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Changes in density of brainstem afferents in ferret primary auditory cortex (AI) during postnatal development

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ABSTRACT

Histochemical methods were used to assess the distribution of 4 neurotransmitters thought to be involved in cortical plasticity. They were measured in the primary auditory cortex of the ferret from just before the onset of hearing. Acetylcholinesterase staining was strongest in layers I, IV and VI and there was a gradual increase in the amount of staining from postnatal day (PND) 21 through to adulthood. Serotonin fibres were located mainly in layers I–III and their density increased gradually over the same time period. Noradrenergic fibres were sparsely scattered throughout the cortex but their density and distribution showed little change over the age range studied. Dopaminergic fibres were densest in layers V and VI at all ages. However, there was a transient doubling in their density that started round about the onset of hearing at PND 28, peaked at PND 35 and had returned to baseline levels by 2 wk later. This transient peak in density did not occur in the adjacent suprasylvian gyrus and did not appear to be a general phenomenon. The local transient increase in dopaminergic fibres implies that they may have an important role during a short period in auditory cortical development. This role may involve modifying the cortical circuitry that is involved in analysing the input from the auditory periphery.

Key words: Cerebral cortex; noradrenaline; dopamine betahydroxylase; acetylcholinesterase; tyrosine hydroxylase; serotonin.

INTRODUCTION

Significant progress has been made in our understanding of the structural and functional organisation of the adult cortex over the past few decades (Scheich, 1991; Schreiner, 1992). Traditionally the cat has been the most widely used species in studies of the mature auditory cortex (Rose, 1949; Reale & Imig, 1980; Wallace et al. 1991*a*). We wished to study the development of the auditory cortex and recently use has been made of the ferret as a convenient model for studying cerebral development. Ferrets are born very immature and with a gestation period 3 wk shorter than the cat, a considerable amount of cortical development occurs postnatally in the ferret (Luskin & Jackson et al. 1989). The ferret is of particular interest as a model for studying auditory cortical plasticity because it is the only mammal which has been used to study developmental plasticity in the auditory system at all levels within the hindbrain and midbrain (Moore, 1991). There have been relatively few studies of the immature auditory cortex in any species and apparently there have been no direct studies of the degree of plasticity occurring during its development. This is in marked contrast to the visual cortex, where critical periods during development have been convincingly demonstrated (Hubel & Wiesel, 1970; Mower, 1991). Because of our interest in cortical plasticity, we wished to describe the normal development of 4 subcortical afferent systems that are thought to be involved in modifying the strength of cortical connections.

It has been suggested that monoamines, particularly noradrenaline (NA), are important agents in this early plasticity of the visual cortex of cats. Local perfusion of kitten visual cortex with 6-hydroxydopamine prevented the effects of monocular deprivation in kittens and reinfusion of NA restored this plasticity (Kasamatsu et al. 1979). In kittens which were deprived of vision until the 28th day of life and then received monocular visual experience for 3 h, there was a transient increase in serotonin levels (Kossut et al. 1981). Serotonin levels have also been shown to peak in layers IV and VI of the rat primary sensory

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cortical areas at postnatal days (PND) 7–17 and it has been suggested that this transient serotonergic innervation may exert a trophic influence on the development of cortical circuitry and thalamocortical connections (D'Amato et al. 1987). Acetylcholinesterase (AChE) levels have also been shown to peak in layers III and IV of the rat primary visual cortex at PND 12–14 (Robertson et al. 1985). This has also been demonstrated in the rat primary auditory cortex at PND 9–12 (Robertson et al. 1991). It has been postulated that this transient AChE activity may serve as a marker for the primary sensory thalamocortical terminal fields and may be characteristic of geniculocortical axon terminals.

We have therefore studied the ferret primary auditory cortex (AI) during normal development to measure the progressive changes in neurochemicals thought to be involved in cortical plasticity. The ferret does not start to hear until between PND 27 and 32 (Moore, 1982; Morey & Carlile, 1990) and the bulk of cell migration in the cortex has ceased by PND 21 (Voigt & De Lima, 1991). Thus we studied ferrets from PND 21 to adult and focused the study on changes in neurochemicals occurring in the weeks immediately after the onset of hearing. In addition to studying serotonin and AChE we also studied tyrosine hydroxylase and dopamine beta hydroxylase as these are the enzymes involved in the synthesis of dopamine and noradrenaline. Some of our results have previously been published in preliminary form (Harper & Wallace, 1993).

MATERIALS AND METHODS

Twenty-three pigmented ferrets, raised in the College animal house, were used. Three animals at PND 21 and 4 animals each at PND 28, 35, 42, 49 and adult were killed by an overdose of sodium pentobarbitone (Sagatal) intraperitoneally and perfused transcardially with 4% paraformaldehyde. Blocks of cortex were then removed and left in phosphate buffer containing 30% sucrose for 24 h at 4 °C for cryoprotection. Cryostat sections were cut at 30 μm through the middle ectosylvian gyrus in the coronal plane. The sections were collected in phosphate buffer and mounted onto gelatin subbed slides. The sections were divided into 5 series for different staining protocols.

Noradrenergic fibres were visualised using an antibody against dopamine beta hydroxylase (DBH). DBH is the enzyme which converts dopamine (DA) to noradrenaline (NA). Dopaminergic fibres were visualised using an antibody against tyrosine hydroxylase (TH). TH is the rate-limiting enzyme in the synthesis of dopamine but is also present in noradrenergic fibres. Thus it is not entirely specific for dopaminergic fibres but it appears that noradrenergic fibres often contain too little TH for it to be demonstrated histochemically (Foote & Morrison, 1987). In our results the large differences in the staining patterns for TH and DBH implied that few if any noradrenergic fibres were stained by the TH antibody. Rabbit polyclonal antibodies against TH (Chemicon) and DBH (Eugene Tech) were used at dilutions of 1:400 and 1:500 respectively. The blocking solution was 0.1 M phosphate buffer, pH 7.4, containing 1% triton X-100 and 5% normal goat serum. Primary and secondary antibodies were diluted in this blocking buffer. Sections were preincubated in blocking solution for 2 h and then incubated overnight with primary antibody. The secondary antibody (goat antirabbit IgG biotinylated (Sigma), used at a 1:200 dilution) was applied for 2 h, and then after 3 washes in phosphate buffer, an avidin-biotin-peroxidase complex (ABC elite) from Vector was applied for 90 min. After washing, the sections were preincubated in 0.5 mg/ml diaminobenzidine for 10 min, then incubated in 50 ml of 0.5 mg/ml diaminobenzidine containing 17 μl of 30% hydrogen peroxide until developed. All incubation steps were performed at room temperature. The use of a relatively high concentration of triton X-100 (1%) ensured the complete penetration of all antibodies used. This was confirmed by focusing down through the section using a × 100 oil immersion lense and identifying well-stained fibres close to the surface of the slide. In sections where an attempt was made to obtain antibody binding in the presence of 0.1% triton X-100, fibres were only adequately stained in the part of the section closest to the coverslip.

Mouse monoclonal antibody against serotonin (Dako) was used at a dilution of 1:200. Exactly the same experimental protocol was followed as above except the blocking buffer was made of 0.1 M phosphate buffer, pH 7.4, containing 1% triton X-100 and 3% normal horse serum and the secondary antibody used was horse antimouse IgG biotinylated (Sigma) used at a dilution of 1:200. As a negative control the primary antibody was omitted in the staining procedure and blocking solution applied on its own for this incubation step. These sections contained no immunoprecipitate. Another series of sections was stained for acetylcholinesterase (AChE) as described previously (Wallace et al. 1991*b*). The 5th series of sections was stained for Nissl substance with thionin.

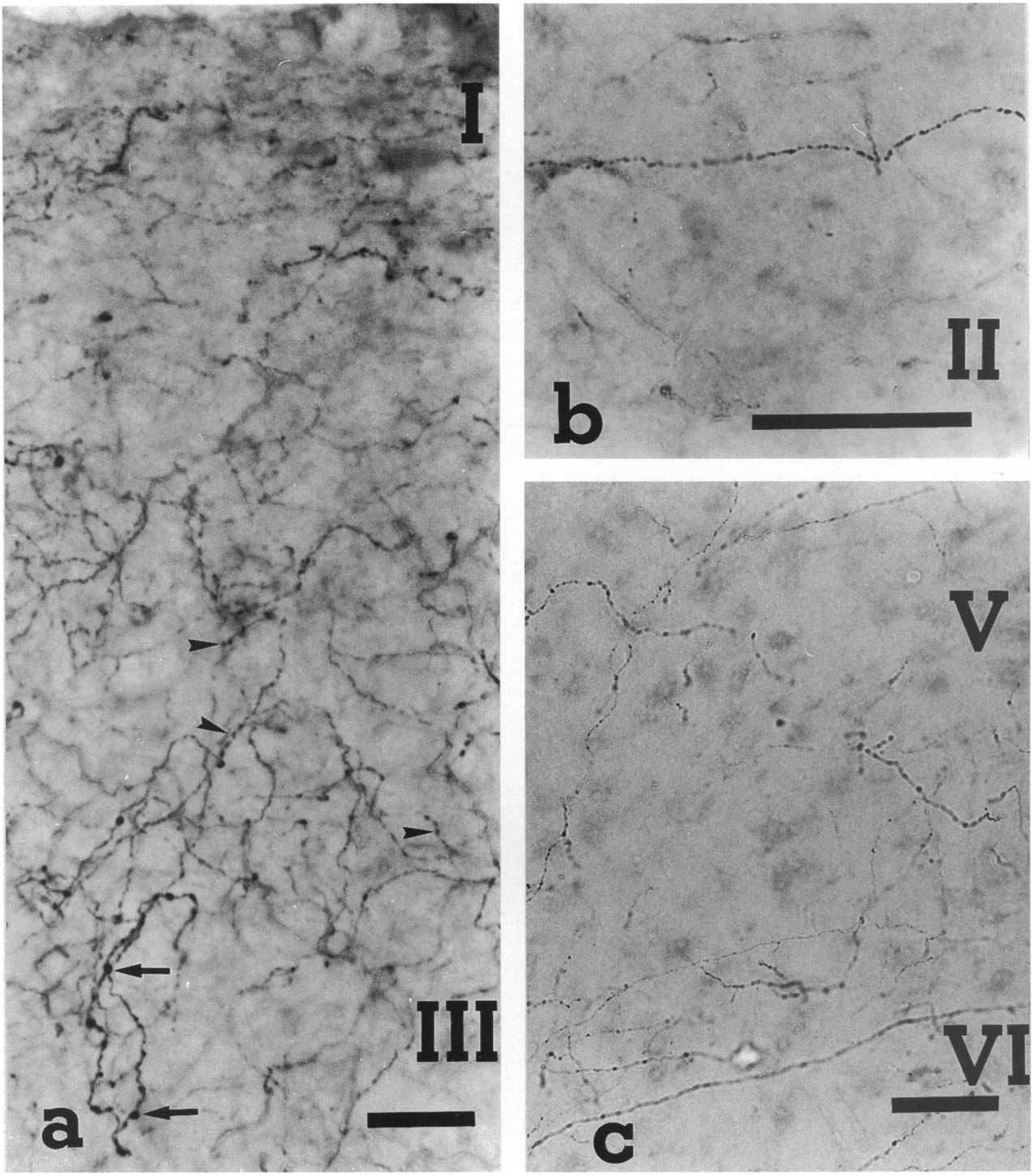

Fig. 1. Examples of immunoreactive fibres in coronal sections through AI. (*a*) Serotonergic fibres in outer 3 layers in the adult. Arrows mark large varicosities on thick fibres. Arrowheads mark varicosities on the much more numerous fine fibres. (*b*) Dopamine beta hydroxylase immunoreactive fibres in layer II at 49 d old. (*c*) Tyrosine hydroxylase immunoreactive fibres in layers V and VI at 35 d old. Bars, 50 μm.

To demonstrate the development of cortical fibres containing TH, DBH and serotonin, drawings were made using a camera lucida attachment and a $\times 20$ objective, or the $\times 40$ objective for verification of fine fibres. Nearby Nissl stained sections were used to mark the layers on these drawings. All drawings were made from the middle of the area bounded by the middle suprasylvian sulcus and the anterior and posterior ectosylvian sulci. These are considered to delineate AI in the ferret (Kelly et al. 1986; Phillips et al. 1988). The position of this area was confirmed by architectonic criteria. AI has a cell-sparse layer V and a blending of layer IV into layers II–III (Rose, 1949; Pallas et al. 1990) as shown by Nissl stains. It has also

been reported that, in the adult ferret, AI can be defined using AChE which marks its boundaries (Pallas et al. 1990; Wallace & Bajwa, 1991).

Using the Cavalieri principle of systematic random sampling, drawings were made from 12 equally spaced (150 μm) sections throughout AI of each hemisphere to give unbiased estimates of fibre density (Mayhew, 1990). The quantitative analysis of fibre density at different postnatal ages was made using a map measurer. This is a small mechanical device used to measure the distance between two points on a map. This method was found to be more accurate and simpler to use than a digitising tablet and computer. Measuring the total length of fibres in part of a section meant that it was comparatively easy to calculate the absolute length of fibres within a given area. Obtaining absolute values allows direct comparison with other cortical areas or other species. The total length of fibres in a 9 cm wide rectangle of grey matter, that included all 6 cortical layers, was measured for each drawing. Each drawing was about 11 cm wide and made at the dorsal pole of the ectosylvian gyrus in the area immediately ventral to where the cortical laminae started to curve towards the suprasylvian sulcus. Nine cm on the drawing represented 450 μm on the tissue section. The values for the total length of stained fibres in each section were averaged to give a collective figure for each hemisphere. The mean value and standard deviation at each age were then calculated and plotted on a graph after converting the arbitrary values into more meaningful units. The units used were total length of fibres in cm, contained in a cuboid of cortex with a surface area of 1 mm^2 that extended through the full depth of the cortex, against age of ferret in days.

RESULTS

Serotonin (5-HT)

The serotonin immunoreactive fibres in the auditory cortex of both young and adult animals consist of both thick and thin fibres. Well over 90% of the fibres present were fine axons with small, sharply defined varicosities (arrowheads in Fig. 1*A*). A very small proportion of the total was composed of thicker fibres, which had large varicosities (arrows in Fig. 1*A*). Occasional thick fibres without any varicosities were rarely observed but these were considered to be segments of the same fibres at a point where they lacked axonal swellings. Although most fibres could be assigned to the fine or thick groups, a few fibres, especially where only a short length appeared in the section, were intermediate in type. This ambiguity, along with the small number of thick fibres, meant that no attempt was made to distinguish between them while making the drawings, or in the subsequent analysis.

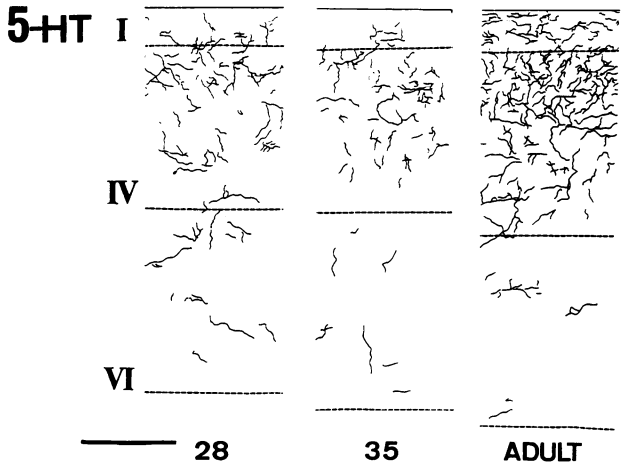

Fig. 2. Representative camera lucida drawings of serotonergic fibres in coronal sections of AI at 3 different ages: 28 d, 35 d and adult. At all ages there is a higher density of fibres in layers I–III than in V and VI. There is a gradual increase in density with increasing age. Bar, 300 μm.

At PND 21 there were very few serotonin immunoreactive fibres in the upper layers of the cortex and almost no immunoreactive fibres in layers V and VI. By PND 28 there was a large increase in the density of immunoreactive fibres in the upper layers where the density was about 18 cm/mm^2 (Fig. 2). There were very few immunoreactive fibres in layers V and VI. Over the next 3 wk there was little change in the distribution of fibres (Fig. 2) but by adulthood the density of immunoreactive fibres had increased further in layers I–IV, to a level of 36 cm/mm^2. In the adult there was a dense plexus of immunoreactive fibres in layers I–III, layer IV was less densely innervated and there were very few immunoreactive fibres in layers V and VI (Fig. 2). All the immunoreactive fibres were oriented randomly.

These results were plotted on a graph to show the density of fibres in layers I–IV and in layers V and VI (Fig. 3*A*).There was considerable variation in values between ferrets of the same age. However, the overall trend was of an increase in the number of fibres in layers I–IV between PND 21–28, then little change between PND 28–49. From PND 49 to adulthood there was a further increase in density in layers I–IV. The increased density in the adult was significant at the $P < 0.02$ level compared with the density at PND 21 using an independent samples t test. In layers V and VI the density of fibres remained low

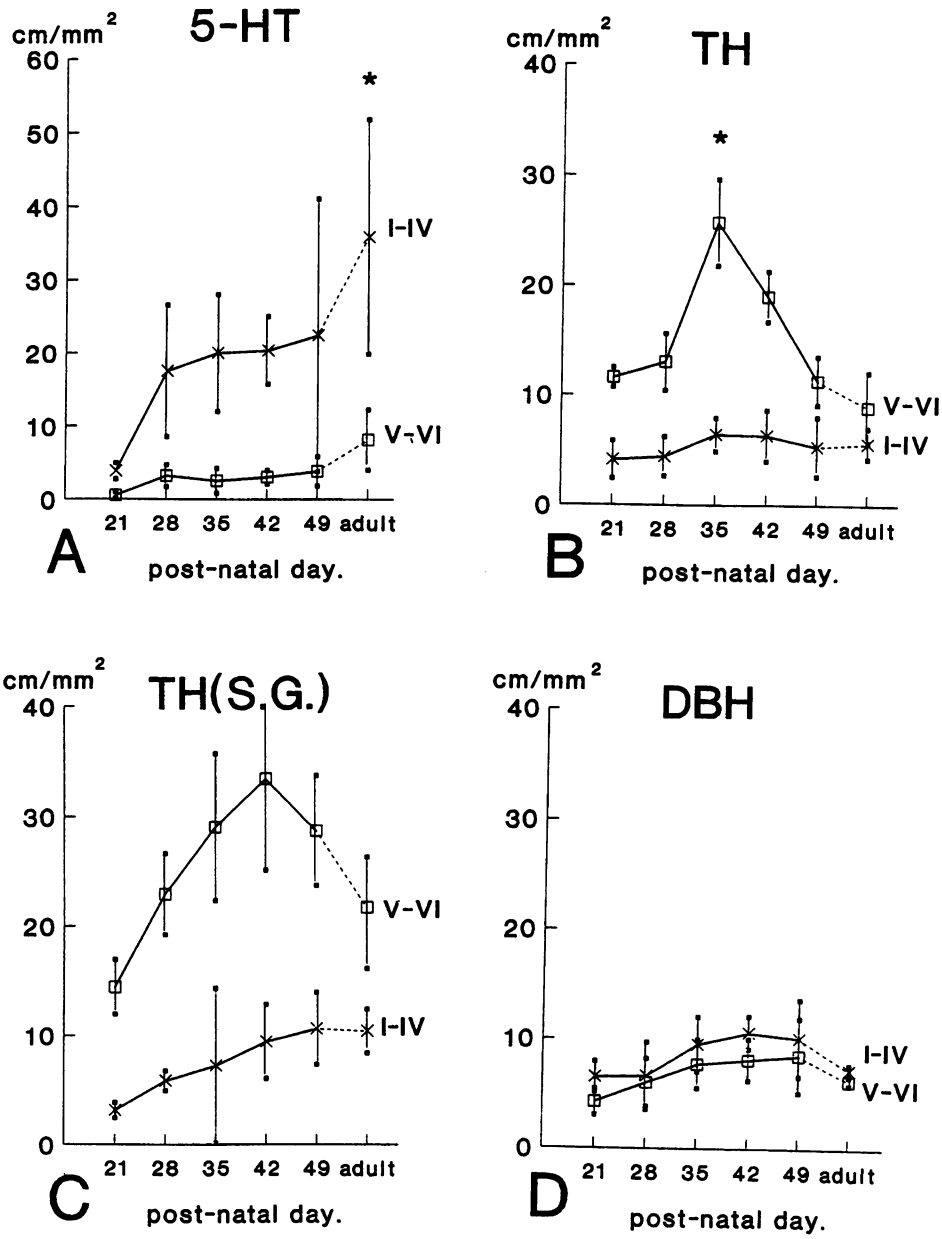

Fig. 3. Graphs showing the density of immunohistochemically stained fibres in layers I–IV and V–VI of ferret cortex at different ages. Measurements were taken from 12 sections per hemisphere at each age and plotted as cm of fibre contained in a cuboid of cortex with a surface area of 1 mm². (*A*) Serotonin (5-HT) immunoreactive fibres in AI. The increase in density in the adult is significant at the $P < 0.02$ level (*) compared with the value at PND 21. (*B*) Tyrosine hydroxylase (TH) immunoreactive fibres in AI. The density of fibres remains fairly constant during development in layers I–IV but shows a prominent peak in layers V and VI at PND 35. This peak is significant at the $P < 0.001$ level (*) compared with values at PND 21 and adult. (*C*) Tyrosine hydroxylase (TH) immunoreactive fibres in the suprasylvian gyrus (S.G.) In layers V–VI there is a rapid rise in density of fibres which peaks around PND 42 and is still high at PND 49. (*D*) Dopamine beta hydroxylase (DBH) immunoreactive fibres in AI. The density in all layers remains low and shows little variation with age.

throughout the time course studied. However there was a small increase in density between PND 21 and the adult.

Tyrosine hydroxylase (TH)

The TH immunoreactive fibres formed a homogeneous population of slender axons with numerous varicosities along their length (Fig. 1 *C*). In contrast to the serotonergic fibres, the TH immunoreactive fibres had a higher density in layers V and VI than in the upper layers. In layers I–IV there were only a few sparsely distributed fibres at all ages studied (Fig. 4). However the density of TH immunoreactive fibres in layers V and VI of the ferret AI was substantially less than the density of serotonin immunoreactive fibres in layers I–III, at each age except PND 21 where the density of serotonin immunoreactive fibres was very

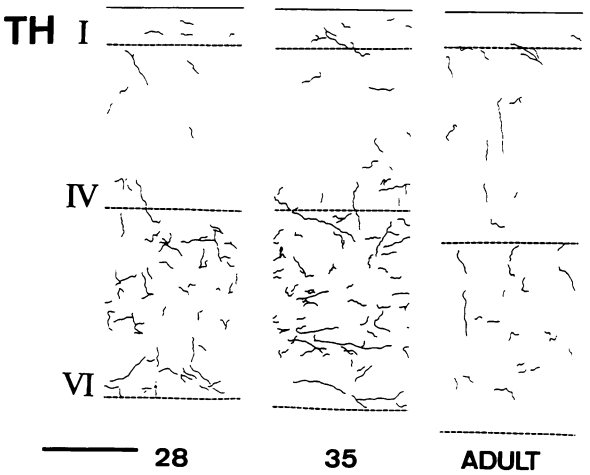

Fig. 4. Representative camera lucida drawings of tyrosine hydroxylase (TH) immunoreactive fibres in ferret AI at 3 different ages. They illustrate the preponderance of fibres in layers V and VI at all ages and the peak in density that occurs at PND 35. Bar, 300 μm.

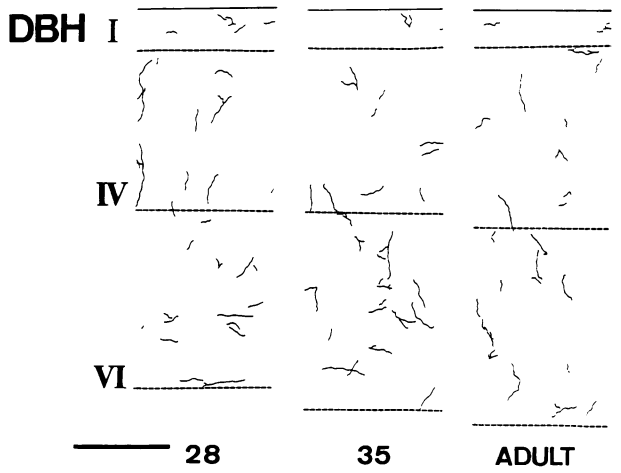

Fig. 5. Representative camera lucida drawings of dopamine beta hydroxylase immunoreactive fibres in ferret AI at 3 different ages. Fibres are scattered randomly throughout the cortex and there is no significant change in density or distribution with increasing age. Bar, 300 μm.

low and at PND 35 where there was a peak in density of TH immunoreactive fibres.

Between PND 21 and PND 28 the density of fibres in the deep layers remained low. However, between PND 28 and PND 35 there was a rapid rise in the density of TH immunoreactive fibres in the deep layers and then an equally rapid fall in density from PND 35 until 49. By PND 49 the adult pattern had been reached where there were only a few immunoreactive fibres throughout each layer of the cortex. The fibres were all randomly oriented. These results were quantified and again plotted on a graph (Fig. 3*B*). The graph shows that between PND 21–35 the density of immunoreactive fibres in layers V and VI had more than doubled from 12 to 26 cm/mm^2. There was then a sharp drop in the density of immunoreactive fibres between PND 35–42. This decrease in density continued and in the adult the density of fibres in layers V and VI was down to about 9 cm/mm^2. The sharp peak in density was significant at the $P < 0.002$ level when the values at PND 35 were compared with those at PND 21 and the adult by using an independent samples t test.

To find out whether this peak in TH immunoreactivity was a general phenomenon occurring throughout the neocortex, or was restricted to certain localities, measurements were also made on a non-auditory area present in the same sections. Thus TH immunoreactive fibres in the adjacent suprasylvian gyrus were drawn and the results quantified and plotted on a graph (Fig. 3*C*). In this part of the cortex the density of immunoreactive fibres in layers V–VI rises quite sharply between PND 21–28 and reaches a peak around PND 42 at a level of 33 cm/mm^2. However, after this there is no rapid fall in fibre density and the level remains above 20 cm/mm^2 throughout the rest of the time course studied. The density of immunoreactive fibres in layers I–IV is lower and increases gradually throughout the time course with the density reaching 10 cm/mm^2.

Dopamine beta hydroxylase (DBH)

The DBH immunoreactive fibres form a population of sparsely distributed axons with a very fine calibre (< 1 μm) and numerous varicosities (Fig. 1*B*). Their density in the outer layers is the same as or slightly higher than that in the deep layers throughout the time course studied (Fig. 5). In both inner and outer layers there was a small increase in the density of fibres in animals aged between 21 and 42 d but there was no evidence of a definite peak in density. The DBH immunoreactive fibres were randomly oriented and sparsely scattered throughout the cortex at each age. These results were quantified and plotted on a graph (Fig. 3*D*). This showed that, in layers I–IV, the density of DBH immunoreactive fibres was slightly higher than the density of TH immunoreactive fibres over the age range studied. By contrast, in layers V and VI, the density of TH positive fibres was much higher than the density of DBH positive fibres at all ages studied.

Acetylcholinesterase (AChE)

At PND 21 there were only occasional AChE-stained fibres and they did not form any discernible pattern. There was no staining associated with cell bodies. By PND 28 there were many more stained fibres, pre-

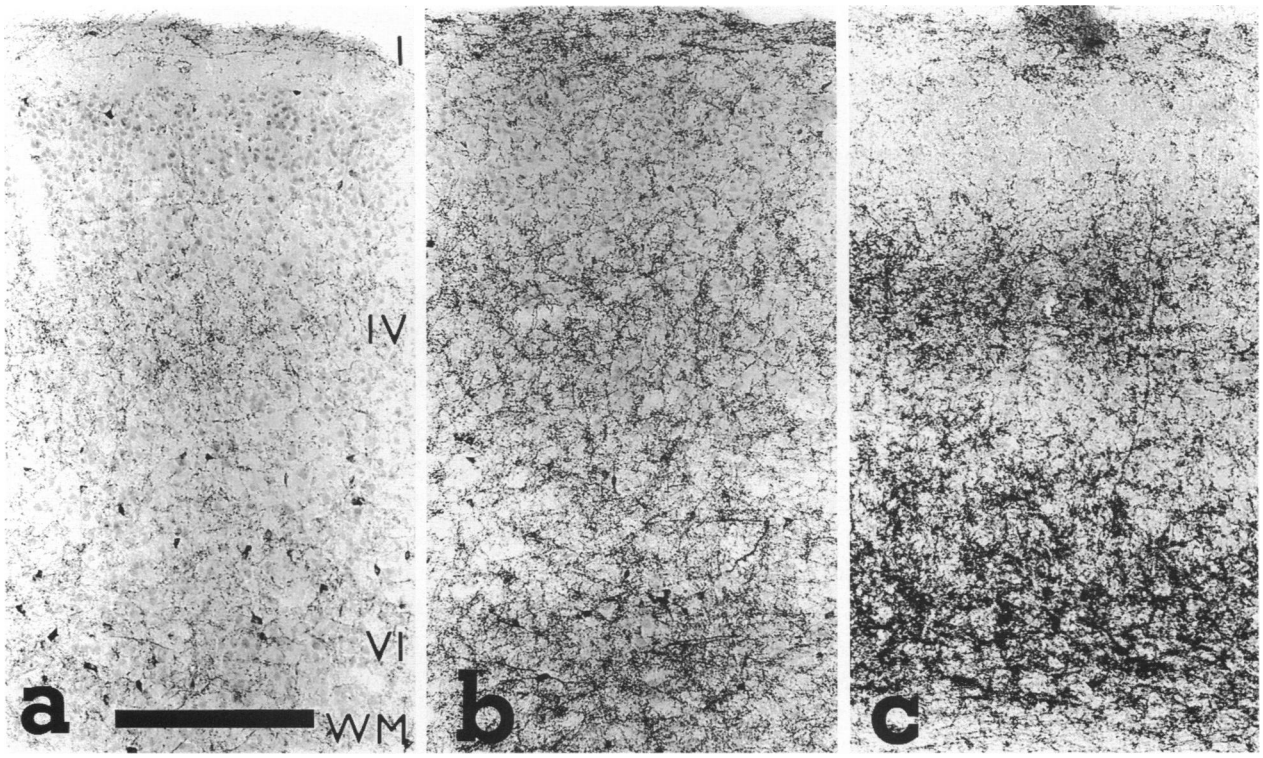

Fig. 6. Coronal sections through centre of ferret AI that illustrate AChE activity at 35 d old (*a*), 49 d old (*b*) and in the adult (*c*). They show the relatively high staining in layers I, IV and VI and the gradual increase in staining with age. Bar, 300 μm.

dominantly in layers I, IV and VI of the cortex (Fig. 6*A*). There was also prominent staining in cell bodies. These were primarily located in layer VI but a few cells also occurred in more superficial layers and in the white matter. Thereafter there was a gradual increase in the level of AChE staining, with the highest levels continuing to be in layers IV and VI and the lowest levels in layer II. At PND 49, there were still some prominently stained cell bodies (Fig. 6*B*) but in the adult, staining of cell bodies, while still present, was masked by the densely stained neuropil. In the older animals and especially in the adult, the dense staining of the neuropil made it difficult to identify individual fibres. As a result, no attempt was made to quantify the number of fibres at different ages. In the younger animals the sparseness of the fibres meant that densitometric measurements were also inappropriate. Despite the absence of quantification, the smooth gradual increase in the AChE staining was clearly discernible. There was no evidence of a peak in AChE containing fibres around the onset of hearing.

DISCUSSION

Significance of AChE staining during development

The neocortex receives a highly divergent afferent input from sources in the basal forebrain and brainstem as well as a specific thalamic input. There is evidence that AChE is a useful marker for studying the development of the thalamic input to the rat auditory cortex (Robertson et al. 1991). However this was not found to be the case in the ferret. We observed a gradual increase in the density of AChE staining in ferret AI during development, with layers I, IV and VI having relatively high levels at all ages. This staining pattern is consistent with the interpretation that the AChE is associated primarily with basal forebrain afferents (Johnston et al. 1981; Bigl et al. 1982; McKinney et al. 1983). There was no indication of a transient expression of AChE activity in layer IV early in development that could be related to the growth of thalamocortical projections into layer IV of the primary auditory cortex. Such a relationship has been observed in the rat auditory cortex where there is dense AChE staining in layer IV during the second postnatal week (Robertson et al. 1991). At later ages lower levels of AChE activity are observed in layer IV and this is associate with the input from the basal forebrain. In this study the AChE levels were not measured directly in the thalamus or basal forebrain and so the relative levels of AChE activity in the projecting neurons cannot be described. However, some sections of the ferret striatum were stained at the same time as the cortex. The dense black reaction product observed confirmed that the AChE staining was working well in the young ferrets.

Role of brainstem afferents during development

Three brainstem afferent systems are known to project to the neocortex. Each of these is associated with a transmitter that has been implicated in synaptic plasticity: (1) serotonergic neurons in the raphe nuclei (Porrino & Goldman-Rakic, 1982; O'Hearn & Molliver, 1984), (2) noradrenergic neurons in the locus coeruleus (Foote et al. 1983) and (3) dopaminergic neurons in the substantia nigra-ventral tegmental area (Foote & Morrison, 1987).

Serotonergic innervation

In the ferret AI serotonin immunoreactive fibres were located mainly in the outer 3 layers of the cortex at all ages studied. There was a large amount of variation in the number of immunoreactive fibres between ferrets of the same age. This was unlikely to have been due to alterations in the affinity of antibody binding caused by different degrees of fixation in different brains. Much smaller amounts of variation were observed in sections stained with antibodies to TH or DBH that were taken from the same blocks. It may be that levels of serotonin vary between different animals of the same age. In general, the density of serotonin immunoreactive fibres increased rapidly between PND 21 and PND 28, and then there was a gradual increase in density from PND 28 through to adulthood. Again this is in contrast to a peak in density observed in layers IV and VI of the primary sensory areas of the rat cortex (D'Amato et al. 1987). Nevertheless, an immunohistochemical study on AI of the cat revealed very similar results to these in the ferret. By PND 21 the distribution of fibres in the cat AI was densest in layers I–III. By PND 56 the fibres had more than tripled their density in the outermost 3 layers since birth. In this study Vu & Törk (1992) suggested that there are two systems of serotonergic axons: the fine axon system characterised by small fusiform varicosities up to 1 μm in diameter, and the beaded axon system, the fibres of which have round varicosities up to 5 μm in diameter. The density of fine axons increased steadily from birth while the beaded axons first appeared in the auditory cortex at PND 21 and gradually increased in number thereafter. In the ferret auditory cortex thick fibres were observed at all ages studied. However, these fibres were in such a small proportion compared with the fine fibres that they were not plotted on a separate graph. They seemed to follow the same pattern as the fine fibres during development and were taken into account in all the measurements. In a related study of the ferret visual cortex performed by Voigt & De Lima (1991) a similar laminar distribution and developmental time scale were reported, with the highest innervation density found within layers I, II and III in the adult cortex. These results could suggest that the main action of serotonin is on the upper layers of the cortex. The gradual increase in the density of serotonergic fibres implies that they do not have any unique role during development.

Specificity of staining for dopaminergic innervation

In this study antibodies to TH and DBH were used to identify noradrenergic and dopaminergic innervation in the cortex. The specificities of anti-TH and anti-DBH antibodies for noradrenaline (NA) and dopamine (DA) have been well documented (Campbell et al. 1987; Gaspar et al. 1989). The enzyme TH is essential for all catecholamine synthesis and is present in neurons containing DA, NA and adrenaline. No adrenergic fibres have been detected in the neocortex (Hökfelt et al. 1974). Hence the antibody to TH may recognise NA and DA positive fibres. However, evidence has been reported to suggest that in the neocortex anti-TH antibodies will predominantly label DA containing fibres. Lewis et al. (1987) observed no change in the pattern of TH immunoreactive fibres after the ascending NA projections of the locus coeruleus were ablated in the squirrel monkey. From this they concluded that only DA axons were visualised with their TH antiserum. An explanation for this apparent selectivity could be that TH is found in lower amounts in NA than in DA containing axon terminals of the cerebral cortex (Schmidt & Bhatnagar, 1979). It may be that NA fibres do not normally contain a sufficient concentration of TH to be detected with immunohistochemical techniques. The antibody to DBH only recognises NA containing fibres (Ericson et al. 1989). Even if all the noradrenergic fibres were stained by our TH antibody, subtracting the pattern of DBH labelling from the pattern of TH labelling would produce a conservative representation of dopaminergic fibres. In this case the peak increase in dopaminergic fibre density at PND 35 would be even more conspicuous than that shown in Figure 3*B*.

Noradrenergic innervation

The density of DBH immunoreactive fibres remained low throughout the age range studied and showed no consistent change in distribution. In a similar study by Foote & Morrison (1984) on the primary visual cortex

of the monkey, they found the density of DBH immunoreactive fibres increased from birth to about 2 months, when they reached an adult pattern. Since this chemical has been implicated in plasticity (Kasamatsu et al. 1979) it might be expected that DBH immunoreactive fibres would peak in density in the cortex around the time of onset of hearing. However no transient increase was observed. This would suggest that although NA may be involved in plasticity it is not particularly important during auditory cortex development in the ferret.

Dopaminergic innervation

In contrast to DBH, TH immunoreactive fibres showed a distinctive peak in density in layers V and VI at PND 35 in the ferret AI. The steep decline in density of fibres after PND 35 did not appear to be a phenomenon widespread throughout the cortex since there was no similar decline in density of TH immunoreactive fibres in the suprasylvian gyrus after PND 35. There may have been peaks in other primary sensory areas but these were not examined in the present study. The transience of the increase in immunoreactive fibres could be due to a proliferation of dopaminergic terminal branches in AI up until PND 35, followed by an elimination of axon branches similar to that seen in the initial exuberance and subsequent pruning of callosal collaterals in the visual cortex of the cat (Innocenti, 1981). Another explanation would be that the peak in density is caused by a temporary increase in enzyme expression during this period that allows previously undetected fibres to be visualised. From this study it is not possible to say which of the two possibilities is correct.

There have been few developmental studies of DA in any primary sensory area of any species. Cambell et al. (1987) reported a significant rostral to caudal gradient of decreasing density of TH immunoreactive fibres in the adult monkey AI. No such gradient was obvious in the ferret AI either in the rostral/caudal or dorsal/ventral directions, but to prevent any small differences arising from a gradient affecting our results, all drawings were made from the middle of the gyrus.

Most studies of the development of cortical dopaminergic innervation have involved the prefrontal cortex. In a study of the rat prefrontal cortex, using an antibody to DA, Kalsbeek et al. (1988) observed DA immunoreactive fibres already present in layers V and VI immediately after birth. The density of these fibres increased until PND 60. In a further study Kalsbeek et al. (1989) depleted the prefrontal cortex of DA innervation from PND 1 onwards by lesions of the DA cell group (A10) in the ventral tegmental area. These DA depleted animals showed a 30% decrease in the total length of the basal dendrites of the pyramidal cells in layer V of the prefrontal cortex. The decreased dendritic length was due mainly to a reduced branching frequency of the basal dendrites. Since dopamine is already thought to have a trophic role in neuronal differentiation of the prefrontal cortex during development, it could have a similar function in the auditory cortex in the 2 wk immediately after the onset of hearing.

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REFERENCES

Bigl V, Woolf NJ, Butcher LL (1982) Cholinergic projections from the basal forebrain to frontal, parietal, temporal, occipital and cingulate cortices: a combined fluorescent tracer and acetylcholinesterase analysis. *Brain Research Bulletin* **8**, 727–749.

Campbell MJ, Lewis DA, Foote SL, Morrison JH (1987) Distribution of choline acetyltransferase-, serotonin-, dopamine-B-hydroxylase-, tyrosine hydroxylase-immunoreactive fibers in monkey primary auditory cortex. *Journal of Comparative Neurology* **261**, 209–220.

D'Amato RJ, Blue ME, Largent BL, Lynch DR, Ledbetter DJ, Molliver ME et al. (1987) Ontogeny of the serotonergic projection to rat neocortex: transient expression of a dense innervation to primary sensory areas. *Proceeding of the National Academy of Sciences of the USA* **84**, 4322–4326.

Ericson H, Blomquist A, Köhler C (1989) Brainstem afferents to the tuberomammillary nucleus in the rat brain with special reference to monoaminergic innervation. *Journal of Comparative Neurology* **281**, 169–192.

Foote SL, Bloom FE, Aston-Jones G (1983) The nucleus locus coeruleus: new evidence of anatomical and physiological specificity. *Physiological Reviews* **63**, 844–914.

Foote SL, Morrison JH (1984) Postnatal development of laminar innervation patterns by monoaminergic fibers in monkey (*Macaca fascicularis*) primary visual cortex. *Journal of Neuroscience* **4**, 2267–2680.

Foote SL, Morrison JH (1987) Extrathalamic modulation of cortical function. *Annual Review of Neuroscience* **10**, 67–95.

Gaspar P, Berger B, Febvret A, Vigny A, Henry JP (1989) Catecholamine innervation of the human cerebral cortex as revealed by comparative immunohistochemistry of tyrosine hydroxylase and dopamine B hydroxylase. *Journal of Comparative Neurology* **279**, 249–271.

Harper MS, Wallace MN (1993) Dopaminergic fibres have a peak density during the onset of hearing in the ferret auditory cortex. *British Journal of Audiology* **27**, 362–363.

Hökfelt T, Fuxe K, Goldstein M, Johansson O (1974) Immunohistochemical evidence for the existence of adrenaline neurons in the rat brain. *Brain Research* **66**, 235–251.

Hubel DH, Wiesel TN (1970) The period of susceptibility to the physiological effects of unilateral eye closure in kittens. *Journal of Physiology* **206**, 419–436.

INNOCENTI GM (1981) Growth and reshaping of axons in the establishment of visual callosal connections. *Science* **212**, 824–826.

JACKSON CA, PEDUZZI JD, HICKEY TL (1989) Visual cortex development in the ferret. I. Genesis and migration of visual cortical neurons. *Journal of Neuroscience* **9**, 1242–1253.

JOHNSTON MV, MCKINNEY M, COYLE JT (1981) Neocortical cholinergic innervation: a description of extrinsic and intrinsic components in the rat. *Experimental Brain Research*, **43**, 159–172.

KALSBEEK A, VOORN P, BUIJS RM, POOL CW, UYLINGS HBM (1988) Development of the dopaminergic innervation in the prefrontal cortex of the rat. *Journal of Comparative Neurology* **269**, 58–72.

KALSBEEK A, MATTHIJSSEN MAH, UYLINGS HBM (1989) Morphometric analysis of prefrontal cortical development following neonatal lesioning of the dopaminergic mesocortical projection. *Experimental Brain Research* **78**, 279–289.

KASAMATSU T, PETTIGREW JD, ARY M (1979) Restoration of visual cortical plasticity by local microperfusion of norepinephrine. *Journal of Comparative Neurology* **185**, 163–182.

KELLY JB, JUDGE PW, PHILLIPS DP (1986) Representation of the cochlea in primary auditory cortex of the ferret (*Mustela putorius*). *Hearing Research* **24**, 111–115.

KOSSUT M, WÓJCIK M, SKANGIEL-KRAMSKA J (1981) Dynamic changes of serotonin levels during the first visual experience of binocularly deprived kittens. *Journal of Neurochemistry* **37**, 1077–1080.

LEWIS DA, CAMBELL MJ, FOOTE SL, GOLDSTEIN M, MORRISON JH (1987) The distribution of tyrosine hydroxylase immunoreactive fibres in primate neocortex is widespread but regionally specific. *Journal of Neuroscience* **7**, 279–290.

LUSKIN MB, SHATZ CJ (1985) Neurogenesis of the cat's primary visual cortex. *Journal of Comparative Neurology* **242**, 611–631.

MAYHEW TM (1990) Efficient and unbiased sampling of nerve fibres for estimating fiber number and size. In *Methods in Neurosciences*, vol. 3. *Quantitative and Qualitative Microscopy* (ed. P. M. Conn), pp. 172–187. San Diego, London: Academic Press.

MCKINNEY M, COYLE JT, HEDREEN JC (1993) Topographic analysis of the innervation of the rat neocortex and hippocampus by the basal forebrain cholinergic system. *Journal of Comparative Neurology* **217**, 103–121.

MOORE DR (1982) Late onset of hearing in the ferret. *Brain Research* **253**, 309–311.

MOORE DR (1991) Development and plasticity of the ferret auditory system. In *Neurobiology of Hearing: The Central Auditory System* (ed. R. A. Altschuler, R. P. Bobbin, B. M. Clopton & D. W. Hoffman), pp. 461–475. New York: Raven Press.

MOREY AL, CARLILE S (1990) Auditory brainstem of the ferret: maturation of the brainstem auditory evoked response. *Developmental Brain Research* **52**, 279–288.

MOWER GD (1991) The effect of dark rearing on the time course of the critical period in cat visual cortex. *Developmental Brain Research* **58**, 151–158.

O'HEARN E, MOLLIVER ME (1984) Organization of raphe-cortical projections in rat: a quantitative retrograde study. *Brain Research Bulletin* **13**, 709–726.

PALLAS SL, ROE AW, SUR M (1990) Visual projections induced into the primary auditory pathway of ferrets. I. Novel inputs to primary auditory cortex (AI) from the LP/pulvinar complex and the topography of the MGN-AI projection. *Journal of Comparative Neurology* **298**, 50–68.

PHILLIPS DP, JUDGE PW, KELLY JB (1988) Primary auditory cortex in the ferret (*Mustela putorius*): neural response properties and topographic organization. *Brain Research* **443**, 281–294.

PORRINO LJ, GOLDMAN-RAKIC PS (1982) Brainstem innervation of prefrontal and anterior cingulate cortex in the rhesus monkey revealed by retrograde transport of HRP. *Journal of Comparative Neurology* **205**, 63–76.

REALE RA, IMIG TJ (1980) Tonotopic organization in auditory cortex of the cat. *Journal of Comparative Neurology* **192**, 265–291.

ROBERTSON RT, TIJERINA AA, GALLIVAN ME (1985) Transient patterns of acetylcholinesterase activity in visual cortex of the rat: normal development and the effects of neonatal monocular enucleation. *Developmental Brain Research* **21**, 203–214.

ROBERTSON RT, MOSTAMAND F, KAGEYAMA GH, GALLARDO KA, YU J (1991) Primary auditory cortex in the rat: transient expression of acetylcholinesterase activity in developing geniculocortical projections. *Developmental Brain Research* **58**, 81–95.

ROSE JE (1949) The cellular structure of the auditory area of the cat. *Journal of Comparative Neurology* **91**, 409–439.

SCHEICH H (1991) Auditory cortex: comparative aspects of maps and plasticity. *Current Opinion in Neurobiology* **1**, 236–247.

SCHMIDT RH, BHATNAGAR RK (1979) Assessment of the effects of neonatal subcutaneous 6-hydroxydopamine on noradrenergic and dopaminergic innervation of the cerebral cortex. *Brain Research* **166**, 309–319.

SCHREINER CE (1992) Functional organization of the auditory cortex: maps and mechanisms. *Current Opinion in Neurobiology* **2**, 516–521.

VOIGT T, DE LIMA AD (1991) Serotoninergic innervation of the ferret cerebral cortex. II. Postnatal development. *Journal of Comparative Neurology* **314**, 415–428.

VU DH, TÖRK I (1992) Differential development of the dual serotoninergic fiber system in the cerebral cortex of the cat. *Journal of Comparative Neurology* **317**, 156–174.

WALLACE MN, KITZES LM, JONES EG (1991*a*) Intrinsic inter- and intralaminar connections and their relationship to the tonotopic map in cat primary auditory cortex. *Experimental Brain Research* **86**, 527–544.

WALLACE MN, KITZES LM, JONES EG (1991*b*) Chemoarchitectonic organization of the cat primary auditory cortex. *Experimental Brain Research* **86**, 518–526.

WALLACE MN, BAJWA S (1991) Patchy intrinsic connections of the ferret primary auditory cortex. *NeuroReport* **2**, 417–420.