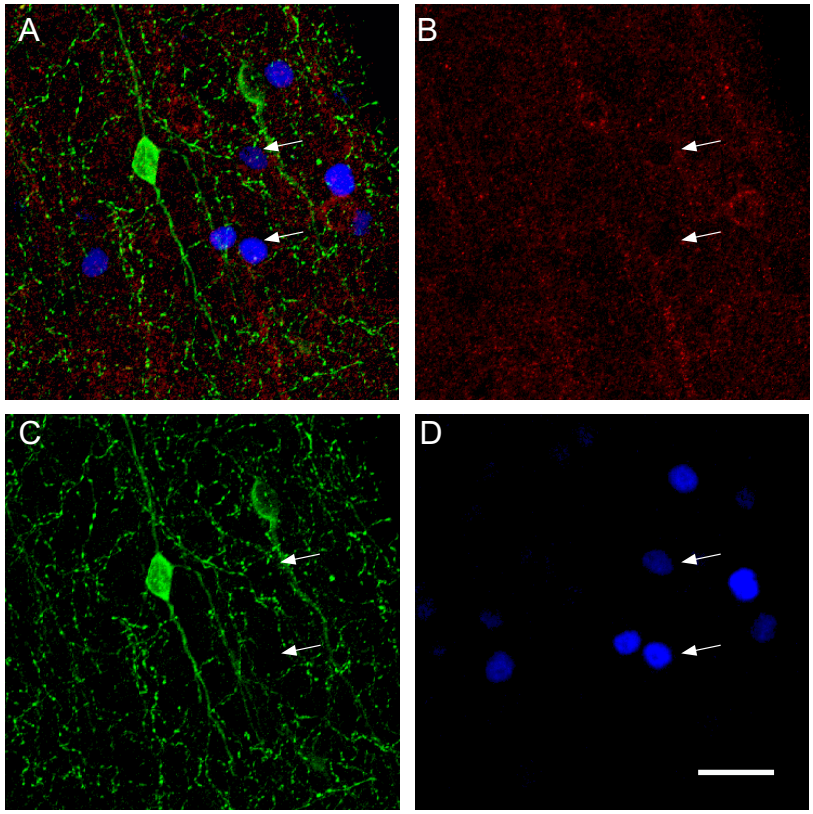

Fig. 5. Microphotographs of triple immunolabeling of brain sections in layer IV of V1 immunostained for c-Fos (blue), GlutT (red) and ChAT (green) obtained by confocal microscopy. (A) Confocal reconstruction from images obtained after scanning the same region at different wavelengths (B–D). ChAT immunoreactive fibers are in close proximity to c-Fos immunoreactive glutamatergic neurons (arrows). Scale bar=70 μm.

Effects of basal forebrain ACh depletion on visual capacities

Previous studies have shown that ACh was released in V1 during visual stimulation (Collier and Mitchell, 1966; Fournier et al., 2004; Laplante et al., 2005) and could modulate visual cortical cell excitability (Kuczewski et al., 2005) and properties (Greuel et al., 1988; Murphy and Sillito, 1991; Roberts et al., 2005; Zinke et al., 2006). However, it was not clear if/how the basalocortical cholinergic system actively participates *in vivo* in cortical processing of visual stimuli. To evaluate the functional impact of the cholinergic control of thalamocortical inputs, we tested the visual acuity and visual learning capacity of IgG Sap rats using the visual water maze (Prusky et al., 2000). Based on the pre- and post-lesion visual acuity task, the complete lesion to the cholinergic system did not affect task retention and post-lesion performance in the pre-lesion learned task. Learning capacity was also preserved in these IgG Sap rats considering that animals were able to learn the new orientation task. However, more trials were needed for the lesioned rats during the acquisition of the new orientation task (for which rats did not received any training before the lesion) as well as to succeed near the orientation discrimination threshold. It is reasonable to assume that this condition requires a higher level of attention to match the difficulty of the discrimination. These data suggest that cholinergic innervation is not necessarily a requisite for vision per se, however an intact cholinergic system maybe necessary for proficient discrimination learning.

The modulatory role of the cholinergic system on learning tasks has been well documented (Fletcher and Wilson, 2002; Sarter et al., 2005; Bennett et al., 2007). Although this system is commonly associated with cognition, memory (Bennett et al., 2007), and attention (Muir et al., 1992; Sarter et al., 2005), the grating discrimination task used in the current study is indicative of a visual learning task (Frost et al., 2000) independent of reward pathways. Since the IgG Sap lesion resulted in substantial cholinergic loss throughout the basal forebrain, a cognitive, memory, and attentional component to these behavioral results cannot be entirely ruled out.

Visually-induced c-Fos expression is modulated by cholinergic transmission

This study further showed by single-cell resolution that endogenous cholinergic transmission especially from the basal forebrain is necessary for a complete stimulus-driven response of the V1 neurons. This was demonstrated by the abolishment of the activity-evoked c-Fos expression following (1) the lesion experiments, showing the specificity of the response to the cholinergic projections from the HDB to V1, and following (2) the scopolamine injection, showing the muscarinic transmission involvement in this effect.

Cholinergic contribution to c-Fos expression has been shown previously in S1HL after scopolamine injection (Weiner et al., 1991; Zimmer et al., 1998; Kocharyan et al., 2005), intracortical infusion of exogenous ACh (Hughes and Dragunow, 1994), and cholinergic agonists (Bernard et al., 1993). The present study further demonstrates a positive correspondence between the strongest density of cholinergic HDB projections in V1 (Eckenstein et al., 1988; Mechawar et al., 2000) and c-Fos immunoreactivity. Moreover it suggests that the scopolamine effect on c-Fos immunoreactivity reported here most likely results from the inhibition of cortical mAChRs, considering that V1 is endowed with postsynaptic M1 and M4 mAChRs (Volpicelli and Levey, 2004) and presynaptic M2 mAChRs (Mrzljak et al., 1998) and that visual cell excitation is impaired in cortical slices of mAChRs knockout mice (Kuczewski et al., 2005). However, it could not be excluded that i.p.-administered scopolamine could also have blocked cholinoceptive subcortical structures including the dorsal geniculate nucleus (McCormick, 1992) or the ascending reticular formation (Lewandowski et al., 1993). Although not tested, nicotinic receptors, which are also enriched in layer IV of the visual cortex (Prusky et al., 1988; Aubert et al., 1995) could also have played a presynaptic excitatory effect on the thalamocortical fibers (Gil et al., 1997) or on GABAergic neurons of layer I (Christophe et al., 2002). Their deactivation after cholinergic fibers lesion could have contributed in the decrease of neuronal activity of the cortical cells. The analysis of the LM area, showing an absence of neuronal activation in cholinergic deficient groups, further suggests that cholinergic deficit influences the visual flow from the first step, and then consequently throughout the visual pathways.

Since thalamic inputs to the visual cortex are glutamatergic (Nahmani and Erisir, 2005) and c-Fos induction is dependent on a rise in intracellular calcium (Kaczmarek and Chaudhuri, 1997; West et al., 2001), the possibility that the c-Fos increase in the current experiment was mediated through NMDA receptor activation was further investigated. c-Fos labeling in layer IV was not affected by CPP administration, indicating that NMDA receptors were not involved in this use-dependent effect. Other studies have reported that NMDA receptor antagonists such as MK-801 often failed to abolish c-Fos expression (Bernard et al., 1993; Soyguder, 2004). These results suggest that the cholinergic-dependent c-Fos induction in the cortical layer IV was not mediated by NMDA transmission and most probably did not result from classical long term potentiation.

Visual cortex circuitry involved in c-Fos induction

It was also not known which neuronal elements of the visual cortex microcircuitry were controlled by the cholinergic fibers. The results here suggest a predominant activity-induced cortical c-Fos response in layer IV, which is in line with previous reports on layer pattern of c-Fos expression evoked by sensory stimulation in visual (Montero and Jian, 1995) or S1HL (Gass et al., 1993; Staiger et al., 2000) of rats. Layer IV of the rat visual cortex is composed of various excitatory neurons (spiny stellate cells and few star pyramidal cells) and inhibitory interneurons (mostly PV basket and chandelier cells) (Peters et al., 1979; Gonchar and Burkhalter, 2003; Markram et al., 2004) as well as feedforward thalamocortical or intracortical projections from layer VI glutamatergic fibers (Stratford et al., 1996). All of these elements were found in close proximity to cholinergic fibers as shown by the triple immunolabeling. It appeared that the most abundant c-Fos reactive neurons were glutamatergic (14%) and to a smaller amount GABAergic (7–8%). Whether this proportion results from a stronger density of glutamatergic than GABA neurons or to a specific activation of glutamatergic neurons remains to be clarified.

The proportion of c-Fos cells that co-localized GAD is consistent with precedent observations made in infra- and subgranular layer of the cat V1 (Van der Gucht et al., 2002) and the common low c-Fos reactivity of the GABAergic cells to the sensory stimulation (Bubser et al., 1998; Staiger et al., 2002). It is known that PV cells represent 72% of the GABAergic neurons of layer IV of the visual cortex (Gonchar and Burkhalter, 1999) and as fast-spiking interneurons, they are powerful modulators to shape the output of the pyramidal cells and spiny stellate cells to provide a fine tuning of the afferent inputs (Swadlow, 2002). Our results showing only 2% of PV staining among c-Fos cells suggest that PV neurons are not a predominant target of the cholinergic-dependent visual activation. This is in line with a recent study showing that the cholinergic modulation of the visual cortex in monkeys does not result in improving the orientation tuning functions in V1 (Zinke et al., 2006).

Neuronal activation detected by c-Fos as a result of the visual stimulation was more frequent in the GlutT neurons of layer IV. Ultrastructural appositions between cholinergic fibers and glutamatergic neurons and fibers have been described in this layer of the visual cortex, at least in the cat (Aoki and Kabak, 1992). In our study, RBPC marker, shown as a good marker for pyramidal cells (Staiger et al., 2002), was however rarely co-localized with c-Fos. It thus appeared that the majority of c-Fos expressing cells belonged to the population of spiny stellate cells. This was confirmed by the morphology of GlutT cells reactive for c-Fos (data not shown). Spiny stellate cells being the main target of thalamocortical pathways (Stratford et al., 1996), this finding suggests that induction of c-Fos in the V1 cortical cells most likely corresponds to the thalamic input on the excitatory neurons of V1.

The diversity of the possible neuronal targets of the cholinergic projections as well as the cholinergic enhancement on the functional activity of the thalamic fiber recipient cortical—layer IV—but not the cortico-cortical connections recipient layers—layer II/III—fits well with the proposed models of the modulatory role of the cholinergic pathways on cortex circuitry. These models suggest that the cortical cholinergic inputs may switch the cortical processing mode from an intracortical to a (sensory) input-processing mode (Gil et al., 1997; Hasselmo, 2005; Yu and Dayan, 2005; Levy et al., 2006; Zinke et

al., 2006) by enhancing the stimulus-driven inputs and inhibiting the feedback cortical control.

CONCLUSION

In conclusion, these results indicate that HDB cholinergic fibers may enhance post-synaptic processing of the visual stimuli in the visual cortex cells. Altogether with the behavioral results, this role could be related to a better efficiency of visual discrimination or learning performance specifically in conditions requiring high levels of attention. These mechanisms would result in the enhancement of the cortical representation of incoming visual inputs, i.e. sensory-induced cortical plasticity.

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REFERENCES

Aoki C, Kabak S (1992) Cholinergic terminals in the cat visual cortex: ultrastructural basis for interaction with glutamate-immunoreactive neurons and other cells. Vis Neurosci 8:177–191.

Arnold HM, Burk JA, Hodgson EM, Sarter M, Bruno JP (2002) Differential cortical acetylcholine release in rats performing a sustained attention task versus behavioral control tasks that do not explicitly tax attention. Neuroscience 114:451–460.

Aubert I, Rowe W, Meaney MJ, Gauthier S, Quirion R (1995) Cholinergic markers in aged cognitively impaired Long-Evans rats. Neuroscience 67:277–292.

Beaver CJ, Mitchell DE, Robertson HA (1993) Immunohistochemical study of the pattern of rapid expression of C-Fos protein in the visual cortex of dark-reared kittens following initial exposure to light. J Comp Neurol 333:469–484.

Bennett BM, Reynolds JN, Prusky GT, Douglas RM, Sutherland RJ, Thatcher GR (2007) Cognitive deficits in rats after forebrain cholinergic depletion are reversed by a novel NO mimetic nitrate ester. Neuropsychopharmacology 32:505–513.

Bernard V, Dumartin B, Lamy E, Bloch B (1993) Fos immunoreactivity after stimulation or inhibition of muscarinic receptors indicates anatomical specificity for cholinergic control of striatal efferent neurons and cortical neurons in the rat. Eur J Neurosci 5: 1218–1225.

Brocher S, Artola A, Singer W (1992) Agonists of cholinergic and noradrenergic receptors facilitate synergistically the induction of long-term potentiation in slices of rat visual cortex. Brain Res 573:27–36.

Bubser M, de Brabander JM, Timmerman W, Feenstra MG, Erdtsieck-Ernste EB, Rinkens A, van Uum JF, Westerink BH (1998) Disinhibition of the mediodorsal thalamus induces fos-like immunoreactivity in both pyramidal and GABA-containing neurons in the medial prefrontal cortex of rats, but does not affect prefrontal extracellular GABA levels. Synapse 30:156–165.

Christophe E, Roebuck A, Staiger JF, Lavery DJ, Charpak S, Audinat E (2002) Two types of nicotinic receptors mediate an excitation of neocortical layer I interneurons. J Neurophysiol 88:1318–1327.

Clapp WC, Eckert MJ, Teyler TJ, Abraham WC (2006) Rapid visual stimulation induces N-methyl-D-aspartate receptor-dependent sensory long-term potentiation in the rat cortex. Neuroreport 17:511–515.

Collier B, Mitchell JF (1966) The central release of acetylcholine during stimulation of the visual pathway. J Physiol 184:239–254.

Correa-Lacarcel J, Pujante MJ, Terol FF, Almenar-Garcia V, Puchades-Orts A, Ballesta JJ, Lloret J, Robles JA, Sanchez-del-Campo F (2000) Stimulus frequency affects c-fos expression in the rat visual system. J Chem Neuroanat 18:135–146.

Dauphin F, Lacombe P, Sercombe R, Hamel E, Seylaz J (1991) Hypercapnia and stimulation of the substantia innominata increase rat frontal cortical blood flow by different cholinergic mechanisms. Brain Res 553:75–83.

Eckenstein FP, Baughman RW, Quinn J (1988) An anatomical study of cholinergic innervation in rat cerebral cortex. Neuroscience 25: 457–474.

Espinoza SG, Thomas HC (1983) Retinotopic organization of striate and extrastriate visual cortex in the hooded rat. Brain Res 272:137–144.

Fletcher ML, Wilson DA (2002) Experience modifies olfactory acuity: acetylcholine-dependent learning decreases behavioral generalization between similar odorants. J Neurosci 22:RC201.

Fournier GN, Semba K, Rasmusson DD (2004) Modality- and region-specific acetylcholine release in the rat neocortex. Neuroscience 126:257–262.

Freund TF, Gulyas AI (1991) GABAergic interneurons containing calbindin D28K or somatostatin are major targets of GABAergic basal forebrain afferents in the rat neocortex. J Comp Neurol 314: 187–199.

Frost DO, Boire D, Gingras G, Ptito M (2000) Surgically created neural pathways mediate visual pattern discrimination. Proc Natl Acad Sci U S A 97:11068–11073.

Gass P, Herdegen T, Bravo R, Kiessling M (1993) Spatiotemporal induction of immediate early genes in the rat brain after limbic seizures: effects of NMDA receptor antagonist MK-801. Eur J Neurosci 5:933–943.

Gaykema RP, Luiten PG, Nyakas C, Traber J (1990) Cortical projection patterns of the medial septum-diagonal band complex. J Comp Neurol 293:103–124.

Gil Z, Connors BW, Amitai Y (1997) Differential regulation of neocortical synapses by neuromodulators and activity. Neuron 19: 679–686.

Gilbert C, Ito M, Kapadia M, Westheimer G (2000) Interactions between attention, context and learning in primary visual cortex. Vision Res 40:1217–1226.

Gonchar Y, Burkhalter A (1999) Differential subcellular localization of forward and feedback interareal inputs to parvalbumin expressing GABAergic neurons in rat visual cortex. J Comp Neurol 406: 346–360.

Gonchar Y, Burkhalter A (2003) Distinct GABAergic targets of feedforward and feedback connections between lower and higher areas of rat visual cortex. J Neurosci 23:10904–10912.

Greuel JM, Luhmann HJ, Singer W (1988) Pharmacological induction of use-dependent receptive field modifications in the visual cortex. Science 242:74–77.

Gu Q (2002) Neuromodulatory transmitter systems in the cortex and their role in cortical plasticity. Neuroscience 111:815–835.

Hasselmo M (2005) Expecting the unexpected: modeling of neuromodulation. Neuron 46:526–528.

Hughes P, Dragunow M (1994) Activation of pirenzepine-sensitive muscarinic receptors induces a specific pattern of immediate-early gene expression in rat brain neurons. Brain Res Mol Brain Res 24:166–178.

Inglis FM, Fibiger HC (1995) Increases in hippocampal and frontal cortical acetylcholine release associated with presentation of sensory stimuli. Neuroscience 66:81–86.

Jimenez-Capdeville ME, Dykes RW, Myasnikov AA (1997) Differential control of cortical activity by the basal forebrain in rats: a role for both cholinergic and inhibitory influences. J Comp Neurol 381: 53–67.

Kaczmarek L, Chaudhuri A (1997) Sensory regulation of immediate-early gene expression in mammalian visual cortex: implications for

functional mapping and neural plasticity. Brain Res Brain Res Rev 23:237–256.
Kilgard MP, Merzenich MM (1998) Cortical map reorganization enabled by nucleus basalis activity. Science 279:1714–1718.
Kimura F, Fukuda M, Tsumoto T (1999) Acetylcholine suppresses the spread of excitation in the visual cortex revealed by optical recording: possible differential effect depending on the source of input. Eur J Neurosci 11:3597–3609.
Kirkwood A, Rozas C, Kirkwood J, Perez F, Bear MF (1999) Modulation of long-term synaptic depression in visual cortex by acetylcholine and norepinephrine. J Neurosci 19:1599–1609.
Kocharyan A, Fernandes P, Serluca N, Vaucher E, Hamel E (2005) Chemical or electrical stimulation of basal forebrain neurons activates specific subsets of cortical GABA-interneurons in parallel with increases in cortical cerebral blood flow. J Cereb Blood Flow Metab 28:221–231.
Kuczewski N, Aztiria E, Gautam D, Wess J, Domenici L (2005) Acetylcholine modulates cortical synaptic transmission via different muscarinic receptors, as studied with receptor knockout mice. J Physiol 566:907–919.
Laplante F, Morin Y, Quirion R, Vaucher E (2005) Acetylcholine release is elicited in the visual cortex, but not in the prefrontal cortex, by patterned visual stimulation: a dual in vivo microdialysis study with functional correlates in the rat brain. Neuroscience 132: 501–510.
Levy RB, Reyes AD, Aoki C (2006) Nicotinic and muscarinic reduction of unitary excitatory postsynaptic potentials in sensory cortex; dual intracellular recording in vitro. J Neurophysiol, in press.
Lewandowski MH, Muller CM, Singer W (1993) Reticular facilitation of cat visual cortical responses is mediated by nicotinic and muscarinic cholinergic mechanisms. Exp Brain Res 96:1–7.
Markram H, Toledo-Rodriguez M, Wang Y, Gupta A, Silberberg G, Wu C (2004) Interneurons of the neocortical inhibitory system. Nat Rev Neurosci 5:793–807.
McCormick DA (1992) Cellular mechanisms underlying cholinergic and noradrenergic modulation of neuronal firing mode in the cat and guinea pig dorsal lateral geniculate nucleus. J Neurosci 12:278–289.
Mechawar N, Cozzari C, Descarries L (2000) Cholinergic innervation in adult rat cerebral cortex: A quantitative immunocytochemical description. J Comp Neurol 428:305–318.
Montero VM, Jian S (1995) Induction of c-fos protein by patterned visual stimulation in central visual pathways of the rat. Brain Res 690:189–199.
Mrzljak L, Levey AI, Belcher S, Goldman-Rakic PS (1998) Localization of the m2 muscarinic acetylcholine receptor protein and mRNA in cortical neurons of the normal and cholinergically deafferented rhesus monkey. JComp Neurol 390:112–132.
Muir JL, Dunnett SB, Robbins TW, Everitt BJ (1992) Attentional functions of the forebrain cholinergic systems: effects of intraventricular hemicholinium, physostigmine, basal forebrain lesions and intracortical grafts on a multiple-choice serial reaction time task. Exp Brain Res 89:611–622.
Murphy PC, Sillito AM (1991) Cholinergic enhancement of direction selectivity in the visual cortex of the cat. Neuroscience 40:13–20.
Nahmani M, Erisir A (2005) VGluT2 immunochemistry identifies thalamocortical terminals in layer 4 of adult and developing visual cortex. J Comp Neurol 484:458–473.
Paxinos G, Watson CR (1995) The rat brain in stereotaxic coordinates. Sydney: Academic Press.
Peters A, Proskauer CC, Feldman ML, Kimerer L (1979) The projection of the lateral geniculate nucleus to area 17 of the rat cerebral cortex. V. Degenerating axon terminals synapsing with Golgi impregnated neurons. J Neurocytol 8:331–357.
Prusky GT, Arbuckle JM, Cynader MS (1988) Transient concordant distributions of nicotinic receptors and acetylcholinesterase activity in infant rat visual cortex. Brain Res 467:154–159.
Prusky GT, West PW, Douglas RM (2000) Behavioral assessment of visual acuity in mice and rats. Vision Res 40:2201–2209.
Roberts MJ, Zinke W, Guo K, Robertson R, McDonald JS, Thiele A (2005) Acetylcholine dynamically controls spatial integration in marmoset primary visual cortex. J Neurophysiol 93:2062–2072.
Sarter M, Hasselmo ME, Bruno JP, Givens B (2005) Unraveling the attentional functions of cortical cholinergic inputs: interactions between signal-driven and cognitive modulation of signal detection. Brain Res Brain Res Rev 48:98–111.
Soyguder Z (2004) NMDA and AMPA/KA receptors are involved in the c-Fos expression following mustard oil activation of C-fibres. J Chem Neuroanat 28:163–169.
Staiger JF, Bisler S, Schleicher A, Gass P, Stehle JH, Zilles K (2000) Exploration of a novel environment leads to the expression of inducible transcription factors in barrel-related columns. Neuroscience 99:7–16.
Staiger JF, Masanneck C, Bisler S, Schleicher A, Zuschratter W, Zilles K (2002) Excitatory and inhibitory neurons express c-Fos in barrel-related columns after exploration of a novel environment. Neuroscience 109:687–699.
Stratford KJ, Tarczy-Hornoch K, Martin KA, Bannister NJ, Jack JJ (1996) Excitatory synaptic inputs to spiny stellate cells in cat visual cortex. Nature 382:258–261.
Swadlow HA (2002) Thalamocortical control of feed-forward inhibition in awake somatosensory "barrel" cortex. Philos Trans R Soc Lond B Biol Sci 357:1717–1727.
Thiel CM, Friston KJ, Dolan RJ (2002) Cholinergic modulation of experience-dependent plasticity in human auditory cortex. Neuron 35:567–574.
Van der Gucht E, Clerens S, Cromphout K, Vandesande F, Arckens L (2002) Differential expression of c-fos in subtypes of GABAergic cells following sensory stimulation in the cat primary visual cortex. Eur J Neurosci 16:1620–1626.
Verdier D, Dykes RW (2001) Long-term cholinergic enhancement of evoked potentials in rat hindlimb somatosensory cortex displays characteristics of long-term potentiation. Exp Brain Res 137: 71–82.
Volpicelli LA, Levey AI (2004) Muscarinic acetylcholine receptor subtypes in cerebral cortex and hippocampus. Prog Brain Res 145:59–66.
Weiner ED, Kalasapudi VD, Papolos DF, Lachman HM (1991) Lithium augments pilocarpine-induced fos gene expression in rat brain. Brain Res 553:117–122.
West AE, Chen WG, Dalva MB, Dolmetsch RE, Kornhauser JM, Shaywitz AJ, Takasu MA, Tao X, Greenberg ME (2001) Calcium regulation of neuronal gene expression. Proc Natl Acad Sci U S A 98:11024–11031.
Yu AJ, Dayan P (2005) Uncertainty, neuromodulation, and attention. Neuron 46:681–692.
Zimmer LA, Ennis M, Wiley RG, Shipley MT (1998) Nerve gas-induced seizures: role of acetylcholine in the rapid induction of Fos and glial fibrillary acidic protein in piriform cortex. J Neurosci 18:3897–3908.
Zinke W, Roberts MJ, Guo K, McDonald JS, Robertson R, Thiele A (2006) Cholinergic modulation of response properties and orientation tuning of neurons in primary visual cortex of anaesthetized marmoset monkeys. Eur J Neurosci 24:314–328.

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