Eur J Anat, 4 (2): 83-94 (2000)

c-Fos activation in the superior olivary complex and nuclei of the lateral lemniscus of sound-stimulated rats

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Summary

The pattern of c-fos expression was analyzed along the superior olivary complex and the nuclei of the lateral lemniscus of rats subjected to different types of sound stimuli. A detailed map was obtained by comparing parallel series stained with Giemsa, acetyl cholinesterase histochemistry, and fos immunocytochemistry. The experimental groups consisted of rats isolated for 24 hours in a soundproof cage and sound-stimulated rats. Sound-stimulated rats received 5 KHz and a combination of 4 tones.

The superior olivary complex was divided into three levels. The rostral level I contained only the rostral periolivary nucleus. In level II, a core and periolivary nuclei were differentiated. The core was composed of the medial supraolivary nucleus and the lateral supraolivary nucleus and was surrounded by the dorsal periolivary nucleus, the superior periolivary nucleus, the nucleus of the trapezoid body and the medial and lateral divisions of the ventral periolivary nucleus. Caudal to level II, level III, only contained the caudal periolivary nucleus. The medial division of the ventral periolivary nucleus and the rostral periolivary nucleus showed c-fos activation in isolated conditions and were strongly activated following any kind of sound stimulus. The rest of the superior olivary nuclei showed no c-fos activation in isolated rats and a weak but consistently constant activation following sound stimulation. No c-fos activation was obtained in the core of the lateral supraolivary nucleus; however, some labeling occurred in the hilus around the nucleus.

The lateral lemniscus complex consisted of the dorsal, intermediate and ventral nuclei. Additionally, the paralemniscal nucleus and the nucleus sagulum were considered. c-fos activation was absent in isolated rats and increased strongly following all types of stimulation in the nuclei of the lateral lemniscus. Labeled nuclei formed bands parallel and perpendicular to the lemniscal fiber tracts. Labeling was scarce in the paralemniscal nucleus and in the nucleus sagulum.

These results lead us to conclude that both the superior olivary complex and the nuclei of the lateral lemniscus respond to sound stimuli by activating the c-fos immediate early gene. c-fos activation may play an important role in processing auditory information in these brainstem steps of the auditory pathway.

Key Words: Immediate early genes - Auditory system - Acetylcholinesterase

Introduction

The auditory information ascending from the cochlear complex to the inferior colliculus is filtered and modulated by several structures of the brainstem; i.e., the superior olivary complex and the nuclei of the lateral lemniscus (Aitkin, 1989).

Submitted: July 3, 2000
Accepted: August 31, 2000

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The superior olivary complex (SOC) is a group of nuclei located ventrally at the level of the caudal pons. The SOC lies just rostral to the facial motor nucleus and between the root of the facial nerve laterally and the pyramidal bundle medially. The SOC is considered to be the first level of the auditory system at which there is integration of information from both ears onto individual neurons. The integrated information is then transferred to other cells within the SOC for further processing and to higher centers, the nuclei of the lateral lemniscus (LL) and inferior colliculus (IC), as well as back down to the cochlear nuclei (CN) and cochlea through the olivocochlear bundle (Schwartz, 1992).

The lateral lemniscus is a tract of fibers running from the lateral side and rostral end of each SOC to the ipsilateral IC. Groups of neurons, the LL nuclei, are located within the LL. Axons from the CN and trapezoid body also contribute information to the LL. The LL nuclei integrate information directly from the CN, from the SOC, and from the contralateral LL before transmitting it to the IC. The LL neurons may also interact with reticular formation pathways (Schwartz, 1992, Bajo et al., 1993, Merchán et al., 1994, Merchán and Berbel, 1996).

One of the problems in understanding the roles of the SOC and LL in auditory processing is the lack of studies correlating structure and function. In recent years, the expression of immediate early genes (IEG) has been used as a functional marker. c-fos is an IEG that is rapidly and transiently induced by extracellular stimulation (for a review, see Sheng et al., 1990; Morgan et al., 1991).

The expression of c-fos can be detected by "in situ" hybridization with mRNA or by immunocytochemistry (ICC) against its protein product. The neurons of the auditory pathway can be induced to express c-fos after sound stimulation. White noise and pure tones are able to induce an increase in fos-like immunoreactivity (FLI) throughout the auditory system. c-fos activation has been found in the auditory pathway from the cochlear nuclei to the temporal auditory cortex (Ehret et al., 1991; Friauf, 1992; Rouiller et al., 1992; Sato et al., 1992, 1993; Adams, 1995; Brown and Liu, 1995; Olucha et al., 1997; Riera Sala et al., 1999).

However, there is as yet no detailed description of the expression of c-fos in the SOC and LL after a variety of sound stimuli involving the comparison of parallel series of c-fos ICC and samples reacted to Nissl or chemical markers. To fill this lacuna we used a variety sound stimuli and revealed consecutive sections of each area with acetylcholinesterase to obtain an accurate location of c-fos activated nuclei.

Material and Methods

The study was based on 16 adult male Sprague-Dawley rats weighing 250-350 gr. maintained on a 12:12 light/dark cycle, with temperature and humidity controlled and free access to food and water. All protocols were approved by the Committee of the Faculty of Medicine of the University of Valencia for Animal Care and Ethics.

Sound stimulation

Rats were randomly separated into three groups: a) control isolated, b) 5 KHz sound-stimulated, and c) random mixture of 4 tones (Table I). In all cases, rats were isolated for 24 hours in a sound-proof cage before starting the stimuli. Mean sound intensity in the cage was less than 30 dB SPL.

Isolated rats (group a) did not receive any sound stimuli in the 24 hours immediately before sacrifice. Rats stimulated with 5 KHz (group b) received 70dB SPL over 1 minute 1 hour before sacrifice. Rats receiving 4 tones (group c) were given the last sound 1 hour before sacrifice. Tones consisted of 4 consecutive pulses of 0.25 KHz, 1 KHz, 8 KHz and 20 KHz; each pulse was 100 msec and the pulses were separated from one another by 200 msec. This sequence was repeated 25 times over 30 sec, followed by a period of silence of 2 min. 30 sec. This set of stimuli was repeated to a total amount of 20 min. All tones were adjusted to an intensity of 80 dB SPL by measuring them with a Brüel & Kaejer Type 2235 sound level meter with a 4176 microphone. Sounds were generated with a sound card connected to a FoneStar amplifier. The amplifier was connected to two loud speakers, one for 0.01-10 KHz tones, and the other for 8-30 KHz tones.

c-fos immunocytochemistry

One hour after stimulation had finished, the animals were anesthetized and perfused transcardially. For anesthesia, the animals received an overdose of Nembutal (100 mg/Kg). The animals were then perfused with saline (0.9%) and fixative (4% PFA in 0.1M phosphate buffer, pH 7.4). The time between the injection of the anesthesia and the perfusion of the fixative was always less than 10 minutes. After the perfusion, the brains were removed from the skulls and postfixed overnight at 4°C in the same fixative. Then, they were immersed in 30% sucrose for 2-3 days for cryoprotection and, finally, 40µm thick coronal frozen sections were collected and free-floating-processed for immunocytochemistry. From each brain, 6 serial frozen sections were obtained and 4 alternate series were revealed either for c-fos immunocytochemistry or for acetylcholinester-

ase. The other 2 series were frozen for additional studies.

For c-fos immunocytochemistry Genosys antisera (catalogue n° OA-11-824) was used as first antibody. Antisera were raised in sheep using a 16 amino-acid synthetic peptide derived from a conserved region of both mouse and human c-fos (Straaten et al., 1983). After endogenous peroxidase reduction with 0.3% H_2O_2 in 0.05M PBS, pH 7.4, sections were incubated in the solution of the first antibody (diluted 1:2,000) for 48 hours at 4°C, followed by the conventional rabbit anti sheep avidin-biotin reaction (Vectastain, Vector). Peroxidase was visualized by DAB reaction intensified by ammonium sulfate in Tris buffer saline at pH 8.0.

Some serial sections underwent the same treatment with the exception of incubation in the first antibody. The sections were mounted in gelatin-coated slides, air dried and coverslipped with Eukitt. Some sections were contrasted with 0.5% neutral red before coverslipping.

Acetylcholinesterase histochemistry

For AChE histochemistry, free-floating sections were rinsed twice in PBS and twice in 0.05M acetate buffer, pH 5. The reaction was carried on by placing the sections in the incubation solution (0.125% acetylthiocholine iodide, 0.3 M cupric sulfate, 0.2 % glycine, 0.05M acetate buffer and 10^{-3} M ethopropazine) for 3 hours at 37°C. After incubation, the sections were rinsed in distilled water and reacted for 3 minutes in 10% potasium ferricyanide. Sections were rinsed again in 0.01M PBS and mounted on gelatin-coated slides.

Data analysis

Drawings were made with a camera lucida tube attached to a ZEISS microscope. For figure designs, the images were captured by means of a Pixera camera (mod. VCS-10132). The camera was attached to a Nikon Eclipse E600 connected to a PC computer. The images thus captured were converted to a grey scale and automatically adjusted for brightness and contrast with the Adobe Photoshop 4.0 and then exported to CorelDraw for labeling and lettering.

Results

Superior Olivary Complex

The superior olivary complex (SOC) of the rats was composed of a group of nuclei located in the ventral region of the caudal pons.

In our samples, the SOC comprised from level —8.00 to level—10.30 caudal to Bregma according to Paxinos and Watson´s (1986) atlas. In frontal sections we have differentiated three levels.

At *level I* (Fig. 1A, 1D), the SOC was composed of the rostral periolivary region (RPO). This region was located ventromedially to the ventral nuclei of the lateral lemniscus (VLL) and dorsolaterally to the longitudinal fasciculus pons (lfp) and the pontine nuclei (Pn). The RPO was easily recognized in AChE samples since it showed an intermediate reaction product between the intense reaction in the pons and the week reaction in the VLL.

At *level II* (Fig. 1B, 1E), we were able to differentiate several nuclei. The lateral superior olive (LSO) had an N-shaped morphology in frontal sections. The LSO was located laterally in the SOC but medially to the rubrospinal (rs) and the 7 nerve tracts. This nucleus contained a dense neuropil and fusiform or tufted cells; a small number of widely scattered multipolar cells were also seen. Medially to the LSO, we found the medial superior olive (MSO) which could be differentiated from the LSO because it showed a markedly more intense AChE reaction than the LSO. The MSO also contained a fine dense neuropil and columns of fusiform cells. Medially to the MSO, a nucleus composed of large cells —the superior paraolivary nucleus (SPO)— was found. The SPO, MSO and LSO were bordered dorsally by the dorsal periolivary region (DPO) and ventrally by the ventral periolivary nuclei (VPO). The VPO could readily be divided into the ventromedial (MVPO) and the ventrolateral (LVPO) subnuclei. The MVPO showed a more intense AChE reaction than the LVPO. Both nuclei were composed of round fusiform cells and a few multipolar cells. Finally, we observed the nucleus of the trapezoid body (Tz) situated laterally to the pyramidal tract (Py) and medially

Table 1.- Parameters of sound stimulation in each rat groups.

		Isolated	5 KHz	4 tones
Number of rats		4	6	6
Sound parameters	Frequency		5 KHz	0.25 KHz 1 KHz 8 KHz 20 KHz
	Intensity	< 30 dB SPL	70 dB	80 dB

to the SPO. The Tz nucleus consisted of a prominent cluster of globular cells.

In *level III* (Fig. 1C, 1F), we found the caudal periolivary nuclei (CPO) dorsally, bordered by the facial nucleus (7), which showed a more intense AChE reaction. The CPO was sandwiched between the 7n nerve laterally and the Py medially.

Tone-stimulated Rats

In isolated rats, some FLI was seen in the SOC. Labeling was widespread in the RPO nucleus of level I and in MVPO nucleus. The rest of the SOC nuclei were free of activated neurons.

In tone-stimulated rats, FLI nuclei appeared widespread in the RPO nucleus at *level I* of the SOC (Fig. 2A, 2D). However, in most cases there was a cluster of labeled nuclei in the lateral half of the nucleus. No differences were appreciated between 4 tone-stimulated rats and 5 kHz-stimulated rats.

At *level II* (Fig. 2B, 2E, and Fig 3), widespread FLI nuclei were seen in the LSO of 4 tone-stimulated rats but was scattered in the 5 KHz tone group and in some cases no labeling was

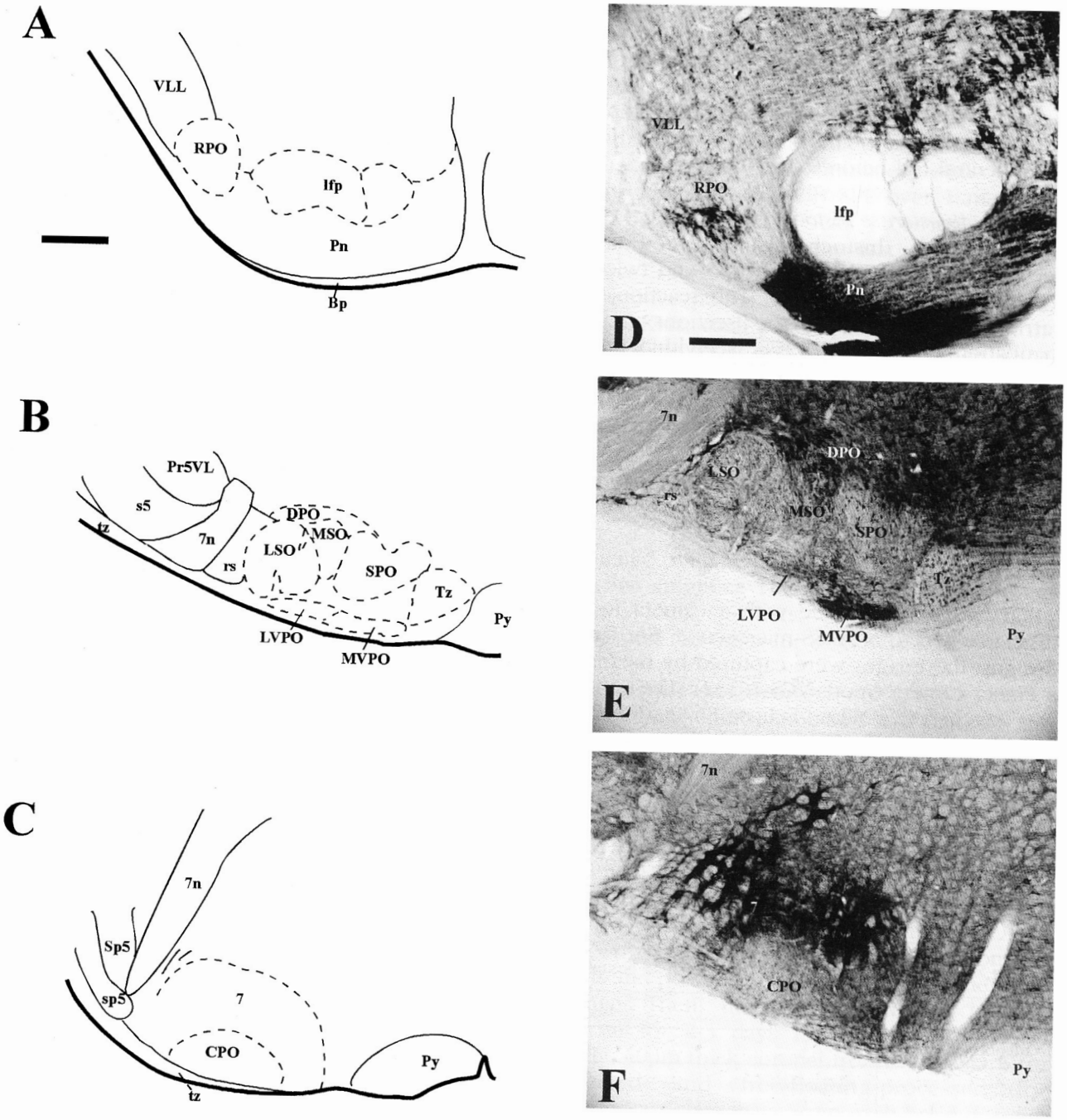

Fig. 1.- Parcellation of SOC according to the Acetylcholinesterase reaction through the 3 rostro-caudal levels: A and D, level I; B and E, level II; and C and F level III. Calibration bar: 0.5 mm.

seen in this nucleus. Some FLI nuclei were seen in the MSO of both 4 tone and 5KHz tone-stimulated rats. This labeling was mainly located in the hilus and in cells surrounding the main columns of both the MSO and LSO. However, the main bipolar cells of these nuclei did not show c-fos activation (Fig 3A). A similar type of labeling was obtained in the DPO nucleus; FLI nuclei did not form clusters and were homogeneously distributed. Scattered labeling was seen in the SPO, most of it located in the dorsomedial corner of the nucleus, leaving the lateral aspect of the nucleus unlabeled. In some 4 tone and 5KHz tone-stimulated rats, no FLI labeling was seen in this nucleus. FLI nuclei were always present in the Tz nucleus of both 5KHz and 4 tone-stimulated rats. In both cases, the labeling was concentrated in the ventrolateral corner of the nucleus. FLI was differently distributed in the two divisions of the VPO following all sound stimuli; labeling was more abundant in the MVPO than in the LVPO in all cases (Fig. 3B).

At *level III* (Fig. 2C, 2F) the FLI-labeled nuclei were preferentially distributed in the ventral aspect of the CPO nucleus.

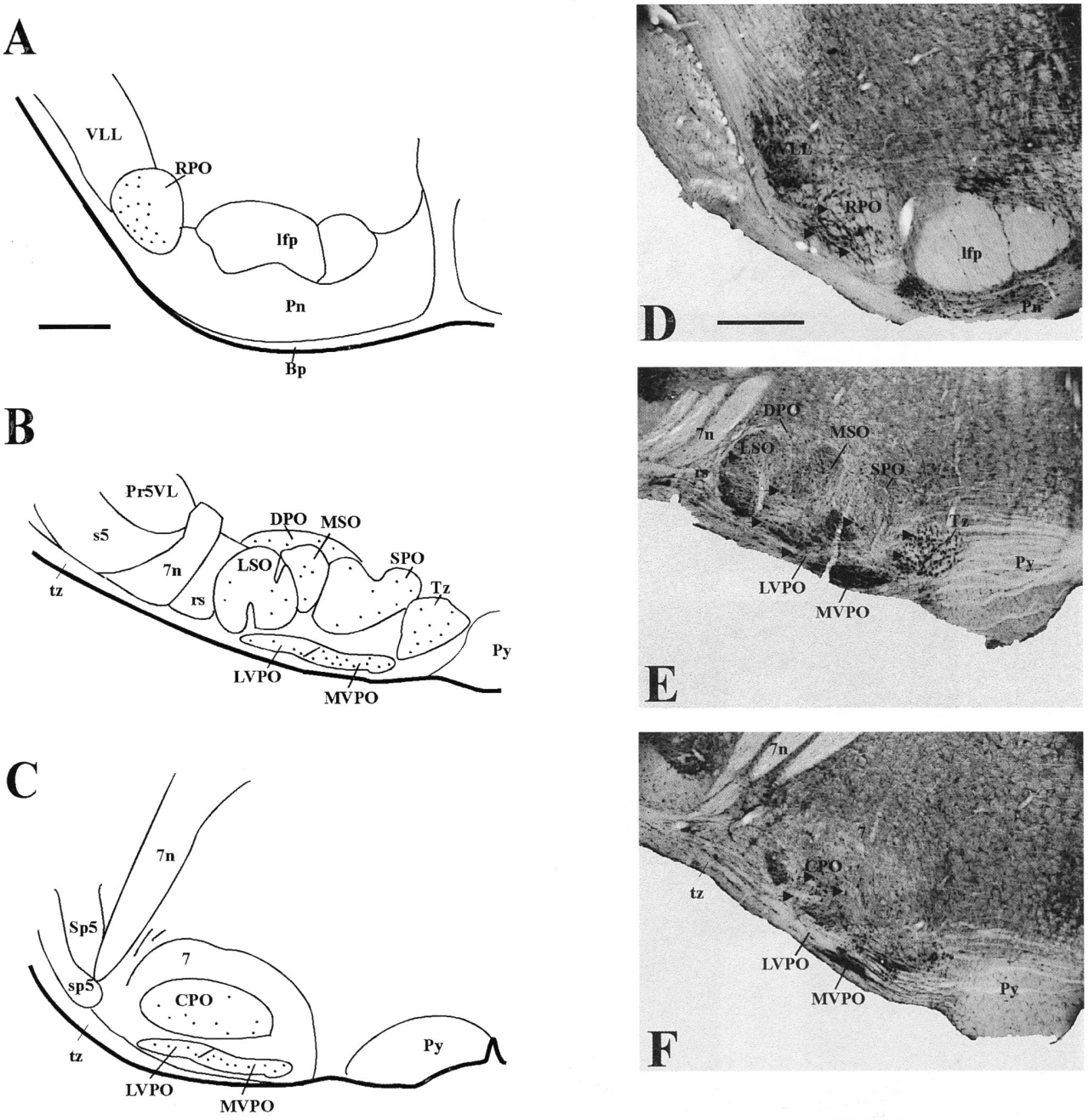

Fig. 2.- Camera lucida drawings (A to C) and computer-captured images (D to F) of the distribution of c-fos activation in the SOC following a 5 KHz tone stimulus. Arrows indicate c-fos activation. Calibration bar: 0.5 mm.

Nuclei of the Lateral Lemniscus

The lateral lemniscus fiber tract travels in a ventro-caudal to dorso-rostral direction, occupying an extreme lateral position in the brain stem. Neurons formed an irregular nuclear complex along the entire length of this tract, the nuclei of the lateral lemniscus (LL).

In our samples, the LL comprised from level —7.64 to level—8.80 caudal to Bregma according to Paxinos and Watson´s (1986) atlas. We differentiated three levels in frontal sections, observing up to 5 nuclei: dorsal nucleus of the lateral lemniscus (DLL), intermediate nucleus of the lateral lemniscus (ILL), ventral nucleus of the lateral lemniscus (VLL), paralemniscal nucleus (PL), and the nucleus sagulum (Sag).

At *level I* (Fig. 4A, 4D), the complex was bounded dorsolaterally by the parabigeminal nucleus (PBG), and dorsomedially by the microcellular tegmental nucleus (MiTg). Medially, the complex was bordered by the pontine reticular formation. Finally, the ventrolateral boundary was comprised by the middle cerebellar peduncle (mcp). At this level, the complex was composed of the ILL dorsally, the VLL ventrally and the PL nucleus medially. The ILL was composed of neurons of different sizes, although the type was very small. The

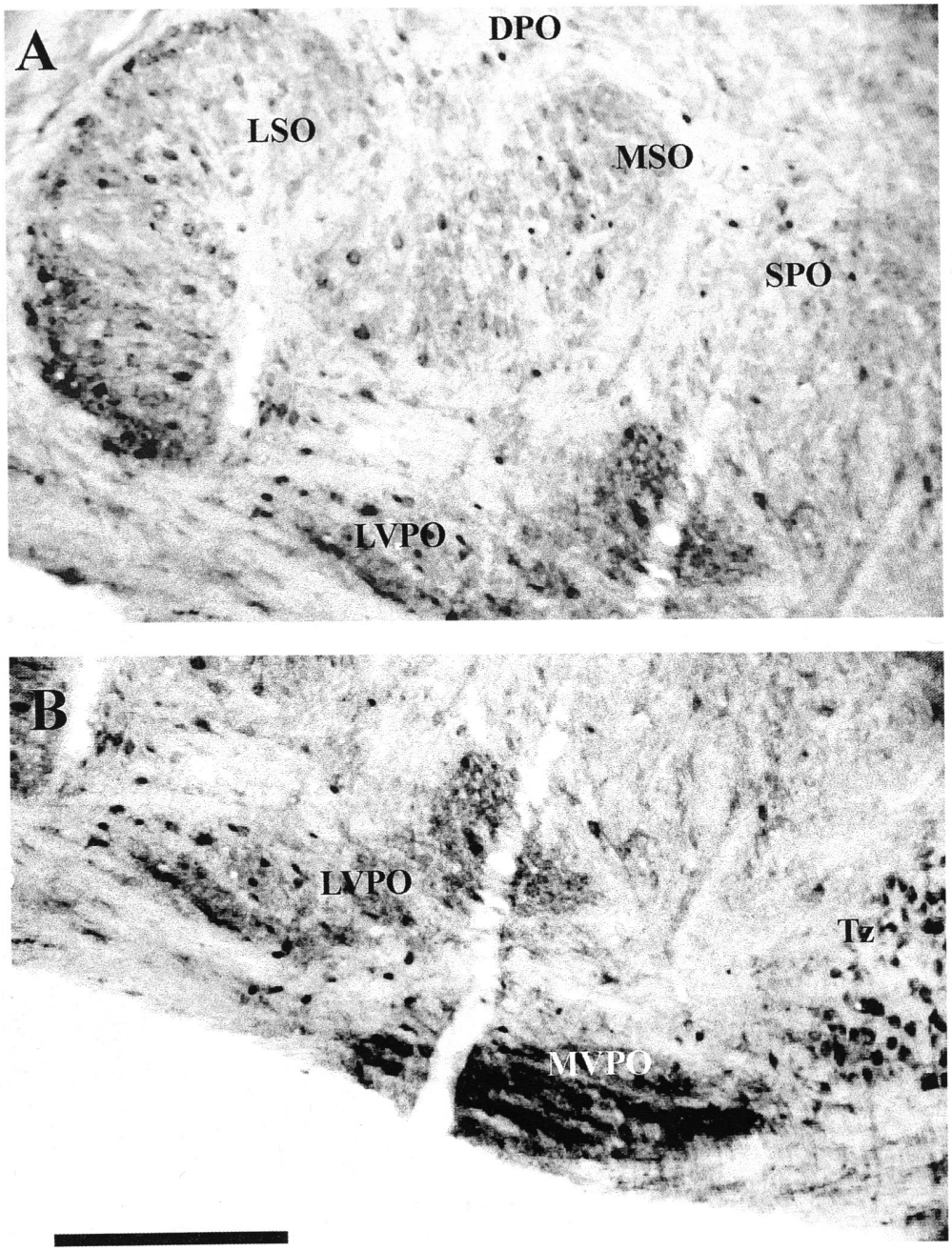

Fig. 3.- Computer-captured images of the distribution of c-fos activation in the core SOC nuclei (A) and in the VPO nuclei (B). Calibration bar: 0.25 mm.

neurons were aligned in vertical rows situated between the tracts of the lateral lemniscus. The VLL neurons were densely packed and were uniformly of medium size and displayed enlarged morphologies with the main axis perpendicular to the tracts of the lateral lemniscus. The PL nucleus was composed of tiny neurons loosely packed in the medial border of both the DLL and the ILL.

At *level II* (Fig. 4B,4E) the nuclei of the lateral lemniscus were bounded by the external cortex of the inferior colliculus (ECIC) dorsally, the cuneiform nucleus (CnF) and the parabrachial nuclei (PB) dorsomedially, the pontine reticular formation medially, and the mcp ventrally. This level was characterized by containing the DLL, the ILL and the VLL. Additionally, this level also contained the Sag and the PL nuclei. The ILL, VLL and PL displayed the same cytoarchitectural features as level I. The DLL was composed of large neurons mainly located in the medial half and smaller neurons in the outer half. Large

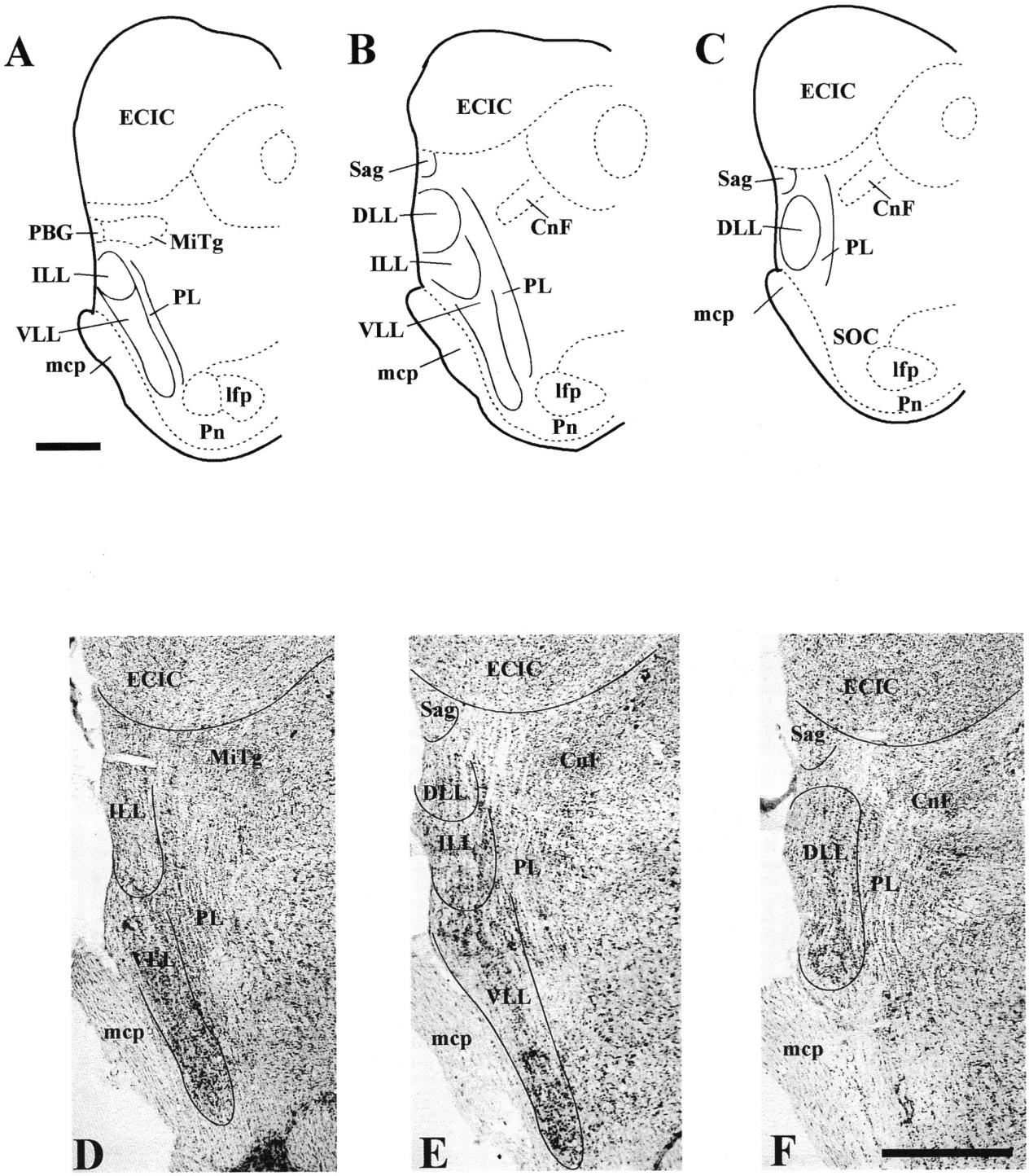

Fig. 4.- Parcellation of the nuclei of the LL according to the Giemsa staining through the 3 rostro-caudal levels considered: A and D, level I; B and E, level II; and C and F, level III. Calibration bar: 1 mm.

neurons had triangular to fusiform morphologies with the main axis perpendicular to the lemniscal bundles. Also, the outer half of the DLL displayed an intense AChE reaction. The Sag was composed of small densely packed neurons.

Level III (Fig. 4C, 4F) was located in the caudal pons. The complex was bounded by the

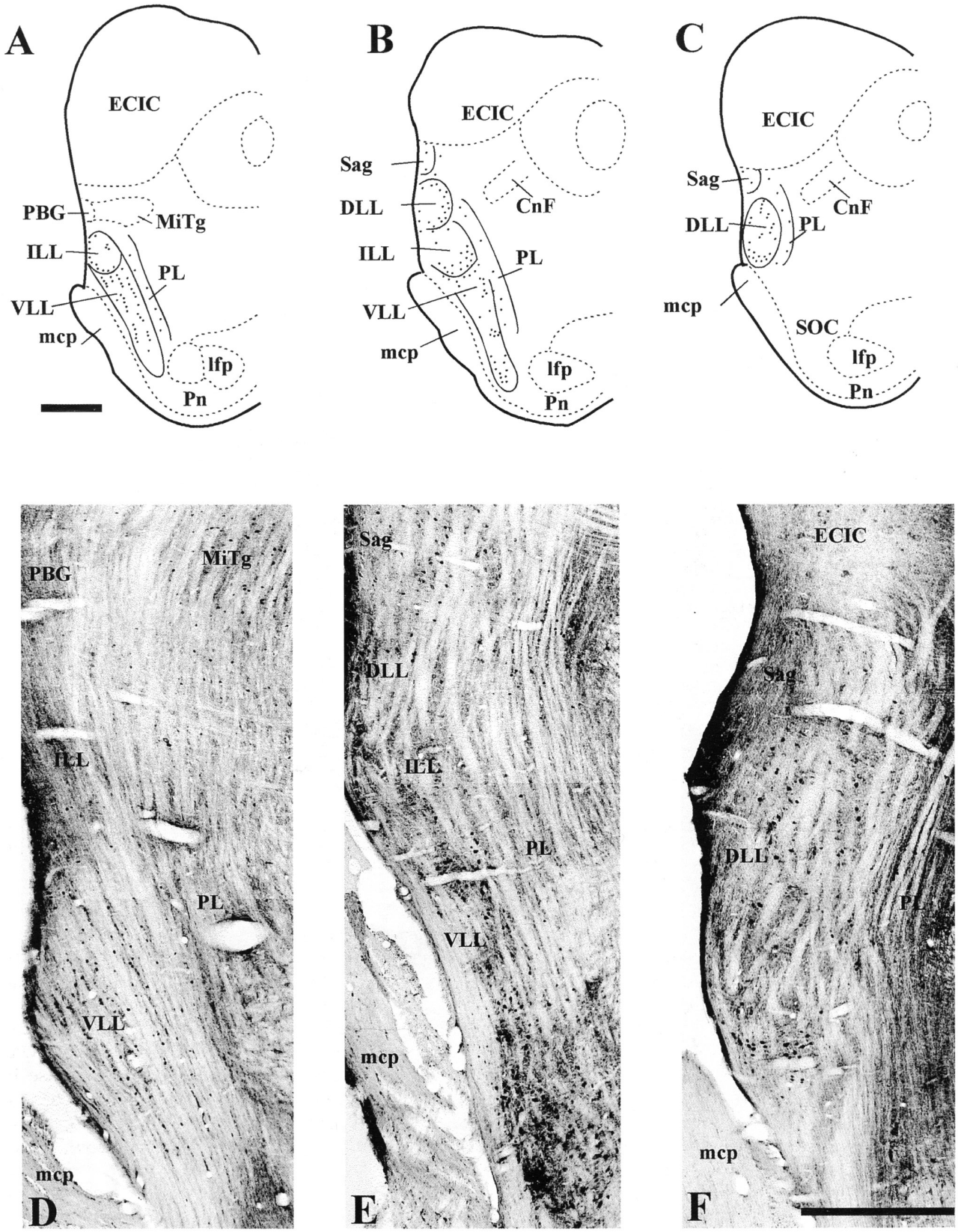

Fig. 5.- Camera lucida drawings (A to C) and computer-captured images (D to F) of the distribution of c-fos activation in the nuclei of the LL following a 5 KHz tone stimulus. Calibration bars: A to C = 1 mm; D to F = 0.5 mm.

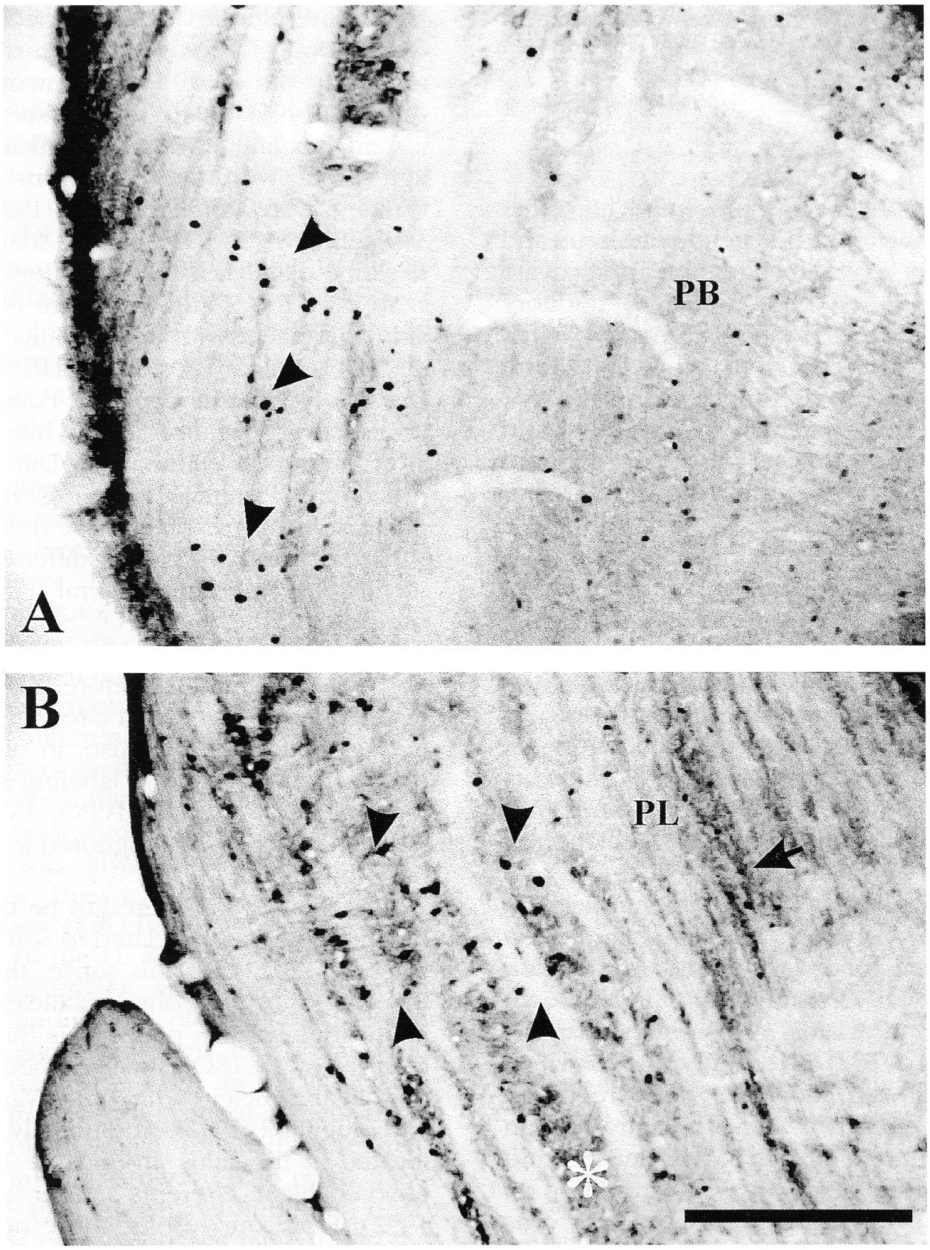

Fig. 6.- Computer-captured images of the distribution of c-fos activation in the nuclei of the DLL (A) and VLL (B). Arrowheads point to horizontal clusters of activated nuclei that appear between unlabeled areas (asterisk). Arrow points to activated nuclei in the paralemniscal area. Nuclei in this area are smaller than in the LL nuclei. Calibration bar: 0.25 mm.

ECIC dorsally, the cuneiform and parabrachial nuclei medially, and the rostral aspects of the superior olivary complex ventrally. At this level, the nuclei of the lateral lemniscus were the DLL, the Sag and the PL nuclei; all of them displayed the same features as the rostral levels.

Tone-stimulated Rats

In isolated rats, labeling was nearly absent in all LL nuclei. All types of sound stimulation gave rise to an important increase in FLI.

In the DLL, FLI nuclei were located preferentially in the periphery of the nucleus. In contrast, labeled nuclei located aligned between the bundles in the core of the DLL (Fig.5A-F). Occasionally, activated nuclei formed bands oriented perpendicularly to the fiber tracts; no activated nuclei were observed in the marginal lateral portion of the DLL (Fig. 6 A and B). FLI nuclei in the ILL were mainly concentrated in the lateral border at level I and in the ventral aspects of the subnucleus in level II. FLI was intense in the VLL, labeled neurons being distributed in rows between the bundles of the LL. Occasionally, groups of FLI neurons were orientated forming horizontal clusters perpendicular to the LL fiber tracts. Some disperse labeled neurons were observed in the PL nucleus (Fig 6B) but labeling in this area was weaker than in the LL nuclei. Scattered and disperse labeling was also found in the Sag nucleus. No differences

were appreciated between 4 tone-stimulated rats and rats stimulated with a single tone of 5 KHz.

Discussion

It is clear from the results presented here that nearly all the nuclei of the lateral lemniscus and superior olivary complex are able to activate IEGs such as c-fos as a result of auditory stimulation. However, the patterns of c-fos activation following different types of sound stimuli were similar in each nucleus, irrespective the type and the parameters of the sound stimulus. The correlation of c-fos activation and AChE histochemistry has thus allowed a better location of activated cells.

Our pattern of parcellation of the SOC corresponds to that described using Nissl and Golgi methods (Harrison and Warr, 1962; Webster, 1995).

c-fos activation in the SOC has been already detected following pure-tone stimuli in rats (Friauf, 1992; Rouiller et al., 1992; Sato et al., 1993) mice (Brown and Liu, 1995) and cats (Adams, 1995). Also, SOC c-fos activation has been reported following electrical stimulation of the rat cochlea (Vischer et al., 1994).

We were unable to observe tonotopicity in the SOC. This is consistent with most studies on c-fos activation following sound stimulation (Rouiller et al., 1992; Sato et al., 1993; Brown and Liu, 1995 and Adams, 1995). In contrast, the study by Friauf (1992) is the only one in which c-fos tonotopic activation has been reported. However, the sensitivity of c-fos activation in each SOC is different in each case. The MSO and LSO only showed disperse c-fos activation in several but not all cases. This labeling was mainly located in the hilus and in cells surrounding the main columns. This variability is in agreement with other reports. Rouiller et al. (1992) found no c-fos activation in the MSO or LSO. Also, Brown and Liu (1995), in mice, and Adams (1995), in cats, found disperse c-fos activation in the LSO. In contrast, Friauf (1992) demonstrated the tonotopic arrangement of the LSO, the high frequencies being located medially and the lower ones laterally. However, no c-fos activation was seen in the MSO.

There is a wide consensus regarding the patterns of c-fos activation in the shell nuclei of the SOC. In the RPO and in Tz nuclei, our results coincide with those obtained by Friauf (1992). In that paper, the author reported that there is a tonotopic c-fos activation in the RPO and in Tz, in such a way that lower frequencies are represented in the lateral part of the nucleus and higher frequencies in the medial one. In our study, we only used lower frequencies and in all cases the labeling was concentrated in the lateral part. Other studies did not specify any special distribution of c-fos activated nuclei (Rouiller et al., 1992; Sato et al., 1993; Brown and Liu, 1995; Adams, 1995). Also, we observed scarce or absent labeling in the SPO nucleus. This is in agreement with the data from other authors (Friauf, 1992; Rouiller et al., 1992; Sato et al., 1993; Brown and Liu, 1995; Adams, 1995). We describe disperse c-fos activation in the DPO of both pure tones and a mixture of 4 tones. This is in agreement with other results in rats (Sato et al., 1993), mice (Brown and Liu, 1995) and cats (Adams, 1995). In contrast, Friauf (1992) only found c-fos activation after white noise but not after pure-tone stimulation. Differences in the procedures, animal handling or unexpected parameters may explain these differences. In the VPO there are two clearly different regions: the medial (MVPO) and lateral (LVPO). In our samples, the MVPO displayed c-fos immunoreactivity even in isolated rats; this has not been referred to before. Tone stimulation resulted in an increase in c-fos activation in both the LVPO and MVPO. Also, in our samples we observed disperse c-fos labeling in the CPO. c-fos activation in the MVPO, LVPO and CPO nuclei has largely been ignored in most previous descriptions.

The SOC is considered to be the first step in binaural integration related to sound localization (Webster, 1977). In this sense, the SOC is also the origin of the olivo-cochlear bundle. The main origin of this pathway is the main bipolar neurons of the LSO as well as medial and ventral periolivary nuclei (White and Warr, 1983, Vetter and Mugnaini, 1992). Additionally, shell neurons located in the hilus around the LSO also send olivo-cochlear efferents (Vetter and Mugnaini, 1992). From our results, these neurons located around the main core of the LSO may be the ones that are c-fos-activated after sound stimulation and may contribute through this pathway to binaural processing.

Some controversies have arisen concerning the parcellation of the nuclei of the lateral lemniscus (Webster, 1995) and their c-fos activation following sound stimulation (Friauf, 1992; Rouiller et al., 1992; Sato et al., 1993; Brown and Liu, 1995; Adams, 1995). The main disagreement regarding the parcellation of the LL comes from the possible existence of an intermediate division between the DLL and VLL. Our cytoarchitectural and c-fos activation results confirm the segregation of the ILL from the dorsal and ventral divisions. The DLL contains a relatively homogeneous population of large cells and is divided into two portions: a lateral portion that is not crossed by lemniscal fibers, and a medial portion that is composed of large cells distributed between vertical lemniscal tracts. In the present study, FLI nuclei located in

the medial portion and occasionally horizontal clusters of activated nuclei were observed. On contrasting these patterns of the DLL, the ILL was found to contain different kinds of neurons. FLI cells were preferentially distributed surrounding the central core in the lateral and ventral margins of the ILL nucleus. FLI was intense in the DLL, this labeling being mainly concentrated in rows running parallel to the LL fiber tracts. Additionally, clusters perpendicular to those fibers were observed. Most studies on c-fos activation have described general activation in the nuclei of the LL (Rouiller et al., 1992; Sato et al., 1993; Brown and Liu, 1995; Adams, 1995). However, Friauf (1992) observed a tonotopic pattern in the DLL but not in the VLL. In our study, most labeling was concentrated to form two clusters perpendicular to the LL fiber tracts, this pattern may correspond to Friauf´s (1992) model. These two clusters are closer in lower frequencies and separated from each other at higher frequencies. We were not able to observe this pattern, also in some samples different (even more than two) horizontal clusters could be observed. Also, in some samples from the VLL nucleus, horizontal clusters could be traced. Additionally, FLI in the PL and Sag was always present in sound-stimulated rats. This activation has been also observed by Sato et al.(1993) and Friauf (1992).

The nuclei of the LL are interposed between the lower brainstem SOC and cochlear nuclei on one side and the inferior colliculus on the other side. It is thought that their role in auditory processing is mainly to exert an excitatory and inhibitory control over the central nucleus of the inferior colliculus, which originates the main ascending lemniscal pathway (Bajo et al., 1993; Merchán et al., 1994; Merchán and Berbel, 1996).

As a general conclusion, we have found that in contrast to the more accurate tonotopic arrangement in c-fos activation in the cochlear nuclei (Riera-Sala et al., 1999), the SOC and nuclei of the LL show a lower accuracy and a more widespread location of c-fos activated cells.

Acknowledgements

We wish to thank Ms. Rocío Muñoz and Ms. Castillo Calatayud for their technical assistance. This work has been supported in part by a grant from the Generalitat Valenciana GV99-121-1-12.

References

Adams JC (1995). Sound stimulation induces Fos-related antigens in cells with common morphological properties throughout the Auditory Brainstem. *J Comp Neurol*, 361: 645-668.

Aitkin LM (1989). The auditory system. Handbook of Chemical Neuroanatomy, Vol 7: Integrated systems of the CNS, Part II. pp 165-218.

Bajo VM, Merchan MA, Lopez de and Rouiller EM (1993). Neuronal morphology and efferent projections of the dorsal nucleus of the lateral lemniscus in the rat. *J Comp Neurol*, 334: 241-262.

Brown MC and Liu TS (1995). Fos-like immunoreactivity in central auditory neurons of the mouse. *J Comp Neurol*, 357: 85-97.

Ehret E and Fischer R (1991). Neuronal activity and tonotopy in the auditory system visualized by c-fos gene expression. *Brain Res*, 567: 350-354.

Friauf E (1992). Tonotopic order in the adult and developing auditory system of the rat as shown by c-fos immunocytochemistry. *Eur J Neurosci*, 4: 798-812.

Harrison JM and Warr WB (1962). A study of the cochlear nucleus and ascending auditory pathways of the medulla. *J Comp Neurol*, 119: 341-380.

Merchan MA, Saldaña E and Plaza I (1994). Dorsal nucleus of the lateral lemniscus in the rat: concentric organization and tonotopic projection to the inferior colliculus. *J Comp Neurol*, 342: 259-278.

Merchan MA and Berbel P (1996). Anatomy of the ventral nucleus of the lateral lemniscus in rats: a nucleus with a concentric laminar organization. *J Comp Neurol*, 372: 245-263.

Morgan JY and Curran T (1991). Stimulus-transcription coupling in the nervous system: involvement of the inducible protooncogenes fos and jun. *Ann Rev Neurosci*, 14: 421-451.

Olucha FE, Molina Mira A, Valenzuela Mendez C, Valverde Navarro AA and Martinez Soriano F (1997). Activation of proto-oncogene c-fos in the auditory tract of rats stimulated with wide-band noise. *Acta Otorrinolaringol Esp*, 48: 85-92.

Paxinos G and Watson C (1986). The rat brain in stereotaxic coordinates. 2nd Ed. Academic Press, San Diego.

Riera Sala C, Martinez Beneyto P, Marco Algarra J, Martinez Soriano F and Olucha FE (1999). A detailed map of c-fos activation in the rat cochlear complex following different kinds of sound stimuli. *Eur J Anat*, 3: 153-162.

Rouiller EM, Wan XST, Moret V and Liang F (1992). Mapping of c-fos expression elicited by pure tones stimulation in the auditory pathways of the rat, with emphasis on the cochlear nucleus. *Neurosci Lett*, 144: 19-24.

Sato K, Houtani T, Ueyama T, Ikeda M, Yamashita T, Kumazawa T and Sugimoto T (1992). Mapping of the cochlear nucleus subregions in the rat with neuronal Fos-protein induced by acoustic stimulation with low tones. *Neurosci Lett*, 142: 48-52.

Sato K, Houtani T, Ueyama T, Ikeda M, Yamashita T, Kumazawa T and Sugimoto T (1993). Identification of rat brainstem sites with neuronal fos protein induced by acoustic stimulation with pure tones. *Acta Otolaryngol* (Stockh) Suppl 500: 18-22.

Schwartz IR (1992). The superior olivary complex and lateral lemniscal nuclei. In: Webster DB, Popper AN and Fay RR (eds.). Springer Handbook of Auditory Research, Vol. 1. *The mammalian auditory pathway: Neuroanatomy*. Springer-Verlag, New York, pp 117-167.

Sheng M and Greenberg ME (1990). The regulation and function of *c-fos* and other immediate early genes in the nervous system. *Neuron*, 4: 477-485.

VETTER DE and MUGNAINI E (1992). Distribution and dendritic features of three groups of rat olivocochlear neurons. A*nat Embryol,* 185: 1-16.

VISCHER MW, HÄUSLER R and ROUILLER EM (1994). Distribution of Fos-like immunoreactivity in the auditory pathway of the Sprague-Dawley rat elicited by cochlear electrical stimulation. *Neurosci Research*, 19: 175-185.

WEBSTER WR (1995). Auditory System. In: Paxinos G (ed.). *The rat nervous system*. 2nd Ed. Academic Press, San Diego, pp 797-831.

WEBSTER WR (1977). Central neural mechanisms of hearing. *Proc Aust Physiol Pharmacol Soc*, 8: 1-7.

WHITE JS and WARR WB (1983). The dual origins of the olivocochlear bundle in the albino rat. *J Comp Neurol*, 219: 203-214.

ABREVIATIONS

AchE	acetylcholinesterase
CN	cochlear nuclei
CnF	cuneiform nucleus
CPO	caudal periolivary nuclei
DLL	dorsal nucleus of the lateral lemniscus
DPO	dorsal periolivary nuclei
ECIC	external cortex of the inferior colliculus
FLI	fos-like immunoreactivity
IC	inferior colliculus
ICC	immunocytochemistry
IEG	immediate early genes
ILL	intermediate nucleus of the lateral lemniscus
lfp	longitudinal fasciculus pons
LL	nuclei of the lateral lemniscus
LSO	lateral superior olive
LVPO	ventrolateral periolivary nuclei
mcp	middle cerebellar peduncle
MiTg	microcellular tegmental nucleus
MSO	medial superior olive
MVPO	ventromedial periolivary nuclei
PB	parabrachial nucleus
PBG	parabigeminal nucleus
PL	paralemniscal nucleus
Pn	pontine nuclei
Pr5VL	principal sensory trigeminal nuclei, ventrolateral part
Py	pyramidal tract
RPO	rostral periolivary region
rs	rubrospinal tract
s5	sensory root of the trigeminal nerve
Sag	nucleus sagulum
SOC	superior olivary complex
sp5	spinal trigeminal tract
SPO	superior paraolivary nucleus
Tz	trapezoid body nucleus
tz	trapezoid body
VLL	ventral nuclei lateral lemniscus
VPO	ventral periolivary nuclei