Anat Embryol (1995) 191:425–443

ORIGINAL ARTICLE

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Somatostatin and leu-enkephalin in the rat auditory brainstem during fetal and postnatal development

Accepted: 8 November 1994

Abstract A transient expression of the neuropeptide somatostatin has been described in several brain areas during early ontogeny and several opioid peptides, such as leu-enkephalin, have also been found in the brain at this stage in development. It is therefore believed that somatostatin and leu-enkephalin may play a role in neural maturation. The aim of the present study was to describe the spatiotemporal pattern of somatostain and leu-enkephalin immunoreactivity in the auditory brainstem nuclei of the developing rat and to correlate it with other developmental events. In order to achieve this goal, we applied peroxidase-antiperoxidase immunocytochemistry to rat brains between embryonic day (E) 17 and adulthood. Somatostatin immunoreactivity (SIR) was found in all nuclei of the auditory brainstem, yet it was temporally restricted in most nuclei. SIR appeared prenatally and reached maximum levels around postnatal day (P) 7, when great numbers of immunoreactive neurons were present in the ventral cochlear nucleus (VCN) and in the lateral lemniscus. At that time relatively low numbers of cells were labeled in the dorsal cochlear nucleus, the lateral superior olive (LSO), and the inferior colliculus (IC). During the same period, when somata in the VCN were somatostatin-immunoreactive (SIR), a dense network of labeled fibers was also present in the LSO, the medial superior olive (MSO), and the medial nucleus of the trapezoid body (MNTB). As these nuclei receive direct input from VCN neurons, and as the distribution and morphology of the somatostatinergic fibers in the superior olivary complex (SOC) was like that of axons from VCN neurons, these findings suggest a transient somatostatinergic connection within the auditory system. Aside from the LSO, MSO, and MNTB, labeled fibers were found to a smaller extent in all other auditory brainstem nuclei. After P7, the SIR decreased and only a few immunoreactive elements were found in the adult auditory brainstem nuclei, indicating that somatostatin is transiently expressed in the rat auditory brainstem. Leu-enkephalin immunoreactivity showed a lower number and weaker intensity of labeled structures as compared to SIR, with E18 being the earliest day at which labeled fibers appeared in the SOC. At birth, immunoreactive fibers were also present in the cochlear nuclear complex and in the IC. Leu-enkephalin immunoreactive somata were found only after P12 in the CN and after P16 in the IC. Leu-enkephalin immunoreactivity was not transient, but increased progressively with age until about P21, when the adult levels were reached. Our results demonstrate somatostatinergic and leu-enkephalinergic inputs onto auditory brainstem neurons during perinatal life, i.e., during a period when the processes of synapse maturation occur. It is thus likely that both neuropeptides may influence the development of synaptic connections in the auditory brainstem.

Key words Immunocytochemistry · Neuropeptide · Transient · Regressive events · Superior olivary complex

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Introduction

Many rodent species, such as rats and gerbils, do not respond to airborne sound shortly after birth. Their outer ear canals are closed until P12, auditory brainstem responses cannot be reliably recorded before P12–P14 (Jewett and Romano 1972; Uziel et al. 1981; Blatchley et al. 1987; Geal-Dor et al. 1993), and cochlear function remains immature until about P20 (Uziel et al. 1981; Puel and Uziel 1987). Such species have therefore been found useful experimental models for studying auditory development. In spite of the late onset of hearing, however, the anatomical connections, as well as synaptic transmission, are already present in the rat's auditory brainstem around birth. For example, the major connections between the cochlear nuclear complex (CN) and its target nuclei appear to be established by E20, which is 2 days before birth (Kandler and Friauf 1993b). Recordings

from neurons in the superior olivary complex (SOC), which were obtained from an in vitro slice preparation, have identified synaptic activity in response to electrical stimulation as early as E18 (Kandler and Friauf 1993a). Similar results were reported in mice and gerbils, indicating the presence of functional synaptic transmission in neonates (Wu and Oertel 1987; Sanes 1993). These and other anatomical and electrophysiological studies have also shown that a considerable amount of synaptic maturation takes place during the first 2 postnatal weeks. Morphological changes, such as refinement of axon terminals, as well as of dendritic arbors, have also been observed in SOC nuclei (Sanes et al. 1989, 1992b; Sanes and Siverls 1991). Functional changes were indicated by shortening of synaptic delay, shortening of the duration of postsynaptic potentials, and modifications of biophysical membrane properties (Wu and Oertel 1987; Kandler and Friauf 1993a). At least some of these maturation processes are likely to require proper synaptic transmission, since reduction of synaptic input after cochlear removal or pharmacological blockade of postsynaptic receptors profoundly disturbs normal development of SOC neurons and of neurons in other auditory brainstem nuclei as well (Moore 1992; Sanes and Chokshi 1992; Sanes et al. 1992a; Sanes and Takacs 1993). At present, it is unknown whether proper synaptic transmission demands the release of co-transmitters in addition to the classical transmitters involved.

Neuropeptides such as somatostatin and leu-enkephalin, well known as cotransmitters in the CNS (reviews: Epelbaum 1986; Olson et al. 1993), are potential candidates for influencing early neuronal development. This assumption is supported by the fact that the decatetrapeptide somatostatin is transiently expressed in several neuronal systems, such as cranial and spinal sensory ganglia (Katz et al. 1992), the cerebellum (Inagaki et al. 1982), and the retina (Ferriero and Sagar 1987) of pre- and neonatal rats, and only a few somatostatin-immunoreactive (SIR) elements persist in the adult rat brain. Further support for the idea that somatostatin may be an important cotransmitter during early development, comes from receptor studies that report the occurrence of somatostatin receptor mRNA in prenatal rat brains (Wulfsen et al. 1993). Interestingly, one of the five somatostatin receptor subtypes identified so far (SSTR1–SSTR5; review: Bell and Reisine 1993), the SSTR4, is expressed only transiently in the brainstem of neonatal rats and successively replaced by SSTR1 (Wulfsen et al. 1993), indicating that somatostatin acts through a specific receptor during that period.

A strong and early appearance of somatostatin has also been described in the rat auditory system, with the maximum immunoreactivity occurring around P0 (Shiosaka et al. 1981; Takatsuki et al. 1981, 1982). However, these authors employed immunofluorescent techniques and found no SIR elements in the adult auditory system. This is in contrast to the report of Johansson et al. (1984), who described SIR in all auditory brainstem nuclei of adult rats, employing the peroxidase-antiperoxidase (PAP) technique. As this technique is more sensitive than immunofluorescence methods, it is possible that Takatsuki and colleagues have underestimated the amount of SIR in the adult, as well as in the developing brain.

Aside from somatostatin, opioid peptides are also found during fetal and early postnatal development of the mammalian brain (review: McDowell and Kitchen 1987). Like somatostatin, they are therefore thought to influence the development of the nervous system (review: Hammer and Hauser 1992). In fact, opioid peptides are known to affect cell proliferation (Vertes et al. 1982; Kornblum et al. 1987; Zagon and McLaughlin 1987), dendritic outgrowth (Hauser et al. 1989; Ricalde and Hammer 1991), and cell density in several neuronal systems (Zagon and McLaughlin 1986). Leu-enkephalin is an opioid pentapeptide that is found in the brainstem of the fetal rat (review: McDowell and Kitchen 1987). To our knowledge, nothing is known about leu-enkephalin expression in the developing rat auditory brainstem nuclei, whereas a large body of literature has been devoted to the presence of leu-enkephalin in the auditory brainstem of adult rats (Sar et al. 1978; Uhl et al. 1979; Finley et al. 1981; Petrusz et al. 1985) and guinea pigs (Altschuler et al. 1983; Safieddine and Eybalin 1992).

The aim of the present study was to map and analyze the spatiotemporal distribution of somatostatin and leu-enkephalin in the auditory brainstem nuclei of the developing rat. To do so, we performed a sensitive immunocytochemistry employing the PAP method. Our interest was focused on auditory nuclei in the hindbrain and the midbrain and on the period between E17 and P16, when important steps of synaptic stabilization, such as synaptogenesis and synaptic refinement, take place. Our results demonstrate a weak expression of leu-enkephalin in perinatal animals, continuously, but only moderately, increasing until about P21. In contrast, somatostatin appears only transiently in the developing auditory system, yet it is strongly present and widely distributed during the first 2 postnatal weeks. As the temporal expression of both neuropeptides correlates with developmental events such as synapse maturation, it is probable that somatostatin and leu-enkephalin both play a role in synaptic modification.

Materials and methods

Tissue preparation

Altogether 48 brains of Sprague-Dawley rats from E17 to adulthood were used (Table 1). The day of birth was taken as P0. For each developmental stage, the animals used came from different litters. Animals were housed in supervised animal facilities and handled according to the Principles of Laboratory Animal Care (NIH Publication) and the German Law on the Protection of Animals (Tierschutzgesetz). Two adult animals were anesthetized with chloralhydrate and received an ejection of colchicine (150 µg in 10 µl saline) into the fourth ventricle 30 h prior to fixation. All brains (fetal and postnatal) were fixed by transcardial perfusion. Postnatal animals were deeply anesthetized with chloralhydrate (1 g/kg body weight), and fetal animals were obtained via Cesare-

Table 1 Number and age of animals used for somatostatin and leu-enkephalin immunocytochemistry. Altogether 47 brains were used, out of which 37 were processed for leu-enkephalin and 39 for somatostatin (some brains were processed for both neuropeptides, others only for one peptide). Two adult animals received no colchicine (Adult), and two animals were treated with colchicine (Adult colch)

Age	E17	E18	E20	P0	P1	P4	P5	P6	P7	P9	P10	P11	P12	P16	P18	P21	Adult	Adult colch	Total
Leu-enkephalin	2	3	2	1	3	6	1	–	2	2	1	2	2	2	2	2	2	2	37
Somatostatin	2	3	3	–	8	6	–	2	3	3	–	3	–	–	2	–	2	2	39
Sum	2	3	3	1	8	6	1	2	3	3	1	3	2	2	2	2	2	2	48

an section from time-pregnant rats, which had been deeply anesthetized by intraperitoneal injections of chloralhydrate (500 mg/kg). After rinsing with 0.9% saline in 0.01 M phosphate buffer, pH 7.4, the animals were fixed with cold 4% paraformaldehyde in 0.1 M sodium acetate buffer, pH 6.5, followed by cold 4% paraformaldehyde in 0.1 M borate buffer, pH 11. Brains were then removed, postfixed for 4 h in the borate-buffered fixative containing 5% sucrose, and subsequently soaked overnight in 30% sucrose in 0.1 M phosphate buffer, pH 7.4, at 4° C. Serial coronal sections of postnatal rat brains were cut on a freezing microtome (50 µm thick), whereas prenatal brains were cryo-sectioned at 14 or 16 µm and collected in TRIS-buffered saline (TBS, pH 7.6). Sections were usually divided into three sets: one series was used for somatostatin and one for leu-enkephalin immunocytochemistry; set three was Nissl-stained to allow for localization and delineation of auditory nuclei.

Immunocytochemistry

Sections of 44 brains were processed immunocytochemically by the PAP technique (Sternberger 1979) in order to visualize the two neuropeptides. Sections from prenatal animals were stained with the on-slide technique. The cryosections were mounted onto gelatin-coated slides, air-dried, fixed with 5% paraformaldehyde for 5 min, and thoroughly washed in TBS. Immunocytochemistry was performed as indicated below. Sections from postnatal rats were processed free floating, while those from fetal animals were incubated on the slide. After thoroughly washing in TBS, sections were preincubated for 1 h with 10% goat serum and 2.5% bovine serum albumin in TBS with Triton-X-100 (0.075% for prenatal and 0.3% for postnatal) and then transferred into the primary antiserum, which contained either rabbit anti-somatostatin (Peninsula Laboratories; RIK 8001/RAS 8001; dilution of 1:1,000) or rabbit anti-leu-enkephalin (Incstar, 1:1,000). Sections were incubated at 4° C overnight, rinsed several times in TBS, and placed in an unlabeled swine-anti-rabbit IgG antiserum (DAKO), diluted 1:50, for 1.5 h at room temperatur. Sections were again thoroughly washed and incubated in a rabbit-PAP-complex (1:150; DAKO) for 1.5 h at room temperature. All antisera were diluted in TBS containing 1% goat serum and 1% bovine serum albumin (and 0.3% Triton-X-100 for postnatal brains). The peroxidase was visualized with diaminobenzidine (0.05%) in the presence of 0.01% hydrogen peroxide (20 min at room temperature). The sections were then thoroughly washed and the free-floating sections were mounted on gelatin-coated slides and allowed to air-dry overnight. All series were dehydrated in an ascending series of ethanols, cleared in xylene, and coverslips were applied using DPX. In control experiments, specificity or antibody labeling was tested by omitting the primary antibody, resulting in no diaminobenzidine reaction product.

Double-labeling experiments

Four brains from P4 animals were used for double-labeling experiments, employing antibodies against somatostatin and the glial fibrillic acidic protein (GFAP), which specifically stains astrocytes and some groups of ependymal cells. These double-labeling experiments were designed to identify the nature of the somatostatin-positive structures (glial or neuronal), since glial cells have been previously described as being able to express somatostatin (Davidson and Gillies 1993). The tissue was processed according to the protocol outlined above, except that bovine serum was used instead of goat serum. The primary antibody against somatostatin (1:200) and a monoclonal mouse-anti-GFAP antibody (Boehringer, diluted 1:5) were used as a cocktail in which sections were incubated for 24 h. For visualization of the immunocomplexes, sections were incubated with a cocktail of fluorescein-labeled swine-anti-rabbit antiserum (Jackson Immuno Research, 1:50) and Cy3-labeled goat-anti-mouse antiserum (Jackson Immuno Research, 1:1,000) for 1.5 h. Washing and mounting procedures were performed as described above.

Data analysis

Representative sections were analyzed under a Reichert-Jung microscope and diaminobenzidine-labeled structures were photographed with Kodak T-Max 100 films. Fluorescent sections were viewed under epifluorescent illumination, employing filter systems for rhodamine (for Cy3-labeled sections) and fluorescein. In the following, we use the abbreviation SIR for "somatostatin immunoreactivity" as well as for "somatostatin immunoreactive". Similarly, the abbreviation LIR is used for "leu-enkephalin immunoreactivity" and for "leu-enkephalin immunoreactive".

Results

This study focused on the development of the auditory nuclei in the pons and the midbrain, comprising the CN, the SOC, the nuclei of the lateral lemniscus (NLL), and the inferior colliculus (IC). We present the data on somatostatin first, starting caudally in the CN and proceeding rostrally towards the IC.

Somatostatin

Staining for somatostatin revealed a strong but transient appearance in all principal nuclei of the auditory brainstem. In fact, the auditory brainstem nuclei, together with the nucleus of the solitary tract and the principal sensory trigeminal nucleus, contained the most intensely labeled structures of all nuclei in the pons and the midbrain. At E17, the earliest stage investigated, SIR neurons were found nowhere in the mes- and myelencephalon, but in the auditory brainstem, where they were restricted to the anlage of the NLL. Five days later, i.e., at birth, SIR was present in all auditory brainstems stations (the CN, the SOC, the NLL, and the IC). By the end of the first post-

Table 2 Developmental changes in the number of SIR elements in auditory brainstem nuclei. Subdivisions within brainstem nuclei could not be distinguished before birth (*S* SIR somata, *F* SIR fibers, – no SIR, *weak, **moderate, ***strong)

		Somatostatin immunoreactivity								
		CN		SOC			LL		IC	
		DCN	VCN	MNTB	MSO	LSO	NLL	PL	ICC	ICDC ICEC
E17	S		–		–		***		–	
	F		–		–		–		–	
E18	S		*		–		***		–	
	F		*		*		*		–	
E20	S		*		–		***		–	
	F		*		*		*		*	
P1	S	**	***	–	–	*	*	***	*	*
	F	*	*	*	*	**	*	–	**	*
P4	S	**	***	–	–	*	*	***	*	*
	F	–	*	***	*	**	**	–	*	*
P6	S	*	***	–	–	*	–	***	*	*
	F	–	*	***	**	***	**	–	*	*
P7	S	*	***	–	–	–	–	***	*	*
	F	–	*	***	***	***	**	–	–	*
P9	S	*	**	–	–	–	–	**	–	*
	F	–	*	**	**	**	**	–	–	*
P11	S	*	**	–	–	–	–	**	–	*
	F	–	*	*	*	*	*	–	–	*
P18	S	–	**	–	–	–	–	*	–	*
	F	–	*	*	–	–	*	–	–	*
Adult	S	–	*	–	–	–	–	*	–	*
	F	–	–	*	–	–	*	–	–	*

natal week, a maximum level or SIR was reached in the auditory brainstem. Subsequently SIR gradually decreased until in the adult auditory brainstem nuclei only a few neurons and fibers remained SIR (Table 2).

Cochlear nuclear complex

Somata. Neurons in the CN showed a very strong labeling with the somatostatin antibody during development. SIR somata could first be seen at E18 in the anlage of the CN. During further fetal development, both the number of labeled neurons and intensity of labeling remained moderate (Fig. 1A), but both increased considerably in the neonates. Between P1 and P7, SIR somata became heavily labeled in all subdivisions of the CN (Figs. 1B, C; 2A), and sometimes even dendrites were labeled in the posterior ventral cochlear nucleus (PVCN; Fig. 1C). The maximum number of labeled neurons in the dorsal cochlear nucleus (DCN) was found at P1, but at P4, a great many of DCN neurons were still intensely stained. Most of them were located in layers 2 and 3 (Fig. 1B, C; cf. Fig. 4B in Hackney et al. 1990). After the first postnatal week, the numbers and intensity of SIR somata decreased in the PVCN and DCN, yet a moderate number of SIR neurons was still present in the PVCN at P11 (Fig. 1D). Many of these neurons were scattered throughout the whole PVCN and had a soma-dendritic morphology reminiscent of multipolar cells (Fig. 1E), but globular cells were also found (not shown). Some neurons remained immunoreactive in the adult PVCN, as illustrated in Fig. 1F. In contrast, most of the SIR neurons in the DCN had disappeared by P11 (Fig. 1D) and virtually no SIR neurons were detectable in the adult DCN (Fig. 1F).

Within the CN and throughout the whole auditory brainstem nuclei, the greatest number of SIR neurons were located in the anterior ventral cochlear nucleus (AVCN; Fig. 2A), where almost all neurons appeared to be SIR during the first postnatal week (Figs. 2A; 3). Due to their location, size, and morphology, many of these SIR neurons could be identified as spherical cells. During the second postnatal week, SIR declined (Fig. 2B), and only weakly labeled AVCN neurons remained into adulthood (Fig. 2C).

Fibers. The most striking result in the CN was the finding of heavily labeled SIR fibers emerging from the VCN and entering into the ventral acoustic stria, which were SIR during the same period as when neuronal somata in the VCN were also SIR (Fig. 3), indicating that they originate from CN neurons. Within the CN, the number of SIR fibers was much lower than in the ventral acoustic stria. A few SIR fibers were detectable in the primordium of the CN as early as E18. The intensity of SIR in fibers in the VCN was consistently weak during development and temporally restricted to a 3-week period lasting from E18 to P18. In the adult VCN, SIR fibers were no longer detectable. Like the VCN, the DCN also showed only a small number of SIR fibers. In contrast to the VCN, however, the occurrence of these DCN fibers was restricted to an earlier and shorter period of development, namely to a 5-day period between E18 and P1 (data not shown).

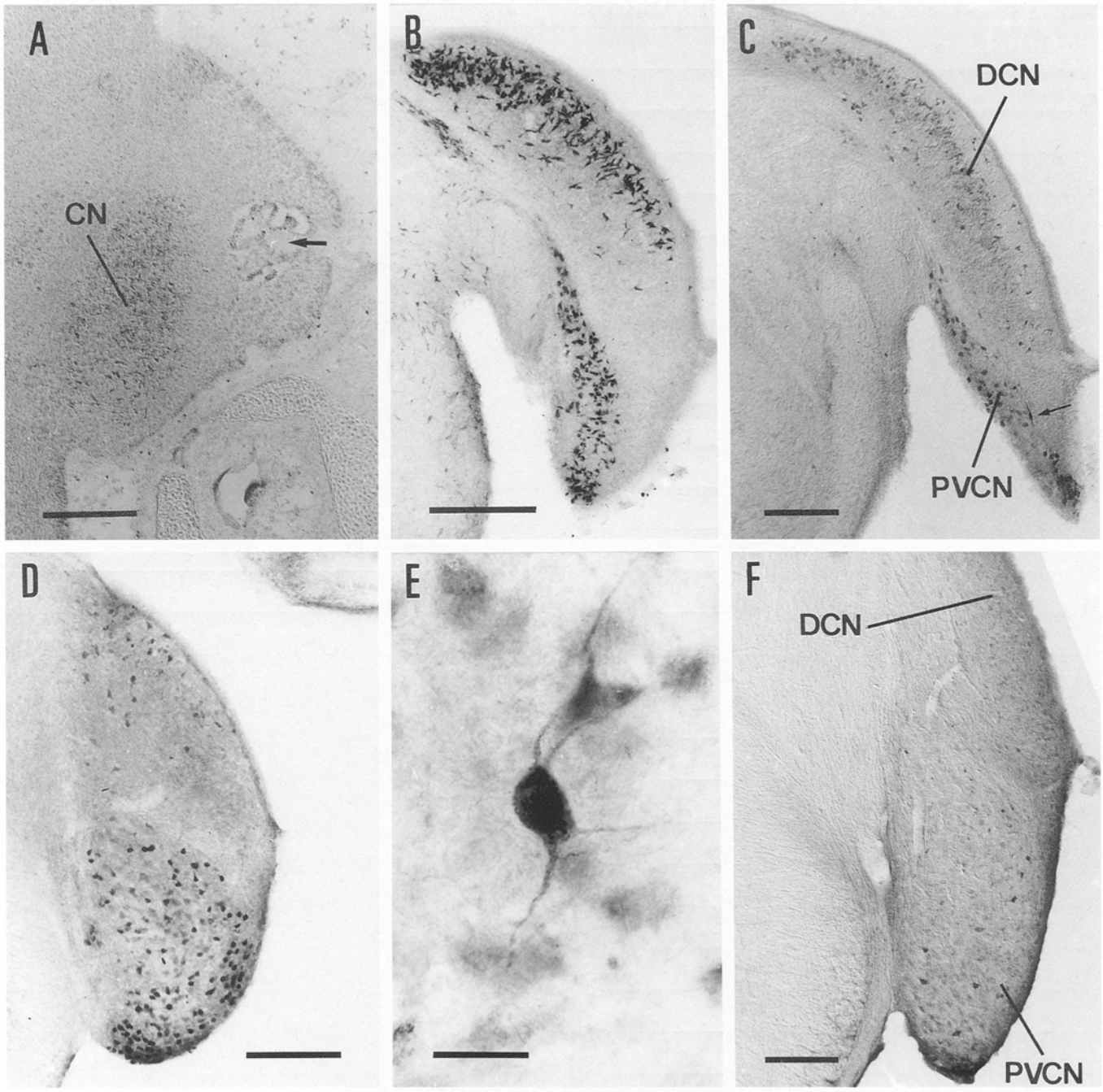

Fig. 1A–F Coronal sections through the right dorsal cochlear nucleus (DCN) and posteroventral cochlear nucleus (PVCN), stained for somatostatin at five different ages between E20 and adulthood. Dorsal is *up* and lateral to the *right* in all panels of this and all following figures. **A** E20. A moderate number of somatostatin immunoreactive (SIR) neurons could already be seen in the anlage of the cochlear nucleus (CN). Notice choroid plexus (*arrow*) which is located immediately lateral to the anlage of the CN and, thus, helped to localize the CN. **B** P4. The DCN, as well as the PVCN, contained a large number of heavily labeled SIR neurons. **C** P7. SIR in the DCN had slightly decreased, but PVCN neurons were still heavily labeled, and SIR dendrites were present in some neurons (*arrow*). **D** P11. SIR had further decreased, and fewer SIR neurons were found in the DCN than in the PVCN. **E** P11. SIR neuron in the PVCN at high magnification, showing the multipolar appearance of the soma-dendritic morphology. **F** Adult. Only a few neurons remained SIR in the PVCN of adult rats, whereas the DCN became virtually devoid of SIR neurons. *Bars*: 250 μm in **A–D**, **F**; 25 μm in **E**

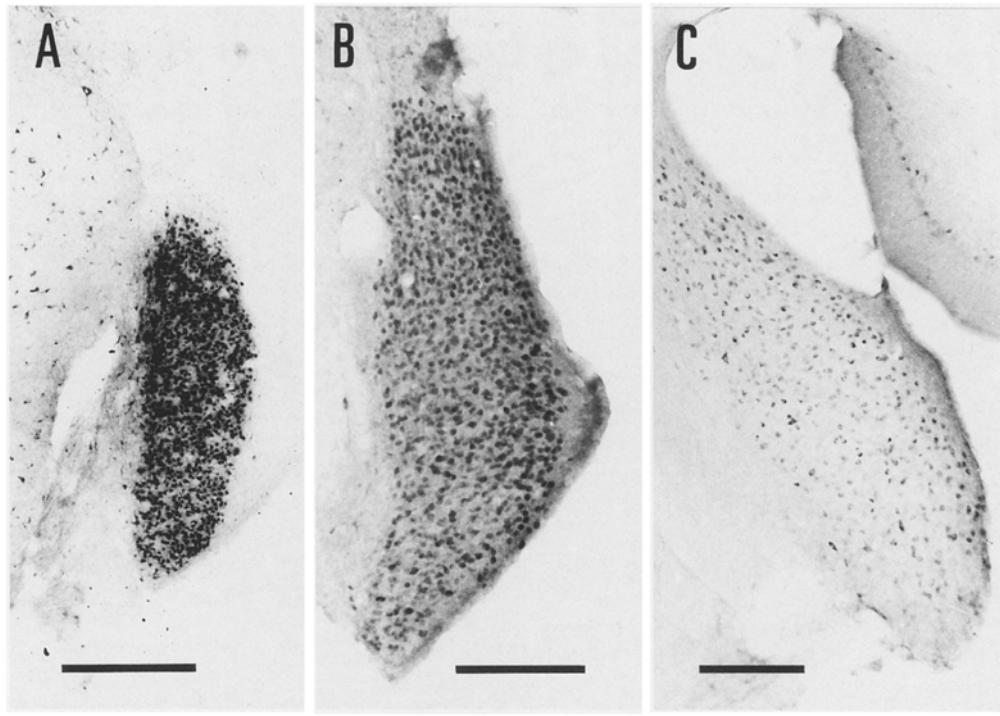

Fig. 2A–C Coronal sections through the right anteroventral cochlear nucleus (AVCN) stained for somatostatin at three different ages. **A** P4. Virtually all AVCN neurons were very heavily labeled. Together with the PL, the AVCN contained the highest density of SIR neurons. **B** P11. SIR neurons were still present throughout the AVCN, but labeling intensity had decreased. **C** Adult. Even after colchicine treatment, only a relatively small number of weakly SIR neurons could be found. *Bars* 250 µm

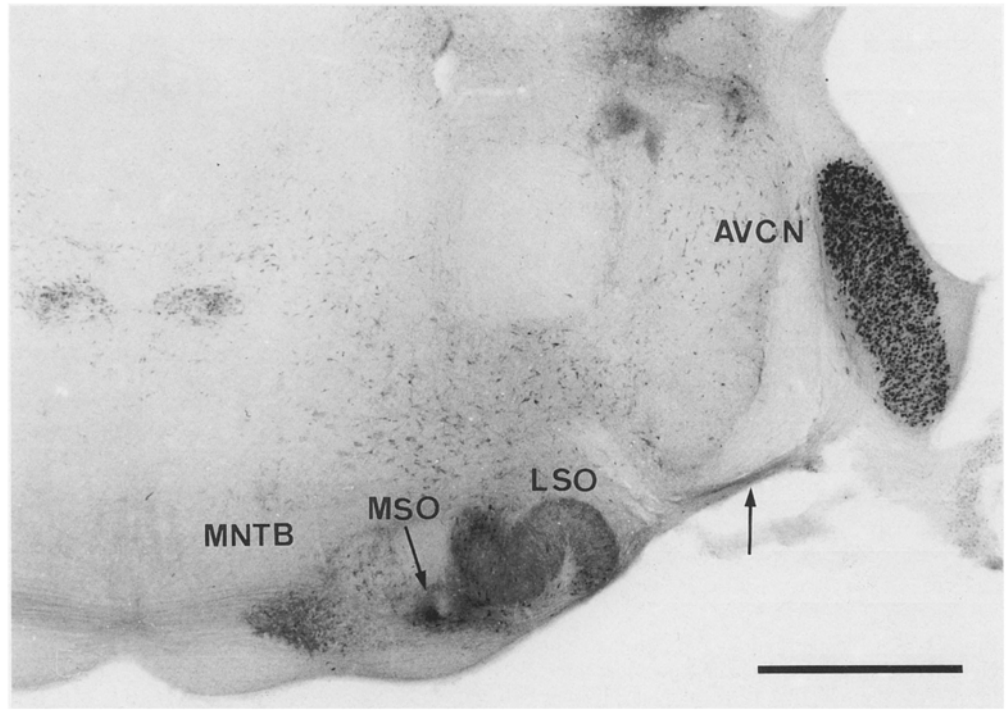

Fig. 3 Coronal section through the right brainstem stained for somatostatin at P6. At this stage, the vast majority of AVCN neurons were SIR. Fibers in the ventral acoustic stria (*arrow*), which originate from VCN neurons, also displayed strong SIR. These fibers can also be seen medial to the medial nucleus of the trapezoid body (MNTB) and their terminals within the lateral superior olive (LSO), medial superior olive (MSO), and MNTB, indicating that the projection from the VCN to the superior olivary complex (SOC) is somatostatinergic at P6. *Bar* 600 µm

Superior olivary complex

Somata. In the SOC, only a few SIR somata appeared during a very restricted period of development and were first detectable 1 day after birth (Fig. 4B). During the following days, they were predominantly located in marginal aspects of the lateral superior olive (LSO) and in the dorsal, lateral, and ventral periolivary nuclei as well. Additionally, moderate numbers of SIR somata were found in the superior paraolivary nucleus (SPN) and very small numbers in the medial superior olive (MSO). The number of SIR somata in the SOC increased until P4, when SIR neurons were unevenly distributed in the LSO, with the majority being located in the lateral aspect of the nucleus (Figs. 4C; 5A). In the other SOC nuclei, the pattern of the distribution appeared to be unchanged, as compared with P1. In animals aged P7 and older, SIR somata were no longer present in the SOC (Figs. 4D–F; 5D–E).

Fibers. We observed a strong occurrence of heavily labeled SIR processes in the SOC, namely in the LSO, the MSO, and the medial nucleus of the trapezoid body (MNTB). This is in clear contrast to the paucity of labeling in SOC somata. SIR fibers were transiently present in the above nuclei, and their appearance covered a relatively wide time span during development. Whereas we did not detect SIR fibers in E17 fetuses, they were found at E18 for the first time. Figure 4A demonstrates a dense network of processes in the area of the developing SOC at E20, when we found it impossible to differentiate between individual nuclei. By P1, the distribution of SIR fibers became more discrete and could be easily ascribed to the MSO, the MNTB, and the LSO, with the highest density appearing in the latter nucleus (Fig. 4B). Aside from these strongly labeled nuclei, only a few SIR processes were visible in the SPN, the dorsal periolivary nucleus, and the lateral and ventral nuclei of the trapezoid body. With increasing age in early postnatal development, the immunoreactivity increased in the SOC. Between P4 and P7 the fiber density, as well as the labeling intensity, became exceedingly rich, reaching a maximum in development at P7 (Fig. 4D). At this age, an extremely dense network of SIR-fibers was present in all limbs of the LSO. The MSO also displayed a very dense network of SIR fibers, but the center of the nucleus, where MSO somata are located, was almost devoid of labeling, and only a few processes were visible (Figs. 4D; 5B). The vast majority of labeled fibers were instead seen along the medial and lateral edges of the MSO, i.e., in areas occupied by the dendrites of MSO neurons. Apparently, all axons in the MNTB, building the calyces of Held, were immunoreactive (Fig. 5C). The fibers running within the trapezoid body also clearly demonstrated SIR (Fig. 4D). In contrast to the high concentration of SIR fibers in the LSO, MSO, and MNTB, the fiber density in the periolivary nuclei and in the SPN was still weak (Fig. 4D).

After P7, the density of SIR fibers decreased rapidly in all SOC nuclei (Fig. 4E, F). Within 10 days most of the SIR had disappeared, and at P18 and in the adult brain, only a few SIR fibers could be found; these were restricted to the MNTB, sometimes resembling calyces of Held (Fig. 5F). No SIR elements were found in the LSO (Fig. 5D) or in the MSO (Fig. 5E).

Nuclei of the lateral lemniscus

Somata. The primordium of the NLL and the hypothalamus were the regions that first contained clusters of SIR somata. At E17, the earliest stage investigated, the anlage of the NLL showed a remarkable number of SIR neurons (Fig. 6A, B). Nuclei within the lateral lemniscus could be differentiated as early as P1. At this age the vast majority of the SIR somata could be attributed to the paralemniscal nucleus (PL), which is located medial to the intermediate NLL (INLL; Figs. 6C; 7A). The number of SIR somata in the PL was consistently high between P1 and P7 (Figs. 6C, D; 7A). After P7, SIR decreased continuously in the lateral lemniscus, yet some neurons in the PL remained labeled into adulthood (Fig. 7C). In addition to the SIR neurons of the PL, some neurons occurred in the ventral NLL (VNLL) and in the INLL between P1 and P4 (Fig. 6C). By contrast, SIR somata were virtually never present in the dorsal NLL proper (DNLL), but they could be found in considerable number surrounding the DNLL (Fig. 6C).

Fibers. SIR processes occurred first at E18 in the anlage of the lateral lemniscus (Fig. 6B). By P1, these fibers could be attributed to the VNLL (Fig. 6C), while the INLL and the DNLL were still devoid of labeling. The number of SIR fibers increased with age in all subdivisions of the NLL, and the maximum fiber density was reached between P4 and P9. Within the INLL and the VNLL, a dense network of SIR processes was present at that time (Fig. 6D). At later stages the density of SIR processes decreased in the lateral lemniscus. Finally, in adulthood there were still some SIR fibers in the NLL (data not shown).

Inferior colliculus

Somata. SIR cells were present in the IC in small numbers at P1. These somata were located in the central nucleus of the IC (ICC), as well as in deeper layers of the external and dorsal cortices of the IC (ICEC and ICDC). The number of SIR neurons was consistently small until P7 and in the ICC SIR cells were absent thereafter. In the ICEC and ICDC, the number of SIR cells decreased more slowly than in the ICC and in the adult brain some weakly labeled neurons were still detectable in these areas (Fig. 7D).

Fibers. In fetal brains some SIR fibers were found at E20 in the anlage of the IC (data not shown). The fiber density in the ICC decreased rapidly during ontogeny. SIR processes were only present until P4 (Fig. 7A, B). In older stages, virtually no SIR fibers were present in the ICC. The distribution of SIR fibers in the ICEC and ICDC changed during development, and more SIR fibers could be seen in superficial layers of the adult ICEC and ICDC than in the deeper layers (Fig. 7C, D). Thus, the pattern of SIR fibers in the adult ICEC and ICDC was complementary to that seen during the first postnatal week (cf. Fig. 7A).

Double-labeling experiments

It has been reported that glia cells of the hypothalamus can express somatostatin during development (Davidson and Gillies 1993). We therefore performed double-labeling experiments and stained for somatostatin and GFAP in four animals aged P4, when SIR was high in most au-

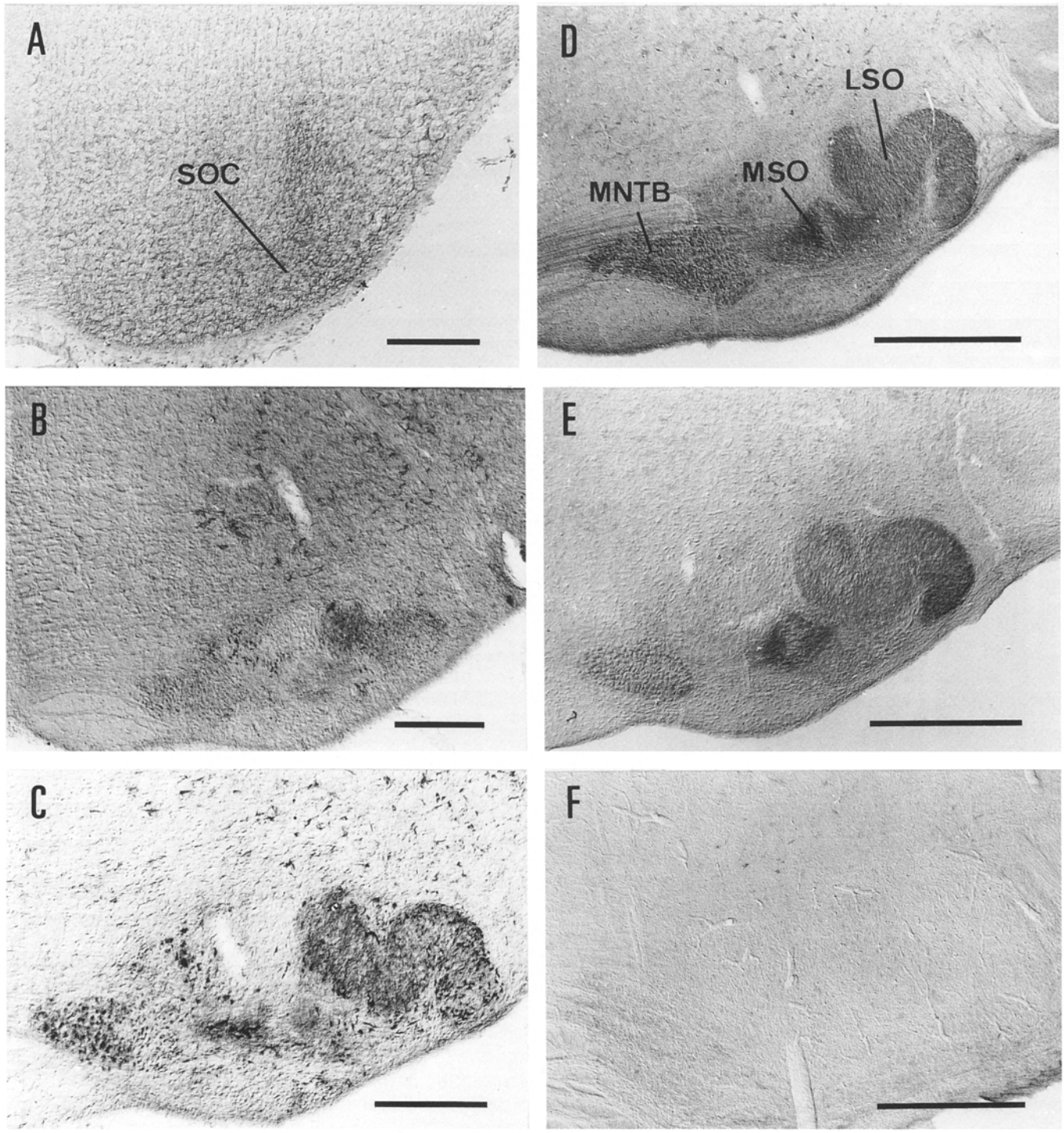

Fig. 4A–F Coronal sections through the right SOC, stained for somatostatin at six different ages between E20 and adulthood. **A** E20. A patch of SIR fibers was clearly present in the anlage of the SOC, but could not be attributed to a distinct nucleus. No SIR somata were seen at this age. **B** P1. SIR fibers could be identified within the nuclear domains of the LSO and the MNTB and within the dendritic region of the MSO. Few SIR somata were present in lateral aspects of the LSO, in the superior paraolivary nucleus (SPN) and in periolivary nuclei. **C** P4. Until this age, the labeling of somata and fibers increased in all of the above nuclei. Notice equal distribution of SIR fibers in the LSO, but a greater concentration of SIR neurons in the lateral than in the medial aspect of the nucleus. **D** P7. SIR somata were not found any more, but labeling of fibers in the LSO, MSO, and MNTB had further increased and the outlines of these nuclei were clearly demarcated. Labeling intensity had reached a maximum, and fibers of the trapezoid body were also strongly SIR. **E** P9. SIR fibers were still present in the same nuclei as those mentioned above, but labeling intensity had begun to decrease. **F** Adult. SIR had decreased dramatically and no labeled structures were visible at low magnification. *Bars*: 250 μm in **A–C**; 500 μm in **D–F**

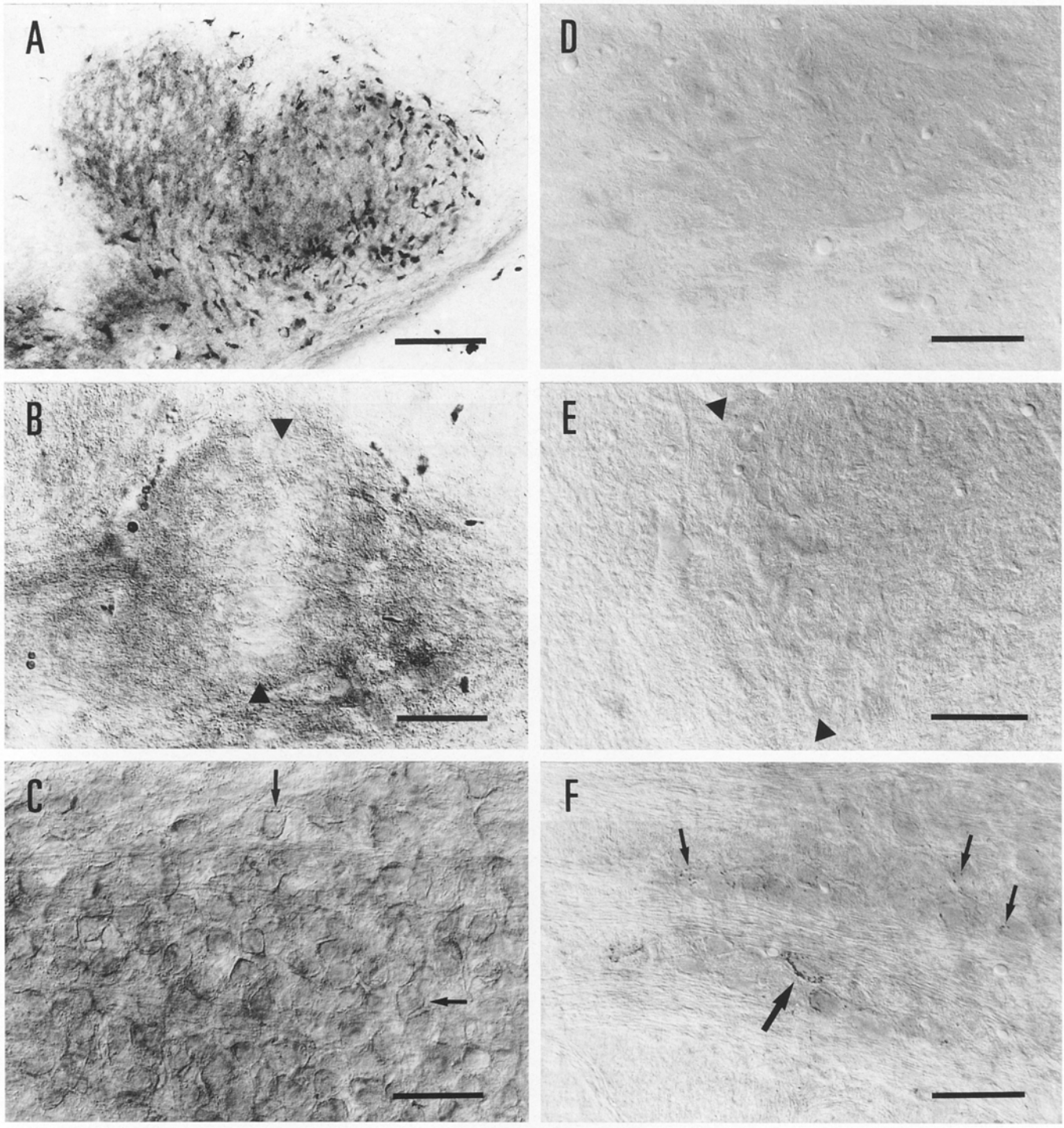

Fig. 5A–F Coronal sections through the developing (**A–C**) and adult (**D–F**) SOC, stained for somatostatin and shown at high magnification in order to illustrate the subnuclear distribution of labeling patterns. **A** LSO at P4. Whereas a dense network of SIR fibers was present in all aspects of the LSO around P4, SIR somata occurred predominantly in lateral aspects of the nucleus. Notice that there are also SIR somata in periolivary nuclei immediately dorsal and ventral to the LSO. **B** MSO at P7. The developing MSO displayed a dense network of SIR fibers in the medial and lateral aspects, which contain the dendritic arbors. SIR fibers were rarely found in the center of the MSO, where the somata are located (*arrowheads*). **C** MNTB at P7. Apparently all axons building the calyces of Held showed SIR, and the immunonegative MNTB somata were surrounded by these SIR calyces (*arrows*). **D** LSO in an adult rat. Neither SIR somata nor SIR fibers were seen in the adult LSO. **E** MSO in an adult rat. As in the LSO, no immunoreactivity was present in the adult MSO (*arrowheads* mark the center of the MSO). **F** MNTB in an adult rat. Only a few SIR fibers remained into adulthood. SIR calyces of Held could barely be identified (*large arrow*), but SIR puncta were still found (*small arrows*). *Bars*: 250 μm in **A**; 50 μm in **B–F**

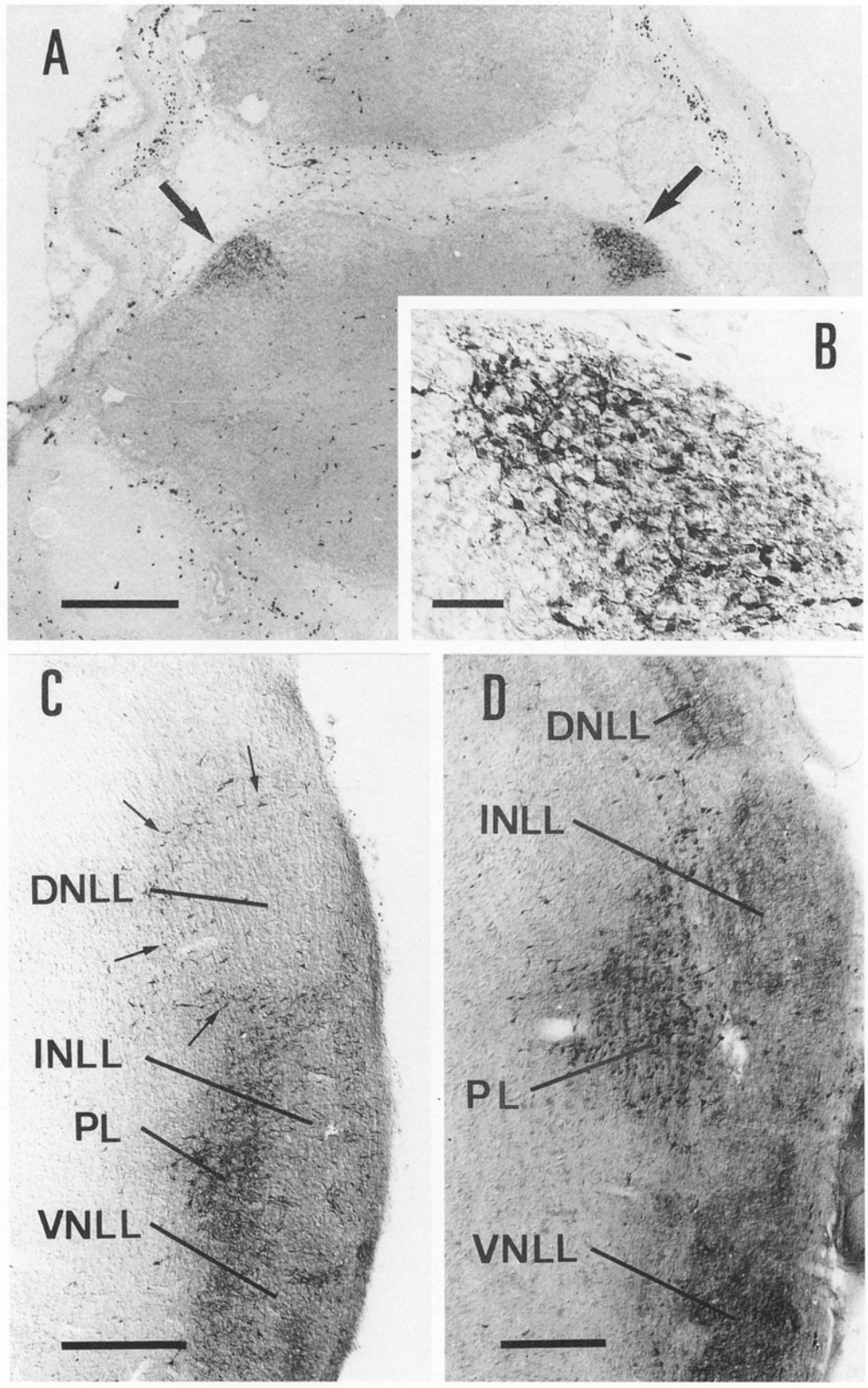

Fig. 6A–D Coronal sections through the nuclei of the lateral lemniscus (NLL), stained for somatostatin at three different ages between E17 and P7. **A** E17. Low-power photomicrograph of a midbrain section that was taken at a level which corresponds to Fig. 19 of Paxinos et al. (1991). Notice the strong SIR which is restricted to the primordium of the NLL on both sides (*arrows*). **B** Right NLL shown in **A** at higher magnification. Heavily labeled somata and SIR processes were already present at E17. **C** P1. The vast majority of SIR somata were present in the paralemniscal nucleus (*PL*). Only a few neurons were found in the VNLL and INLL and none in the DNLL proper. The surrounding of the DNLL, however, contained a considerable number of SRI somata (*arrows*). Patches of SIR fibers were present in the VNLL, whereas the INLL and DNLL were devoid of labeled fibers. **D** P7. Around this age, the maximum of immunoreactivity was reached, with the greatest number of SIR somata seen in the PL, and labeled fibers appearing in all nuclei of the lateral lemniscus. *Bars*: 500 μm in **A**, **C**, **D**; 50 μm in **B**

ditory nuclei (Table 2), in order to investigate if the SIR cells in the auditory brainstem were possibly astrocytes. The experiments revealed that none of the SIR elements in the auditory brainstem were also GFAP-immunoreactive, indicating that they were not astrocytes, but probably of neuronal origin (not shown).

Figure 8 provides a summary of the temporal and regional distribution of somatostatin in the rat auditory brainstem nuclei. Somatostatin shows a strong, but transient, appearance in most of these nuclei. The highest number of heavily SIR somata was found in the lateral leminiscus in fetuses and in the VCN in P6 animals. The

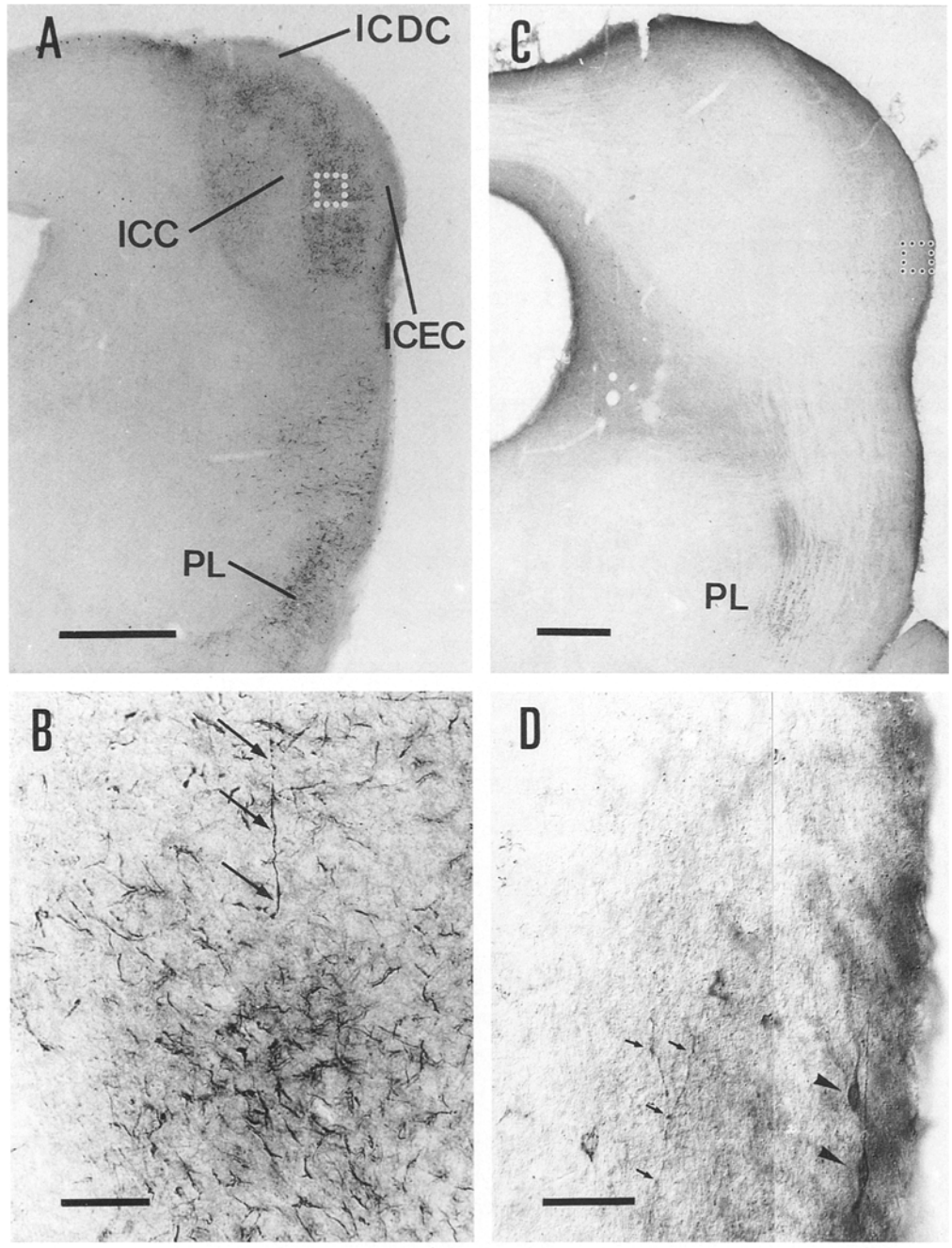

Fig. 7A–D Coronal sections through the right inferior colliculus (IC), stained for somatostatin at P1 and in an adult animal. **A** P1. Remarkable numbers of SIR somata and fibers were present throughout most areas of the IC, with the exception of superficial layers of the dorsal and external cortices (*ICDC* and *ICEC*) and a medio-ventral region within the central nucleus (ICC). Notice labeled neurons also in the PL. **B** *Framed area* shown in **A** at higher power, illustrating strongly labeled SIR structures that only occasionally resembled axons (*arrows*). Double-labeling experiments for somatostatin and GFAP, however, revealed that none of the SIR structures were of astrocytic origin. **C** Adult. The number of SIR neurons and fibers had become low and particularly the ICC was almost devoid of labeling. In contrast, the PL was still filled with SIR somata. **D** High-power microphotograph of the *framed area* in **C**, showing that a few neurons (*arrowheads*) and fibers (*arrows*) in the ICEC were still SIR in the adult. *Bars*: 500 μm in **A**, **C**; 50 μm in **B**, **D**

highest concentration of SIR fibers and terminals was seen in the LSO and the MNTB, where labeling peaked around P6, i.e., at the stage when somata in the VCN also reached a maximum level of immunoreactivity. These results, combined with previous findings, suggest that the projection from the VCN to the SOC is heavily somatostatinergic during the first postnatal week.

Leu-enkephalin

During both early development and in the adult brain, LIR elements were present in all principal auditory brainstem nuclei. Compared with the abundance of SIR elements, however, the density of LIR elements was quite low and neurons were often only weakly labeled. LIR somata occurred relatively late, i.e., after P12. During subsequent development, the number of labeled somata increased only slightly and reached a constant, albeit low, level after P21. In contrast to the late appearance of LIR somata, LIR fibers occurred prenatally in the developing CN, SOC, and lateral lemniscus, indicating that they possibly originated from sources outside the auditory system. At birth, LIR fibers were present in all principal auditory brainstem nuclei, but they were only weakly labeled. During postnatal development the number of LIR fibers increased progressively, but only very slowly, in most auditory brainstem nuclei (e.g., period between P1– P11; see Table 3). No transient appearance of LIR elements was observed between E17 and adulthood (Table 3).

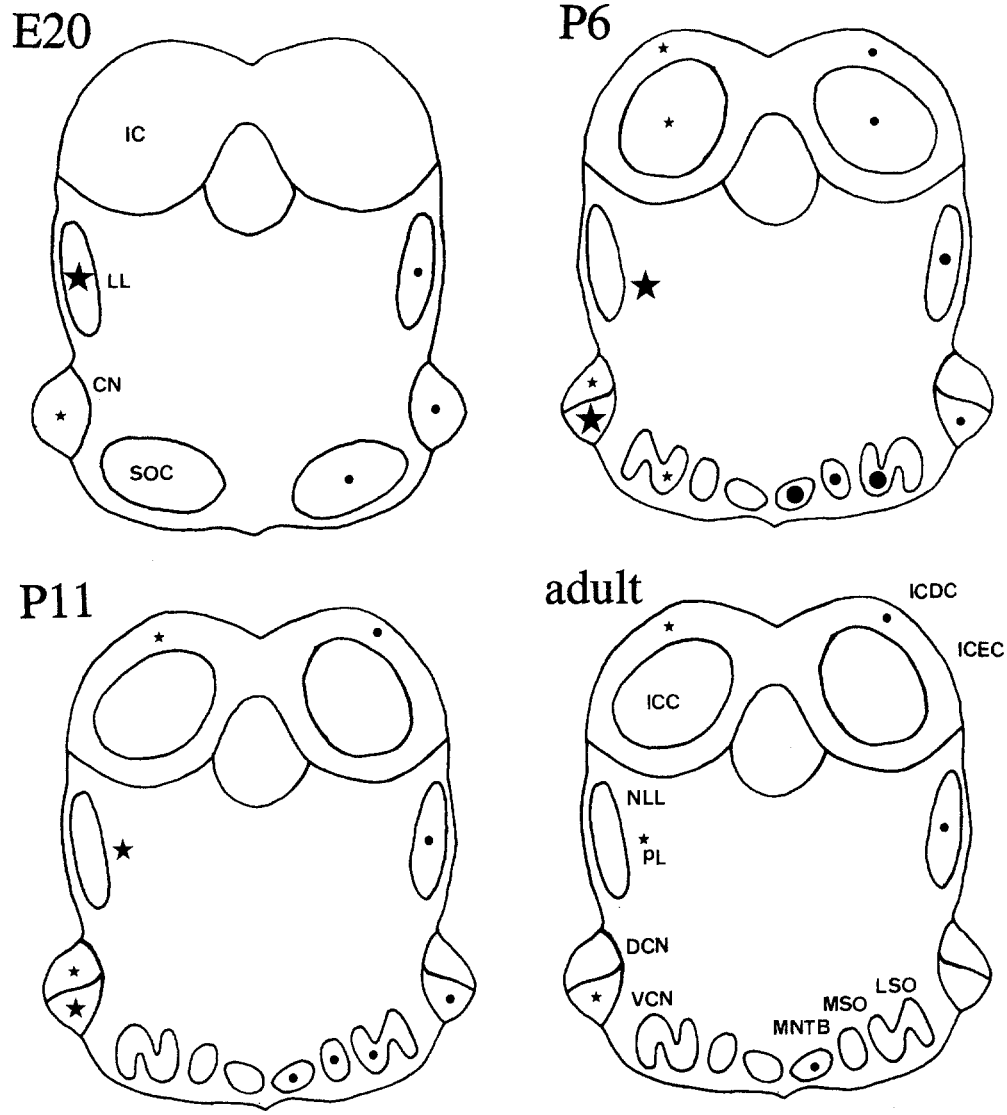

Fig. 8 Semischematic diagram summarizing the development of SIR somata and fibers in principal nuclei of the rat auditory brainstem (E20 to adult). The *left side* of each panel illustrates SIR somata (depicted by *stars*), whereas the *right side* shows SIR fibers (depicted by *dots*). The number of SIR somata and fibers is indicated by the different sizes of the stars and dots: *small symbols* indicate low SIR, *medium-sized symbols* indicate medium SIR, and *large symbols* indicate strong SIR. Notice that the greatest amount of SIR in *somata* occurs in the lateral lemniscus (*LL*) and the VCN, whereas the greatest amount in *fibers* occurs in the LSO and the MNTB

Cochlear nuclear complex

Somata. Very few LIR somata were first found in the VCN at P12. With increasing age, we observed a gradual increase in the number of LIR neurons, and in the adult VCN a moderate number of weakly labeled neurons was present (Table 3). In the DCN, LIR neurons began to appear relatively late in postnatal life; a few LIR neurons were first seen at P21 and were visible thereafter. However, their number remained small throughout ontogeny and labeling was always weak.

Fibers. In contrast to the late appearance of LIR somata, LIR fibers in the CN were present in fetal rats. At E20, few labeled fibers were detectable along the medial border of the anlage of the CN. At birth, when subdivisions of the CN could be demarcated, LIR fibers were present in the DCN, yet only to a small extent. During postnatal ontogeny, the density of LIR fibers in the DCN remained low. LIR fibers were never present in the VCN (Table 3).

Superior olivary complex

Somata. In the SOC, no LIR somata were found prior to adulthood. However, in the adult, colchicine-treated brains, a few labeled neurons were found. These neurons were restricted to the LSO and the ventral nucleus of the trapezoid body (VNTB). The somata in the VNTB were heavily stained, whereas LIR neurons in the LSO showed only a very weak labeling intensity.

Fibers. In contrast to the absence of LIR somata, LIR fibers were found in the developing SOC. These fibers appeared in low density already as early as E18 and became clearly labeled by E20 (Fig. 9A, D). In P0 animals, LIR fibers could be attributed to the periolivary nuclei, where they occurred in all aspects. Labeling intensity of LIR fibers in the periolivary nuclei increased in early postnatal development (Fig. 9B, E) and immunoreactivity persisted into adulthood (Fig. 9C, F). Individual fibers were originally incompletely labeled (Fig. 9D), and

Table 3 Developmental changes in the number of leu-enkephalin immunoreactive (LIR) elements in auditory brainstem nuclei in the rat. As there was no change in the labeling pattern and intensity between P1 and P11, the data from P1, P4, P5, P7, P9, P10, and P11 animals were pooled. The same holds for the period between P16 and P18. Subdivisions within brainstem nuclei could not be distinguished before birth (*S* LIR somata, *F* LIR fibers, – no LIR, *weak LIR, **moderate LIR)

		Leu-enkephalin immunoreactivity					
		CN		SOC perioli	NLL	IC	
		DCN	VCN			ICC	ICDC ICEC
E17	S	–		–	–	–	
	F	–		–	–	–	
E18	S	–		–	–	–	
	F	–		*	*	–	
E20	S	–		–	–	–	
	F	*		*	*	–	
P0	S	–	–	–	–	–	–
	F	*	–	*	*	–	*
P1–P11	S	–	–	–	–	–	–
	F	*	–	**	*	–	*
P12	S	–	*	–	–	–	*
	F	*	–	**	*	–	*
P16–P18	S	–	*	–	–	*	*
	F	*	–	**	*	–	*
P21	S	*	*	–	–	*	*
	F	*	–	**	*	–	*
Adult	S	*	*	*	*	*	*
	F	*	–	**	*	–	*

beaded fibers with growing numbers of boutons matured only at later stages (Fig. 9E, F).

Nuclei of the lateral lemniscus

Somata. LIR neurons in the NLL were detectable in the adult brain only after colchicine treatment. These somata were restricted to the VNLL (Fig. 10C). Although the number of labeled neurons was quite small, the staining was very intense. At all other ages and in all other subdivisions of the NLL, LIR neurons were not seen.

Fibers. In contrast to the lack of LIR somata in the lateral lemniscus, LIR processes were present from fetal ages until adulthood in all subdivisions of the NLL (Fig. 10A, B). At E18 and E20, moderate numbers of LIR fibers were present in the anlage of the lateral lemniscus (Fig. 10A); the moderate fiber density persisted into adulthood.

Inferior colliculus

Somata. LIR somata in the IC were found only in low numbers and only at later developmental stages (Table 3). A few LIR somata occurred at P12 in the ICDC and ICEC and from P16 onwards in the ICC. These neurons were still detectable at later ages, including adulthood.

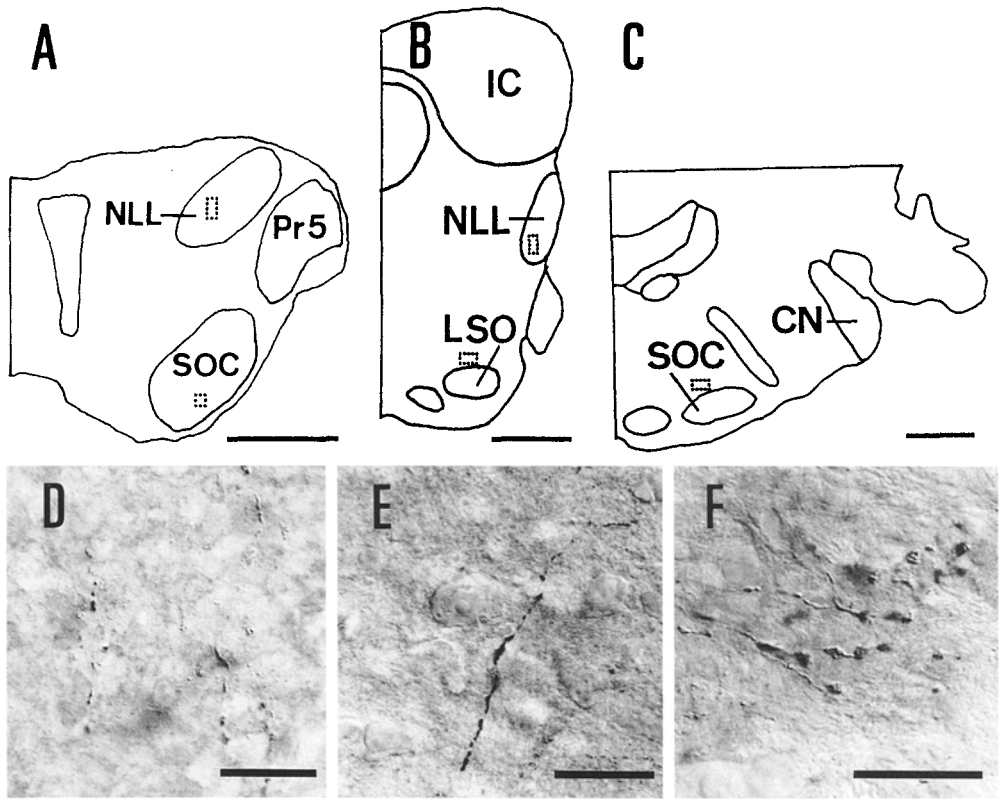

Fig. 9A–F Drawings of the right brainstem and corresponding photomicrographs of the SOC stained for leu-enkephalin between E20 and adulthood. **A–C** Drawings of coronal sections through the right brainstem at E20, P5, and in adulthood, respectively. *Framed areas* within SOC in **A**, **B**, **C** are shown in photomicrographs in **D**, **E**, **F**, respectively. *Framed areas* within NLL in **A**, **B** are shown in photomicrographs in Fig. 10A, B, respectively. **D** High-power photomicrograph of the *framed SOC area* shown in **A**, illustrating a few LIR fibers present in the anlage of the SOC at E20. **E** High-power photomicrograph of the *framed area dorsal to the LSO shown in* **B**, illustrating a LIR fiber in the dorsal periolivary region at P4. **F** High-power photomicrograph of the *framed area shown in* **C**, illustrating LIR fibers with numerous en passent boutons in the adult dorsal periolivary region. *Bars*: 500 μm in **A**; 1 mm in **B**, **C**; 20 μm in **D–F** (*Pr5* principal sensory trigeminal nucleus). The *framed areas in the NLL* (**A**, **B**) are further depicted in Fig. 10A, B

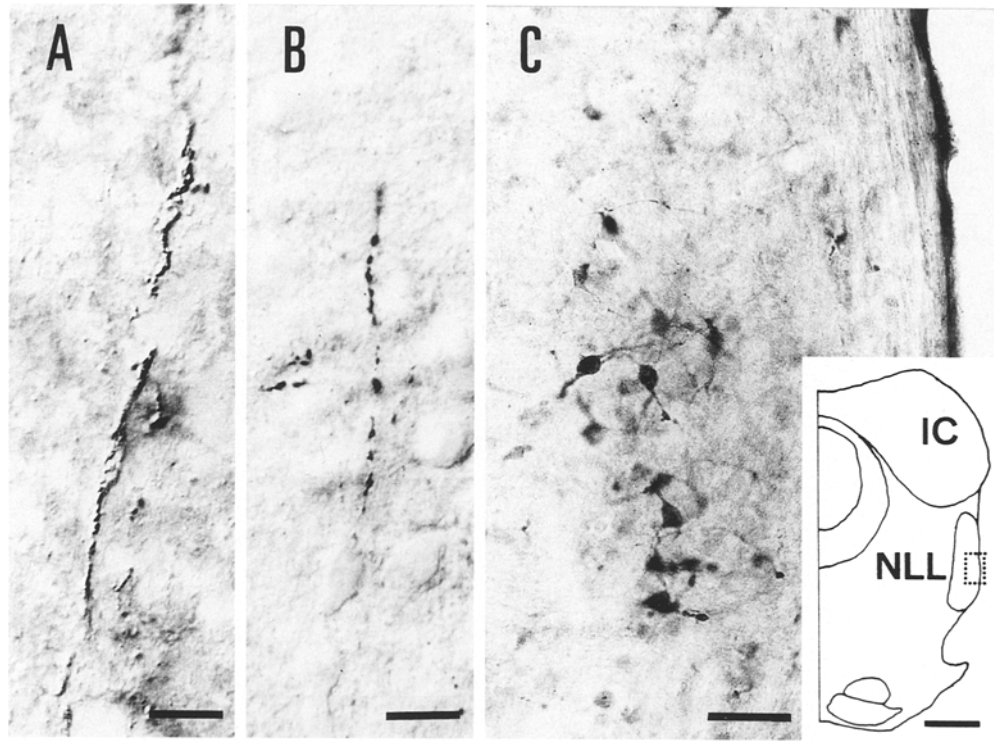

Fig. 10A–C Coronal sections through the right NLL, stained for leu-enkephalin between E20 and adulthood. **A** At E20, LIR fibers were found in the anlage of the NLL. Notice that the photomicrograph corresponds with the *framed area in the NLL shown in Fig. 9A*. **B** LIR fiber in the NLL at P4. Notice that the photomicrograph corresponds with the *framed area in the NLL shown in Fig. 9B*. **C** LIR somata were not seen before adulthood in the NLL; they then became visible in the INLL in colchicine-treated animals. *Insert* shows the location of the high-power photomicrograph. *Bars*: 10 μm in **A**, **B** 50 μm in **C**; 1 mm in *insert*

Fibers. In comparison to the late appearance of labeled neurons in the IC, LIR fibers were present in the ICDC and ICEC during all postnatal stages (Table 3). A negligible number of LIR processes was first found at P0. Low numbers of LIR fibers were present in all the following stages, including adulthood. We never found LIR fibers in the ICC.

Discussion

The present study was undertaken to investigate the precise distribution and the time course of somatostatin- and leu-enkephalin-positive elements in auditory brainstem nuclei of fetal and postnatal rats by using sensitive PAP immunocytochemistry.

Somatostatin

Within the nuclei in the pontine and mesencephalic brainstem and throughout development, heavy SIR labeling was restricted to auditory nuclei, the nucleus of the solitary tract, and the principal sensory trigeminal nucleus. Our results reveal a strong, yet transient, expression of somatostatin in the developing auditory brainstem. SIR-positive neurons can already be found in fetal rats (at least as early as E18) and become present in all principal auditory nuclei before birth. Immunoreactivity increases until about P7, when the maximum of both number of labeled neurons and labeling intensity is reached, and then subsequently decreases. Only a few SIR somata and fibers persist in the adult auditory brainstem.

We found a remarkable coincidence between the SIR within neurons of the CN and that within fibers in those SOC nuclei that are known to receive input from CN neurons, such as the MSO, the LSO, and the MNTB. The beginning of SIR, the peak of immunoreactivity, as well as its decline, all correlated very well within the above nuclei, indicating that the SIR fibers seen in the SOC originate from CN neurons. Further support for this conclusion comes from the fact the ventral acoustic stria, which contains the axons of CN neurons projecting towards the SOC (Adams and Warr 1976; reviews: Cant 1991; Helfert et al. 1991), is SIR during the period when CN neurons and fibers in the SOC are immunoreactive. Moreover, the distribution pattern of SIR axon terminals in the SOC nuclei (e.g., within the MSO, see Fig. 5B) and their morphology (e.g.; terminals in the MNTB resembling calyxes of Held, see Fig. 5C) also provide compelling evidence that the somatostatinergic input to the SOC nuclei originates from neurons in the VCN and is thus an intrinsic, system-immanent projection. Finally, our result that SIR neurons in the VCN most probably belong to spherical AVCN cells as well as multipolar and globular PVCN cells is in accord with previous studies demonstrating that these cell types project into the SOC (Oertel et al. 1990; Thompson and Thompson 1991; review: Helfert et al. 1991). Taken together, these data suggest that the projections between the CN and its target nuclei in the SOC develop under the influence of somatostatin, as discussed below.

Immunoreactivity of SIR terminals in the NLL increased and decreased during the stages in which equivalent changes were observed in VCN somata. Weak labeling in both VCN somata and NLL terminals was first

present at E18, increased during the first postnatal week and declined thereafter. These data suggest that VCN neurons, known to project into the NLL (Glendenning et al. 1981; Friauf and Ostwald 1988; Covey 1993), are the origin of the SIR terminals seen in the NLL.

SIR fibers in the ICC could be seen between E20 and P6, and moderate numbers of SIR neurons in the DCN were found between P1 and P4. Thus, there is some correlation between the appearance of SIR somata in the DCN and terminals in the ICC. As DCN neurons project into the ICC (review: Helfert et al. 1991), we therefore conclude that there may be a somatostatinergic projection between DCN and ICC in perinatal rats.

A great many SIR somata were present in the lateral lemniscus in fetal animals. In contrast, there were no SIR fibers or only a few in the IC, which initially seemed surprising, because of the well-documented projection from NLL neurons to the IC (Adams and Mugnaini 1984; Shneiderman et al. 1988; Saint Marie and Baker 1990). However, although we could not unequivocally distinguish between NLL and PL in prenatal animals, we think that SIR neurons in the lateral lemniscus were located in the anlage of the PL rather than in the NLL. This may explain why we did not see SIR fibers in correspondence with the strong appearance of SIR somata in the lateral lemniscus.

Comparison with other studies

Expression of the somatostatin peptide and mRNA in the developing auditory brainstem of rats has been reported previously by Takatsuki and coworkers (Shiosaka et al. 1981; Takatsuki et al. 1981, 1982) and by Fitzpatrick-McElligott et al. (1991), respectively. Unfortunately, the studies by Takatsuki et al. only investigated "very young rats" between P0 and P7 and did not differentiate between different ages. In contrast, the paper by Shiosaka et al. (1981) reports on the time course of somatostatin expression, thereby emphasizing the fetal period. It does not, however, focus attention on auditory structures and, therefore, provides only general information about the SIR distribution in auditory brainstem nuclei. In comparison with the studies by Takatsuki and coworkers, the present paper reveals some different results, both in the temporal and spatial distribution of SIR elements. First, the largest number of SIR structures was found occurring between E20 and E22 in the study by Shiosaka et al. (1981) and decreased by P5. In contrast, our study indicates that the maximum of SIR, as determined by the number and intensity of labeled structures, was reached around P7. Second, Shiosaka et al. (1981) never found SIR elements in the adult auditory brainstem, whereas we found SIR somata in the VCN, the paralemniscal region, and in the cortices of the IC, as well as SIR fibers in the MNTB, the NLL, and in the cortices of the IC in the adult rat. Our findings are consistent with those of Johannson et al. (1984), who also described SIR elements in these auditory nuclei. Further evidence for SIR in the adult VCN comes from hybridization histochemistry, showing that somatostatin mRNA is detectable in the VCN of adult rats (Fitzpatrick-McElligott et al. 1991). Third, Takatsuki and coworkers found a large number of SIR fibers in the NLL and a comparatively small density of SIR fibers in the SOC, these being restricted to the LSO and MSO. We also found many SIR fibers in the NLL, but in contrast, we identified an even stronger density of heavily labeled fibers in the LSO, the MSO, and the MNTB, which in fact formed the nuclei with the most intensely labeled fibers in the lower brainstem during early postnatal development. Most of the differences between our findings and those of Takatsuki and coworkers appear to result from using different techniques. First, as already mentioned, we used the PAP technique, whereas Takatsuki and coworkers used the less sensitive immunofluorescence technique. Second, the primary antibodies against somatostatin were different. While the specificity of the somatostatin antibody used by Takatsuki and coworkers was not reported, the antibody we used shows full cross-reactivity against somatostatin-14 and somatostatin-28 (data sheet from Peninsula). Because of these differences, it is possible that we detected both cleavage products of the prosomatostatin peptide in the auditory brainstem nuclei and not only one cleavage product, such as somatostatin-14.

Somatostatin immunoreactivity in other neuronal systems

There is a growing body of literature indicating that the transient expression of somatostatin is not restricted to developing auditory structures, but is rather a feature of several brain areas, most of them being of sensory nature. In the rat, virtually all cranial and spinal sensory ganglion cells are SIR at E12, whereas only a few SIR cells are found in the adult (Katz et al. 1992; Maubert et al. 1992). SIR neurons appear also transiently during ontogeny in the retina (Ferriero 1992) and the cerebellum (Inagaki et al. 1982). Circumstantial evidence of the transient appearance of somatostatin is further provided by studies that describe a transient expression of somatostatin receptors during development in the visual system (Bodenant et al. 1991, 1993; Ferriero 1992), as well as in the cerebellum (Gonzalez et al. 1988, 1989). Furthermore, the fact that in these systems the transient expression of somatostatin is associated with the transient appearance of somatostatin receptors in target regions reinforces the idea that somatostatin is indeed acting transsynaptically like a transmitter and may thus regulate the maturation of target cells that express somatostatin receptors.

Physiological effects of somatostatin

Despite the great number of anatomical studies on the development of somatostatin expression in the brain, al-

most nothing is known about its function during ontogeny. To our knowledge there is only one study that has investigated the physiological action of somatostatin during neuronal development in mammals. Gonzales et al. (1992) showed that pressure-injected somatostatin causes a marked reduction of the concentration of intracellular Ca^{2+} and cAMP in cultured, immature cerebellar granular cells. As the inhibitory effect on cAMP production was prevented by pertussis toxin, somatostatin activity in this case most probably involves inhibition of a G protein-coupled adenylyl cyclase. The significance of these effects, however, is still obscure.

In contrast to the paucity of developmental studies, several papers have reported on the physiological action of somatostatin on adult neurons. For example, somatostatin was reported to inhibit Ca^{2+} channels in various cell types, some of them in a voltage-dependent way (Bean 1989; Ikeda and Schofield 1989; Wang et al. 1990; Kleuss et al. 1991; Golard and Siegelbaum 1993), others in a voltage-independent manner (Surprenant et al. 1990). Somatostatin was further found to hyperpolarize hippocampal pyramidal neurons (Pittman and Siggins 1981), probably by augmenting the M-current (Jacquin et al. 1988; Moore et al. 1988; Schweitzer et al. 1993). The M-current is a time- and voltage-dependent potassium current and thought to clamp the membrane potentials near rest, thus inhibiting neurons (Adams et al. 1982, Brown 1988). Aside from the inhibitory effects of somatostatin mentioned above, excitatory action of somatostatin was also described. For example, somatostatin depolarized pyramidal neurons in the hippocampus (Dodd and Kelly 1978), augmented long-term potentation in the hippocampus (Matsuoka et al. 1993), and enhanced glutamate-induced depolarizations in brainstem motoneurons (Wang et al. 1993). Taken together, all of these physiological studies show that somatostatin can have diverse, and even contrasting, effects on various neurons. This is not surprising in light of the results that five different somatostatin receptor subtypes (SSTR1-SSTR5) have so far been identified by molecular cloning techniques (review: Bell and Reisine 1993). These receptor subtypes can now be distinguished pharmacologically (Rens-Domiano et al. 1992; Raynor et al. 1992; Coy et al. 1993) and can act via several different signal transduction pathways (Raynor et al. 1991; Rens-Domiano et al. 1992; Buscail et al. 1994; Hershberger et al. 1994). Furthermore, recent in situ hybridization studies have shown that somatostatin receptor subtypes are distinctly expressed within brain regions, both regionally and temporally (Kaupmann et al. 1993; Kong et al. 1994; Pérez et al. 1994). We think that this may explain the different physiological effects of somatostatin described in the literature.

Functional implications for development

Except for the report by Gonzalez et al. (1992), we are unaware of any other investigation of the possible role of somatostatin during development. Therefore, one can only speculate about the significance of the transient expression of somatostatin in the developing auditory system. Nevertheless, on the basis of our anatomical and physiological knowledge concerning the development of the rat's SOC, we suggest that somatostatin could participate in morphogenetic processes such as synapse stabilization, rather than being involved in processes like neurogenesis. The reason for this is twofold. First, neurogenesis of SOC cells takes place prenatally in the rat and predominantly between E11 and E14 (Altman and Bayer 1980; Weber et al. 1991), i.e., at a time when somatostatin mRNA is still undetectable in the auditory brainstem (Fitzpatrick-McElligott et al. 1991). Second, the transient expression of somatostatin coincides with the period during which fibers from CN neurons have arrived in the SOC and are now forming axon collaterals and end branches (Kandler and Friauf 1993b).

The number of SIR fibers in the SOC is highest at P7, when several other developmental processes occur. For example, glycine, the major inhibitory transmitter in the SOC of adult rats (review: Wenthold 1991), is depolarizing until P7 and becomes hyperpolarizing thereafter (Kandler and Friauf 1993a, c). Also at P7, the calcium-binding protein calbindin-D_{28k}, which is transiently present in LSO neurons of rats, reaches its maximum expression level (Friauf 1993). In gerbils, the morphological development of axon arborizations of neurons in the medial nucleus of the trapezoid body is particularly rapid around P7 (Sanes and Siverls 1991) and an activity-dependent process of synaptic refinement appears to be present in the LSO after P7 (Sanes and Takacs 1993). Finally, unilateral cochlea removal leads to a reduction in neuron numbers in the ipsilateral AVCN, which is most profound when surgery is performed at P7 (Hashisaki and Rubel 1989). Taken together, these data indicate that important anatomical, physiological, and biochemical events occur around the end of the first postnatal week in the rat's and gerbil's auditory brainstem. By the time rats begin to hear, which is around P12 (Jewett and Romano 1972; Uziel et al. 1981; Blatchley et al. 1987; Geal-Dor et al. 1993), the amount of SIR has dramatically declined in the principal auditory brainstem nuclei, except for the VCN. The cause of the decline of SIR and the mechanisms underlying the loss of SIR remain unknown. It is unlikely that degeneration of neurons plays a role, since neuron death in the SOC occurs earlier, i.e., around P3 (Harvey et al. 1990). Further biochemical and electrophysiological studies are required before we can understand the significance of the transient expression of somatostatin and its cellular and molecular function during development. Selective ligands will be needed for this endeavor because of the fact that different somatostatin receptor subtypes are regionally and temporally expressed in the brain. For example, SSTR3 mRNA is mainly expressed within the CN, the SOC, and the IC of adult rats (Pérez et al. 1994), whereas during development, SSTR4 mRNA is abundant in the brainstem (Wulfsen et al. 1993).

In conclusion, we propose that the auditory brainstem is a suitable system to investigate the action and function of somatostatin within a sensory system and during ontogeny. We are able to reach this conclusion because our results show not only a transient expression of somatostatin, but they also indicate that somatostatin is within projection neurons from the CN to the SOC and, therefore, intrinsic to the auditory system.

Leu-enkephalin

Our results show that somatostatin and leu-enkephalin are both present in the rat auditory brainstem during early development, yet we found some remarkable differences between the appearance of the two neuropeptides. First, while somatostatin is only transiently present in the auditory brainstem, leu-enkephalin expression increases continuously during development, reaching adult levels at about P21. Second, the number and labeling intensity of LIR-positive elements are considerably lower than for somatostatin. Third, the LIR fibers in the auditory brainstem nuclei possibly originate from extrinsic sources and not from other auditory nuclei, since we found LIR fibers before we could see any LIR somata in auditory nuclei.

Comparison with other studies

Several studies have demonstrated that leu-enkephalin is present in all principal auditory brainstem nuclei of the adult rat (Sar et al 1978; Uhl et al. 1979; Finley et al. 1981; Petrusz et al. 1985). Our results from adult rats confirm these findings. However, the present paper is, to our knowledge, the first *developmental* study on the expression of leu-enkephalin in the auditory brainstem. Immunocytochemical studies have shown that leu-enkephalin is first present in the rat lower brainstem at E16 (Senba et al. 1982); as we found LIR fibers in the SOC and NLL at E18, our data are in general agreement with these studies. Furthermore, our results showing that the maximum, adult-like level of LIR is reached around P21 confirm findings by Bayon et al. (1979), who reported that the maximum level of leu-enkephalin immunoreactivity in the medulla and midbrain occurs around P25. As ligand-binding for leu-enkephalin at δ-receptors becomes adult-like between P21 and P25 in the rat (McDowell and Kitchen 1986), this is further, indirect evidence that the leu-enkephalin system reaches maturity only after about the 3rd postnatal week.

Compared with somatostatin, which in auditory brainstem nuclei reaches maximal levels after the first postnatal week and thus well before the onset of hearing, leu-enkephalin appears to become prominent relatively late in the auditory system and does not undergo dramatic changes. Moreover, while the somatostatinergic system appears to develop progressively and regressively, leu-enkephalin increases steadily until about the end of the 3rd postnatal week. As with somatostatin, further biochemical and electrophysiological experiments are necessary in order to understand the function of leu-enkephalin in the developing auditory system.

Acknowledgements We wish to thank Dr. Christian Müller for his kind donation of GFAP antibody, Drs. Horst Herbert and Michael Koch for valuable comments on the manuscript, and Gwynn Goldring for correcting our English. Financial support was provided by a grant from the Deutsche Forschungsgemeinschaft to E.F. (Fr 772/1-3) and by a fellowship from the Graduiertenkolleg Neurobiologie Tübingen (M.K.).

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