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Aminergic receptors during the development of the human brain: the contribution of in vitro imaging techniques

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Abstract

The development of the human brain is a complex process and, in this regard, the maturation of neurotransmitter systems and their receptors is of special interest. The study of these systems requires methodological approaches with powerful anatomical resolution. In this paper we review the application of visualization procedures to the fine localization, pattern of appearance and functional relevance of monoaminergic receptors in postmortem human brain samples corresponding to different stages of development (fetal, neonatal, infant). Data obtained by using mostly in vitro autoradiography but also in situ hybridization and, very recently, second messenger labeling, are discussed, including the methodological limitations inherent in working with inmature human tissue. From these studies, several conclusions were made. (1) It is possible to visualize, in the human brain with high resolution, the presence of neuroreceptors at early prenatal stages. (2) The anatomical distribution of monoaminergic receptors in the developing human brain is, in general terms, comparable to that found in the adult. (3) During the developmental process, some receptors, which are early and sometimes transiently expressed, play important thophic roles in the regulation of neuronal development: this is the case with the serotonin 5-HT_{1A} receptors, which attain peak levels of hyperexpression over the hippocampus (dentate gyrus, dendritic areas of CA fields) and the raphe nuclei and show a transient expression in the cerebellum, around the 25 week of gestational age. (4) Different patterns of ontogenetic appearance for human receptors have been identified: dopamine D_2-like (caudate, putamen, nigra) and 5-HT_{1A} receptors are good examples of prenatal development, while 5-HT_{1B} sites (basal ganglia, neocortex) present a mainly postnatal pattern of appearance. (5) Neurotransmitter receptors at human fetal stages are already functional from the point of view of transducing response.

Keywords: Autoradiography; Development; Human brain; In situ hybridization; Prenatal; Receptors; Trophic effects

1. Introduction

Receptors for neurotransmitters are membrane-localized proteins that have the dual job of recognizing a ligand with exquisite sensitivity and chemical selectivity and then converting the process of recognition into a signal that results in a cellular response. The interaction of neurotransmitters with their receptors results in a molecular change in the receptor, such as an altered configuration, and thereby triggers a chain of events leading to a response (Ross, 1996). These receptors can be assigned to several functional families whose members share both common mechanisms of transduction and homologous structures: (a) receptors with autoenzymatic activity; (b) receptors linked to ion channels; and (c) G-protein coupled receptors. Receptors for several peptide hormones belong to the first family, many of them regulating growth and development. Receptors for several aminergic and aminoacidic neurotransmitters (nicotinic cholinergic, $GABA_A$, glutamate) form agonist-regulated ion selective channels which convey their signals by altering the ionic composition. Finally, G-protein coupled receptors act by facilitating the binding of GTP to specific G proteins, which in turn can regulate the activity of specific effectors. A large group of receptors for biogenic amines and many peptide hormones belong to this family (Holz and Fisher, 1998; Schwartz and Kandel, 1991). In fact, most of the results discussed herein refer to G-protein coupled receptors.

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The knowledge of the distribution of receptors in an anatomically complex organ, such as the brain, is crucial for the understanding of chemical neurotransmission in the central nervous system. It is also of great relevance from the point of view of the putative development of drugs acting at this level. The methods for the study of neurotransmitter receptors have continuously been improved. Many of them involve radiometric procedures, i.e., the use of radiolabelled drugs to bind the receptor with high affinity (Bylund and Yamamura, 1990). Regarding fine localization, autoradiographical, immunohistochemical and other histochemical techniques are now feasible. It is also possible to visualize anatomically the level of expression of the mRNA encoding a particular receptor type (Polak and McGee, 1990).

Autoradiographic labelling is the usual procedure for localizing receptors. The development of this technique has provided an useful tool to identify and visualize neurotransmitter binding sites at the light microscopic level (Beaudet, 1993; Kuhar and Unnerstall, 1990). It combines the advantages of protein biochemistry with those of gross and fine anatomy. Thus, it adds to the study of the kinetic properties of the receptor, a high level of anatomical resolution. Much more recently, it has been possible to localize the degree of coupling to signalling mechanisms, following the activation of a given G protein-linked receptor, using [^{35}S]GTPγS as a radiotracer. This procedure provides anatomical information on the functionality of neurotransmitter receptors (Sim et al., 1997).

All these techniques have been applied, not only to the study of neurotransmitter receptors in laboratory animals, but also in human postmortem brain. So, receptors (protein and mRNA) for many neurotransmitters have been mapped in detail in adult human brain (Cortés et al., 1987; Landwehrmeyer et al., 1993; Mengod et al. 1996; Palacios et al., 1986; Pascual et al., 1992; Pazos et al., 1991). The information obtained in these studies has dramatically improved our knowledge of the central chemical neurotransmission in the human species. It has also contributed to the identification of receptor changes in neuropathological diseases (Arango et al., 1995; Cortés et al., 1989; Pascual et al., 1991; Rodríguez-Puertas et al., 1997). Regarding the autoradiographic visualization of the receptor-G protein coupling process, the application of these approaches to human brain tissue is extremely recent (Rodríguez-Puertas et al., 2000).

The development of the central nervous system, including the maturity of neurotransmission systems, is a complex process (Jacobson, 1991; Jessell, 1991). It is important to study the pattern of ontogeny of the different neurotransmitter receptors in order to identify their time of appearance, as well as to detect their distribution and density during development. A number of studies have been devoted to these topics in the rat (Bennett-Clark et al., 1993; Daval et al., 1987; Murrin et al., 1985; Palacios et al., 1988; Schlumpf et al., 1991). On the other hand, in the immature mammalian brain many neurotransmitters play an entirely different role from that which they play in the mature brain, acting as developmental signals or regulators. In fact, many of the major monoaminergic systems as well as some neuropeptides have been shown to exert this function, i.e., to regulate the development of the central nervous system (Whitaker-Azmitia, 1991; Whitaker-Azmitia et al., 1996). Several studies have demonstrated the existence of differential localizations for many receptors during the development of the animal brain (Daval et al., 1987; González et al., 1988; Matthiessen et al., 1992; Prusky et al., 1988).

In contrast, data available about receptor development in the human brain are still limited. So far only a few developmental studies on receptor sites have been done using protein autoradiographic labeling (Bar-Pellet et al., 1991; Glass et al., 1997; Schlumpf and Lichtensteiger, 1987). So far, immunocytochemical procedures have not been applied to developmental human tissue. During the last few years, we have focused our research efforts on this issue, specially on the ontogeny of monoamine receptors (Olmo et al., 1994, 1996, 1998; Pazos et al., 1992). In this paper, we will review several aspects of these studies, including methodological questions, anatomical patterns of distribution, as well as the relevance of the results obtained for the comprehension of neural development.

2. Material and methods

2.1. General procedures of labelling

Briefly, autoradiographic procedures are based on the incubation of tissue sections with appropiate radiotracers, in order to generate autoradiograms (Wharton et al., 1993). In many cases, these images can be readily quantified with a microscopic level of resolution. The handling and storage of human tissue for autoradiographic purposes present a number of difficulties. Our standard procedure is described in Pazos et al. (1991). Immediately after autopsy, brain hemispheres are separated on ice. Large blood vessels and meninges are then removed, and the brain tissue is promptly cut into several blocks containing the different regions and nuclei and stored at -80°C. Sections (20 μm) are cut on the cryostat, and then mounted on gelatin-coated microscope slides and stored at -20°C.

In order to visualize the receptor protein, slides are then incubated with appropriate radiolabeled ligands (usually tritiated or iodinated), agonists or antagonists specific for the receptor studied, under selected condi-

tions of time, buffer composition, temperature and washing procedures. An accurate determination of the level of nonspecific binding is required. Table 1 summarizes the experimental conditions used to visualize several monoaminergic receptors in the developing human brain. A number of general considerations about the experimental procedures of autoradiographic labelling also apply to ontogenetic studies, i.e., the need for a preincubation phase in order to remove some endogenous ligands; or the estimation of maximal densities of receptors when using a single concentration of radioligand: as it is difficult to carry out full saturation autoradiographic assays (in order to calculate K_d values) in human tissue samples, the usual procedure is to take affinity values obtained from membrane assays.

When in situ hybridization is carried out, the tissue sections, after an adequate pretreatment, are incubated overnight in a solution containing an oligonucleotide probe complementary to the mRNA coding for the receptor, labelled with [^{32}P]a-dATP(Polak and McGee, 1990). Finally, visualization of the receptor-induced G protein activation is achieved by the incubation of the tissues with [^{35}S]GTPγS in a medium containing GDP, and the subsequent addition of critical concentrations of unlabelled selective agonists to trigger the transduction system (Rodríguez-Puertas et al., 2000).

After a drying process, tissue sections are exposed to radiosensitive emulsions for variable periods of time. A microscopic analysis of the autoradiograms generated reveals the anatomical distribution of the labelling. In the case of protein receptor and [^{35}S]GTPγS autoradiography, a full quantitation can be carried out. In general, grain densities and optical densities do not increase as rapidly as tissue radioactivity increases, so the quantitative interpretation of autoradiograms will require the use of standards along with experimental tissues (Kuhar and Unnerstall, 1985). The quantitation procedure involves the use of computerized densitometric image analysis systems.

2.2. Some methodological questions

Receptor autoradiographic procedures share similar problems with other neurochemical assays when performed in humans. Factors such as the age of patients, agonal state, premortem conditions, post mortem delay or drug treatment history may produce modifications either in the density of receptors or in the level of mRNA signal (see Pazos and Palacios, 1989). Some of them are of special relevance in developmental studies. The cause of death and the maternal ingest of drugs could affect the levels of receptor density throughout the brain. The analysis of the influence of prenatal distress — a very frequent cause of perinatal death — on the characteristics of neuroreceptors would be of great interest. Regarding the ingest of drugs, it could be of relevance if they present any affinity for the receptor studied, although it has to be taken into account that drugs ingest is usually restricted during pregnancy. Other factors could be post mortem alterations; however neurotransmitter receptors are relatively stable and resistant under long postmortem delays, also in prenatal cases. In the case of the studies addressing the analysis of the level of G protein activation, the freezing storage period, i.e., the time elapsed between the freezing of the sample and the labelling process, is a limiting factor in obtaining a high level of functional response. It is very important to characterize, as much as possible, the sources of postmortem tissue in terms of all these variables.

In addition to these factors, the use of immature human tissue involves additional limitations. First of all, it has to be taken into account that the requirement of using unfixed material in autoradiographic studies usually results in an impaired morphology. This factor is specially detrimental when handling fetal or neonatal brain tissue, which is very friable and hydrated. Thus

Table 1
Autoradiographic localization of receptors during human development: ligands and incubation conditions

	5-HT$_{1A}$	Muscarinic cholinergic	α_2-adrenergic	D$_2$ dopaminergic
Ligand	[^{3}H]8-OH-DPAT	[^{3}H]N-methyl-scopolamine	[^{3}H]UK 14304	[^{3}H]spiperone
Preincubation	30 min at room temperature	–	15 min at room temperature	–
Incubation conditions	0.17 M Tris–HCl 4 mM $CaCl_2$ 0.1% ascorbic acid 60 min at room temperature	Na^+ Phosphate 0.3 M 60 min at room temperature	0.05 M Tris–HCl 0.1 mM $MgCl_2$ 0.1% ascorbic acid 90 min room temperature	0.17 M Tris–HCl 120 mM NaCl 0.01% ascorbic acid 60 min room temperature
Washing	2×5 min 4°C	2×5 min 4°C	2×1 min 4°C	2×5 min 4°C
Exposure	45 days	21 days	60 days	60 days
Nonspecific	10^{-5} M 5-HT	10^{-5}M atropine	10^{-5} M adrenaline	10^{-6}M haloperidol

the anatomical quality of the autoradiographic images obtained from prenatal brains is clearly lower than that observed in adult tissues. The use of 20 μm thick sections is really required in this case. Secondly, the level of immaturity of the nervous system, in terms of cell migration and synaptic connections, makes it also difficult to identify and delineate a particular area or structure in early prenatal human sections in the process of quantitation. This adds a certain degree of caution when ascribing an accumulation of autoradiographic grains to a particular brain nucleus, as defined in the adult. Although the use of atlases of developing human brain (Feess-Higgins and Larroche, 1987) may help in the interpretation of the autoradiographic data, it is not easy to fully overcome that limitation. Third, one of the relevant methodological problems in quantitative autoradiography is encountered when tritiated ligands are used, because there is a significant degree of tissue absorption of the beta rays, and this absorption will vary depending on the density of the tissue and of the material used (Kuhar and Unnerstall, 1985): taking into account that the chemical composition of fetal/neonatal brain tissue is different than that of the adult, the percentage of water and lipids being higher, the different regional tissue quenching might make a direct comparison of density values obtained in immature and adult tissues difficult. Thus, an absolute comparison between both types of values is not feasible when tritiated ligands are used: it should be kept in mind that densities measured in fetal/neonatal brains are underestimated with respect to the adult stage.

Taking into account these limitations, we will discuss the results of several autoradiographic studies on the distribution and properties of monoaminergic receptors in the developmental human brain. These studies have been carried out in tissue sections from a large series of brains obtained at autopsy from three fetuses, 13 newborns and six infants and children (for further details, see Olmo et al., 1996). Causes of death in newborns, infants and children did not involve direct brain disfunction. Gestational ages of fetuses and newborns ranged from 19 to 40 weeks (see Table 2). A clear limitation in these studies is the difficulty of the availability of samples from early fetal cases. Because of that, the results obtained in all fetal and newborn brains are analyzed together. Brain tissues from adults who died without evidence of neuropsychiatric disorders were used for comparison. In all the brains studied, postmortem delay ranged from 6 to 45 h. The procurement and handling of these tissues were carried out under approval of the corresponding ethical committees. The analysis of these studies will allow the discussion of several critical issues, when visualizing both receptors and their mechanisms of cellular activation at the ontogenetic level.

Table 2
Sources of fetal/neonatal brain tissue

Case	Sex	Age[a]	Postmortem[b]	Cause of death
A	F	19	–	Death at birth
B	M	22	17	Prenatal distress
C	M	26	15	Prenatal distress
D	M	27	20	Prenatal distress
E	M	29	11	Prenatal distress
F	M	29	6	Prenatal distress
G	M	34	15	Congenital cardiopathy
H	F	34	20	Kernicterus
I	M	35	–	Death at birth
J	M	36	22	Congenital cardiopathy
K	F	38	18	Septic shock
L	M	39	–	Death at birth
M	M	40	19	Unknown
N	F	40	11	Prenatal distress
O	F	40	19	Congenital cardiopathy
P	M	40	25	Rubella

[a] Weeks of gestational age.
[b] Postmortem delay is expressed in hours.

3. Results

3.1. Properties and localization of human neurotransmitter receptors during ontogeny

When analyzing the results obtained so far in studies of visualization of brain receptors during human development, special attention must be paid to the information regarding three different aspects: (1) the pharmacological properties of the 'developing' receptor; (2) the anatomical localization, allowing the obtention of densitometric maps; and (3) the temporal sequence of appearance.

3.1.1. Pharmacological characteristics (affinity profile)

Affinity studies carried out in developing human tissues have demonstrated that the pharmacological profile of the receptors in fetal/neonatal brain is comparable to that found in the adult brain. As an example of that, we have found for 5-HT_{1A} receptors in neonatal brain the following pharmacological profile: 5-CT, 8-OH-DPAT, 5-HT > buspirone, SDZ(-)21009 > sumatriptan, mianserine (not shown). This pattern of affinities is the same as previously reported in adult brain.

3.2. Anatomical localization of receptors: fetal versus adult

The anatomical distribution of neurotransmitter receptors in the developing human brain is, in general terms, in good agreement with that found in the adult. However, different patterns of distribution can be found, both in terms of exact localization and time of appearance. Muscarinic cholinergic and dopamine D_2-like receptors both localize in very high densities over

the basal ganglia (caudate, putamen) at the fetal and neonatal stages, in good agreement with their anatomical distribution in the adult brain (Fig. 1). A similar pattern of correspondence can be found for 5-HT_{1A} serotonergic and α_2-adrenergic receptors in the hippocampus (lacunosum-moleculare stratum), although the level of labeling is higher in fetal stages in the case of 5-HT receptors (Fig. 2). In this case, a high level of the mRNA signal is seen over the hippocampus, but is concentrated in the stratum pyramidalis (Fig. 3). The human cerebellum also shows a good level of matching with regard to the distribution of α_2-adrenoceptors, but not 5-HT_{1A} receptors, during human development (Fig. 4).

In contrast with these data, autoradiographic labelling of receptors during development has also re-

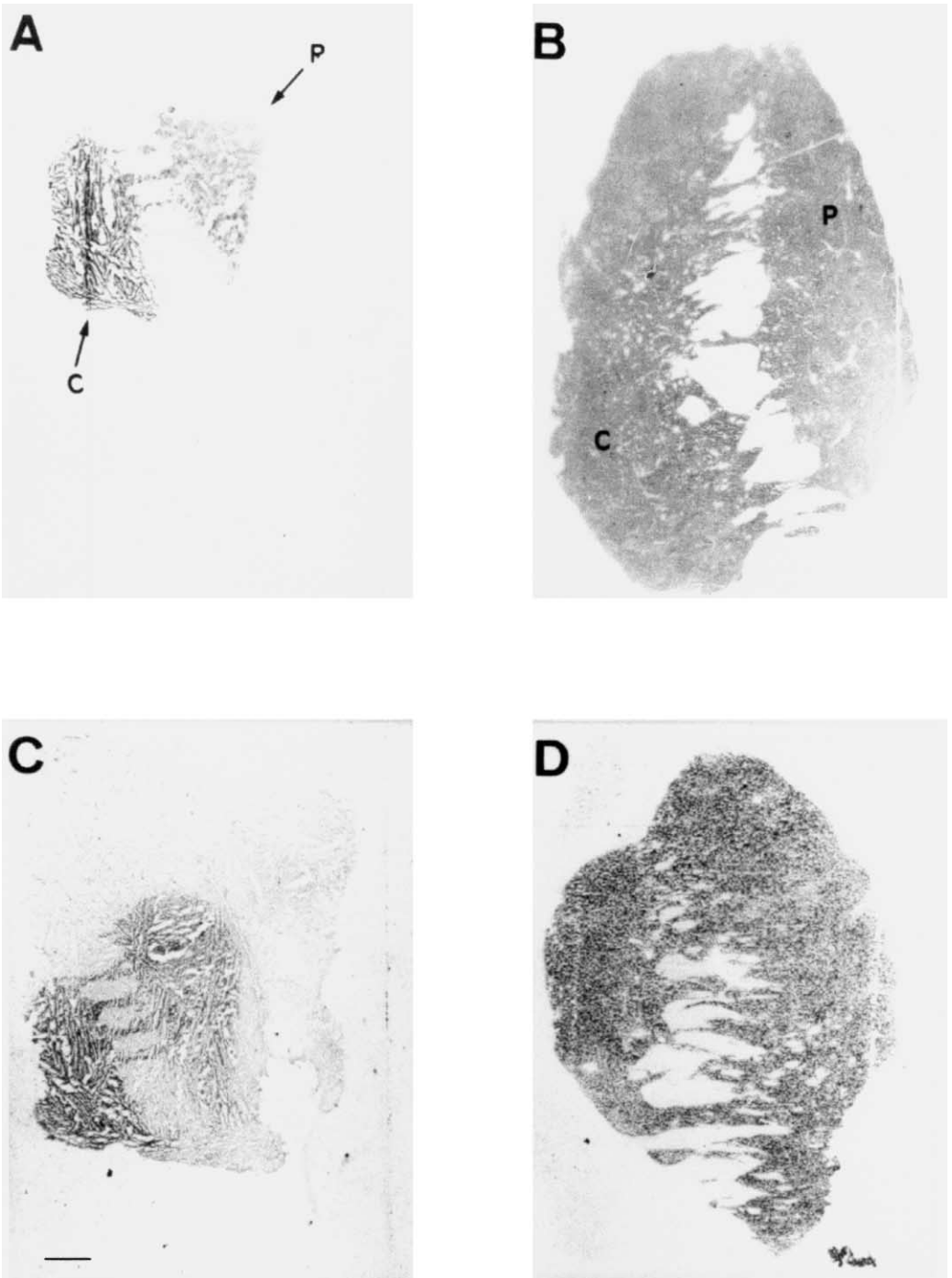

Fig. 1. Autoradiographic illustration of D_2-like dopaminergic receptors (A and B) ([^{3}H]spiperone binding, 2 nM) and cholinergic muscarinic receptors (C and D) ([^{3}H]NMS binding, 1 nM) over the human basal ganglia from a neonatal case of a gestational age of 29 weeks (A–C) and from an adult (B–D). C: caudate; P: putamen. Dark areas are those enriched in radiometric labeling. Bar = 2 mm.

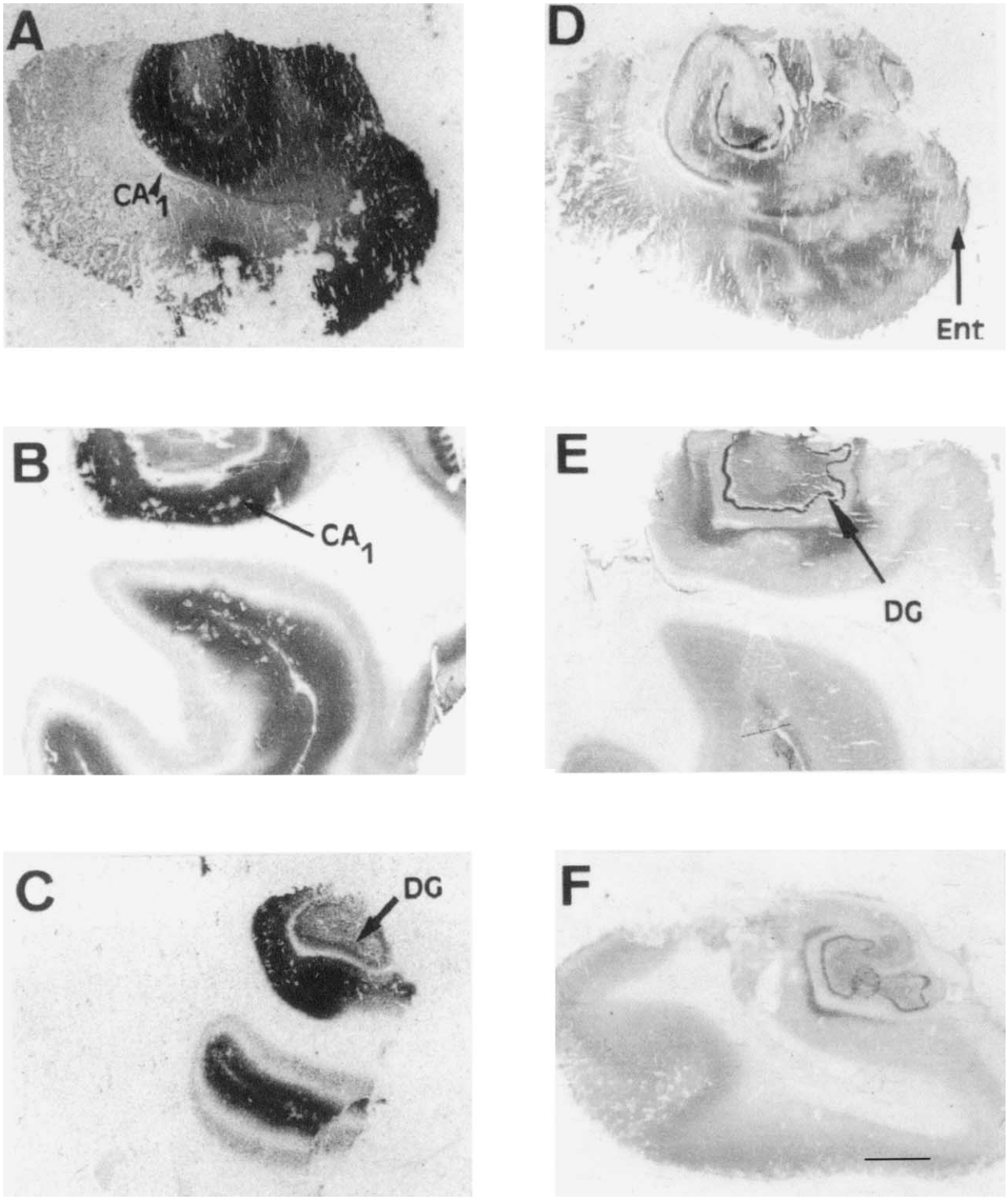

Fig. 2. Autoradiographic illustration of the distribution of [^{3}H]8-OH-DPAT (2 nM) (5-HT$_{1A}$ receptors) (left panel) and [^{3}H]UK14304 (1 nM) (α2-adrenergic receptors) (right panel) binding sites over the human hippocampus during development. A, D: neonatal case of a gestational age of 29 weeks; B, E: 2 year-old child; C, F: adult. CA$_1$: CA$_1$ field of the hippocampus; DG: dentate gyrus; Ent: enthorinal cortex. Bar = 2 mm.

vealed the existence of striking differences in anatomical localization. Some of these differences can be easily explained by the events in neural maturation. In this regard, the process of cortical lamination is not completed till the 1st year of life (Jacobson, 1991), clearly after neuronal migration. This could explain the differences in the fine lamination of most receptors over the cortical areas (see Fig. 5 for the case of 5-HT$_{1B/D}$ receptors). The development of 5-HT$_{1B/D}$ receptors also provides a particular example of maturation-based dif-

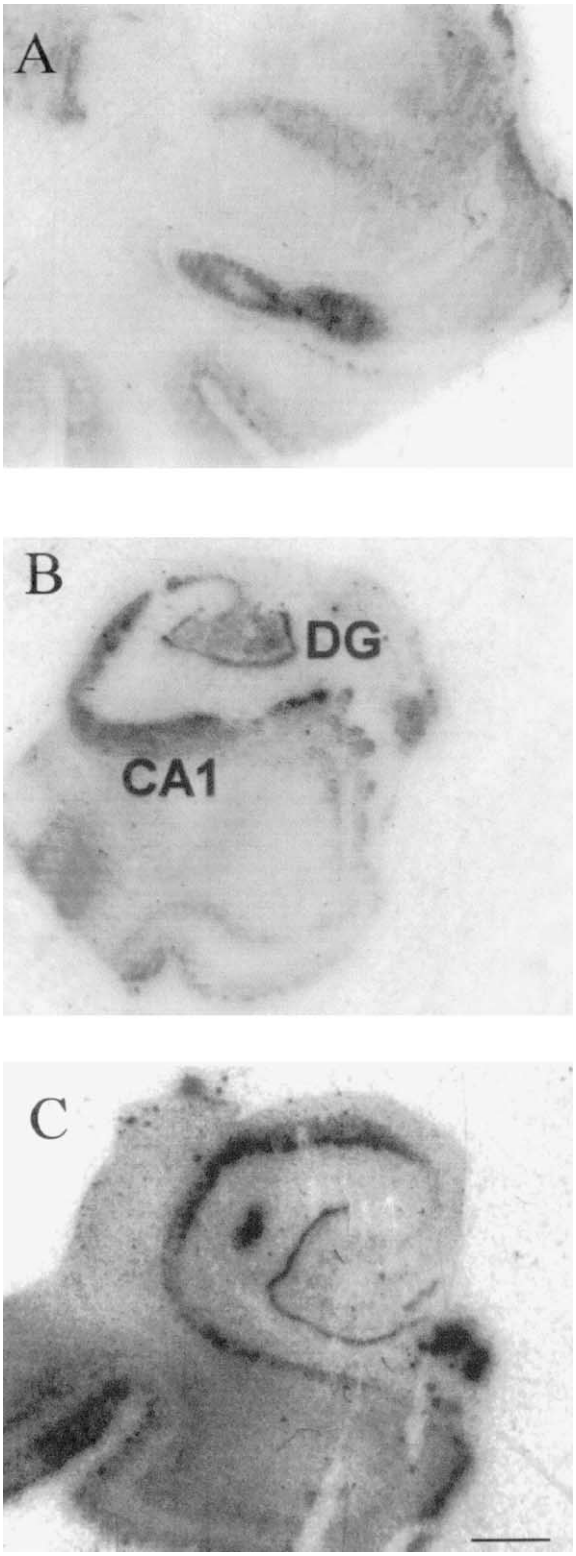

Fig. 3. Regional distribution of 5-HT_{1A} receptors mRNA in the developing human hippocampus. Tissue sections correspond to a neonate (gestational age 40 weeks) (A), an infant (9 months old) (B) and an adult (C). In situ hybridization was carried out by using an oligonucleotide probe complementary to the mRNA coding for the human 5-HT_{1A} receptor corresponding to bases 858-904, labelled with [^{32}P] a-dATP CA_1; CA_1 field of the hippocampus; DG: dentate gyrus. (Modified from Olmo et al., 1998). Bar = 2 mm.

ferential localization: the presence of high densities of these receptors over the medial medullary lamina, indicating their transit through striatofugal pathways to the globus pallidus (Fig. 6).

Much more interesting are those cases where a receptor appears during development in a brain area which is devoid of such a protein at the adult stage. An example of that is the transient and very high expression of 5-HT_{1A} receptors, both protein and mRNA, in the human cerebellar cortex (Fig. 4). These phenomena of transient appearance, as well as those of relative overexpression, are also reported for other receptors such as dopamine D_2 (Noisin and Thomas, 1988) or somatostatin sites (González et al., 1988) in the rat strongly indicate that the identified receptor plays a regulatory role in development (see below), as in the case of 5-HT. This issue is further discussed elsewhere.

The use of computerized densitometry allows a full quantitation of neurotransmitter receptors throughout the brain, with a high level of anatomical resolution. This approach allows the development of detailed quantitative anatomical maps at the different stages of development (Table 3).

Some preliminary data are available on the ontogeny of receptor-induced G protein activation in the human brain. In general terms, the localization of stimulated GTPγS binding levels, induced by selective agonists, reveals an anatomical distribution similar to that obtained by radioligand autoradiography, and with a relatively comparable temporal pattern of appearance . Fig. 7 illustrates the degree of activation of G proteins induced by increasing concentrations of the 5-HT_{1A} agonist 8-OH-DPAT in the human hippocampus at the fetal and infant ages.

3.2.1. Temporal patterns of appearance

In general terms, neurotransmitter receptors have been found to be present earlier in fetal and neonatal brain than are the neurotransmiters. Usually areas maturing first, in terms of differentiation, are also the regions first acquiring receptors so, in the rat, brainstem receptors are at birth more mature than cortical receptors. In addition, two differents patterns of appearance of receptors, prenatal or postnatal, can be found in developing human brain. 5-HT_{1A} and D_2 dopaminergic receptors are basically of prenatal appearance. We have found relevant densities of these receptors similar to or even higher than those present in adults, even at the 26th week of gestation (Figs. 1–4). In contrast, the development of 5-$HT_{1B/1D}$ receptors in the human brain is mainly postnatal: although this receptor subtype is already detectable at earlier developmental stages, high densities are not found before perinatal stages of development (Figs. 5 and 6).

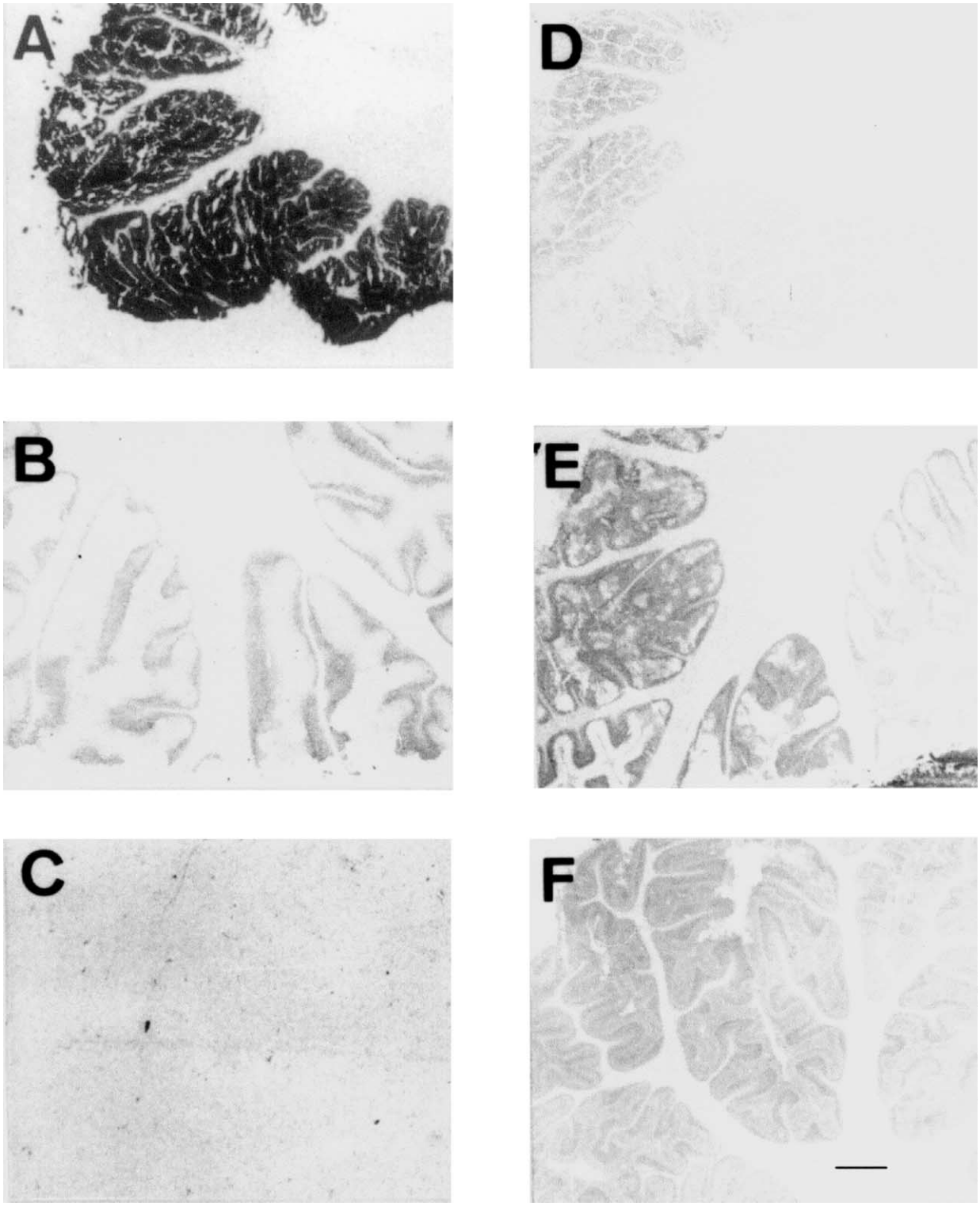

Fig. 4. Autoradiographic illustration of the distribution of [^{3}H]8-OH-DPAT (5-HT_{1A} receptors) (left panel) and [^{3}H]UK14304 (α_2-adrenergic receptors) (right panel) binding sites over the human cerebellum during development. A, D: neonatal case of a gestational age of 29 weeks; B, E: 2 year old child; C, F: adult. Bar = 2 mm.

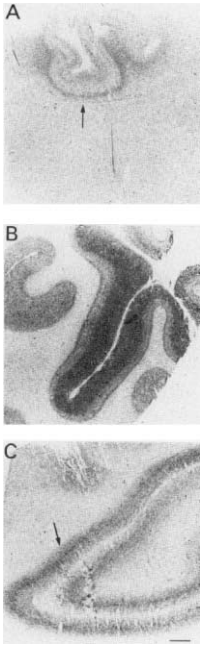

Fig. 5. Autoradiographic illustration of [^{125}I]GTI binding sites (5-$HT_{1B/D}$ receptors) in human striate cortex during development. A: neonatal case of a gestational age of 40 weeks; B: 2 year-old child; C: adult. The arrows indicate the most labelled layers (taken from Olmo et al., 1996). Bar = 2 mm.

4. Discussion

4.1. The anatomical distribution of brain neurotransmitter receptors during ontogeny: a comparison between rat and human data

The densities of neurotransmitter receptors in human brain appear to be generally lower than that in rat brain. Autoradiographic results from tissue sections are in agreement on this point with measurements in membrane preparations (binding). These differences probably reflect the lower density of neuronal cell bodies in humans compared to rats. Besides that, the distribution of receptors in the developing human brain is in good agreement with that found in the developing rat brain. A similar anatomical expression has been found in both rats and humans for dopamine D_2-like sites during development, with high densities over the basal ganglia and substantia nigra, also presenting a conparable temporal pattern (Murrin et al., 1985; present results). In a similar way, data available on the ontogeny of 5-HT_{1A} receptors in human brain, in terms of both protein and mRNA, demonstrate a close parallel with those previously reported in the rat, even those referring to their transient expression (Daval et al., 1987; Matthiessen et al., 1992; Miquel et al., 1994; Olmo et al. 1994, 1996, 1998).

However, the dynamics of the regional development in the human brain does not always correspond to that

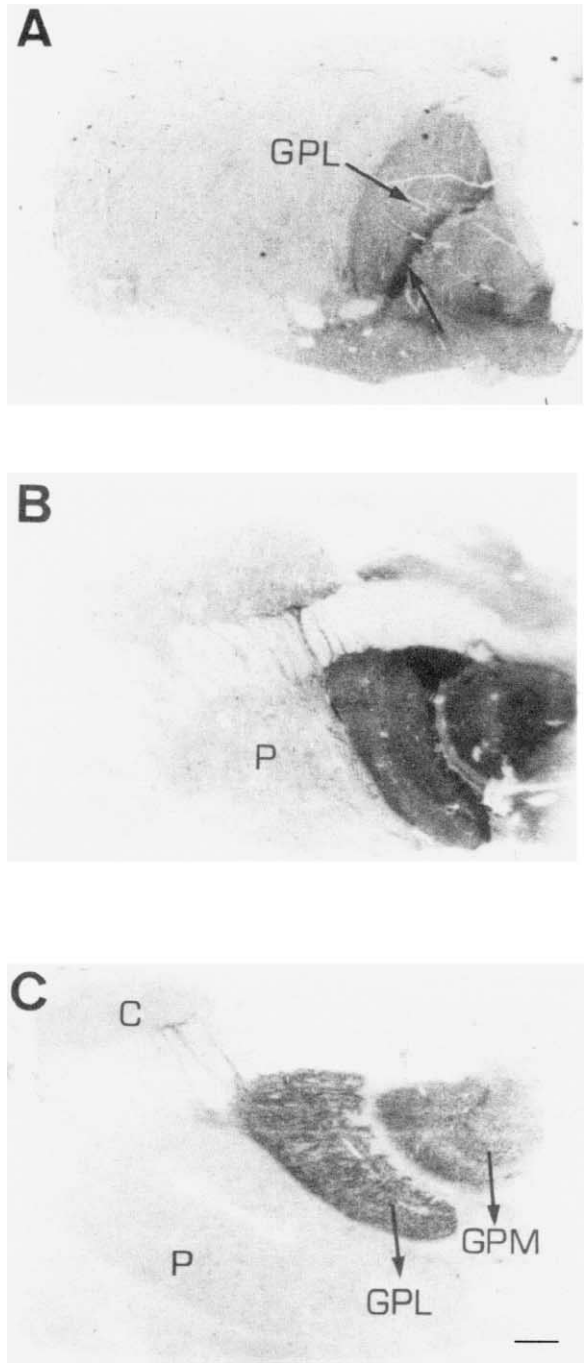

Fig. 6. Autoradiographic illustration of [^{125}I]GTI binding sites (5-$HT_{1B/D}$ receptors) in the human brain at the level of the posterior basal ganglia during development. A: neonatal case of a gestational age of 40 weeks; B: 2 year old child; C: adult. GPL: globus pallidus lateral; GPM: globus pallidus medial. Arrows: medial medullary lamina. (Taken from Olmo et al., 1996). Bar = 2 mm.

of the rodent brain (Dobbing and Sands, 1979). This fact explains the lack of a complete matching between both species regarding receptor appearance. For example, 5-HT receptors located on the cerebellum and appearing at birth in the rat, are already present in the human species around the 30th week of gestation (Matthiessen et al., 1992).

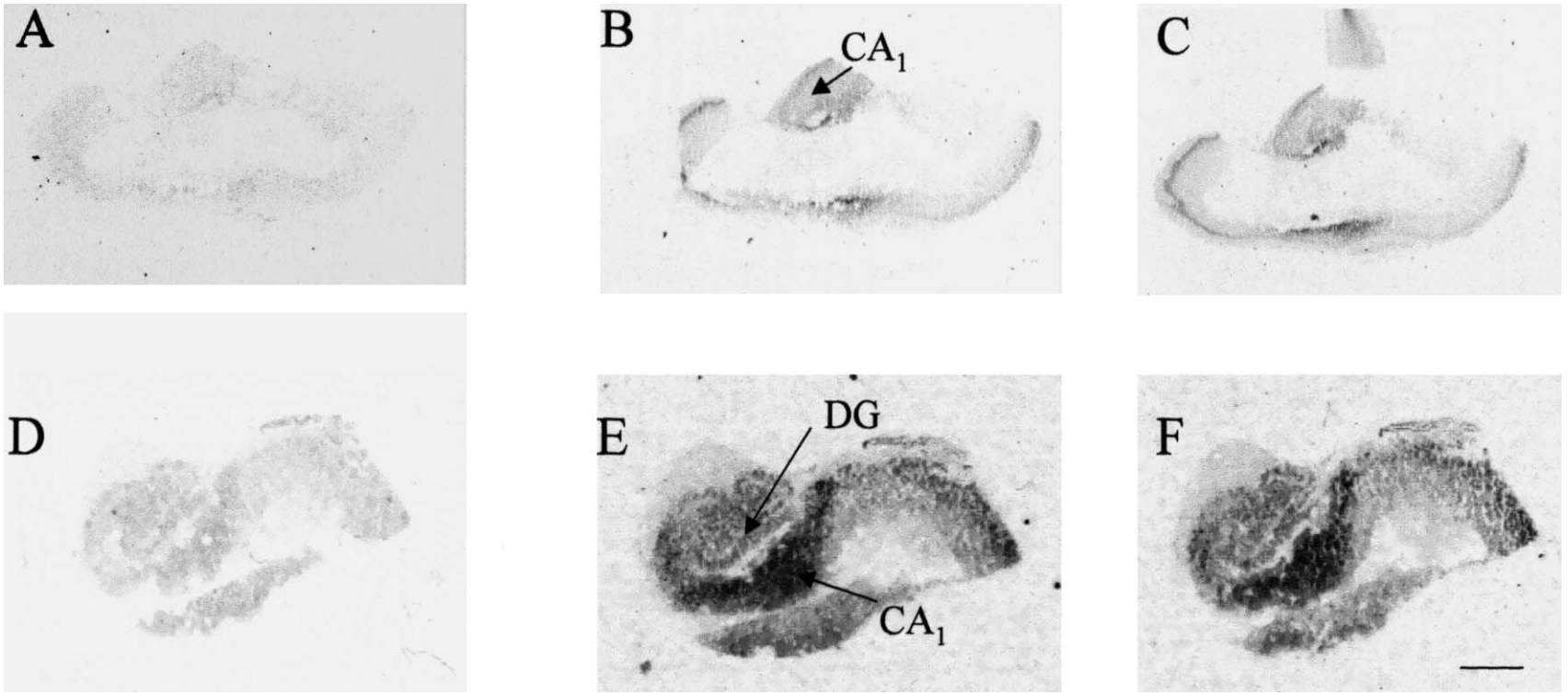

Fig. 7. Autoradiographic images of consecutive tissue sections from the human hippocampus from a fetal case (19 weeks of gestational age) (A–C) and an infant (9 months old) (D–F), illustrating [^{35}S]GTPγS binding (0.05 nM) A and D correspond to basal binding; B and E illustrate activation induced by 10^{-5} M 8-OH-DPAT (a 5-HT_{1A} agonist), C and E show [^{35}S]GTPγS binding activation after coincubation with 3×10^{-6}M 8-OH-DPAT. $CA_1 = CA_1$ field of the hippocampus; DG = dentate gyrus. Bar = 2 mm.

Autoradiographic techniques allow a detailed localization of receptors, which is in contrast with the limited resolution of studies performed in tissue homogenates. However, it is not always possible to ascribe binding sites to a particular cellular population. The combination of light (and electron) microscopic autoradiography, in situ hybridization and inmunocytochemical procedures is the best way to approach this issue. In fact, our results for protein and mRNA visualization of 5-HT_{1A} receptors in the same group of tissues illustrate the complementarity of both procedures. The distribution and abundance of 5-HT_{1A} receptor mRNAs are in good agreement with the pattern found for the protein, although some differences, dependent on the nature of each procedure, are evident: in the dentate gyrus of the hippocampus, the hybridization signal is observed over the granular cell layer (somatic) while the receptor is preferentially localized over the molecular cell layer (dendritic). Regarding brain ontogeny, receptor inmmunocytochemistry has been applied in rat tissue, but not in human samples (Kia et al., 1996). The use of this procedure will provide, in the near future, very valuable information to complete our understanding of the fine localization of receptors during development. The type of cells bearing receptors during development is a matter of great interest: in fact, it has been proposed that astroglial cells frequently express neurotransmitter receptors in a transient way (see Whitaker-Azmitia, 1991). This is not surprising, as it is well known that these cells produce important amounts of neurotrophic factors (Alexander and De Vellis, 1981).

Table 3
Densities of [^{125}I]GTI binding sites (5-HT_{1B} and 5-HT_{1D} receptors) in the developing human brain determined by autoradiography[a] (modified from Olmo et al., 1996)

Area	Fetal/neonatal	Infant/children	Adult
Frontal cortex	2.7 ± 0.9	2.7 ± 0.9	4.2 ± 1.0
Striate cortex	4.5 ± 1.3	21.8 ± 2.7**	5.4 ± 1.0∇∇
Basal ganglia			
Nucleus caudatus	4.4 ± 1.2	9.7 ± 3.9	6.7 ± 1.9
Nucleus putamen	4.4 ± 0.9	9.3 ± 2.2*	3.4 ± 0.5∇
Globus pallidus lateralis	9.5 ± 1.4	36.7 ± 6.8**	23.9 ± 1.6**∇
Claustrum	ND[b]	10.1 ± 1.7**	4.5 ± 1.7*
Hippocampus			
CA_1 field, lac-moleculare	1.1 ± 0.3	4.5 ± 0.3**	3.8 ± 1.4**
Dorsal subiculum	1.0 ± 0.4	4.1 ± 1.6**	2.4 ± 1.4**
Midbrain			
Substantia nigra	16.9 ± 3.1	35.4 ± 3.9**	19.5 ± 3.7∇
Nucleus raphe dorsalis	6.4 ± 1.5	18.6 ± 8.4*	3.9 ± 0.8∇

[a] Values are expressed as B_{max} densities (fmol/mg tissue, mean ± SEM).

[b] ND, not detectable.

* $P<0.05$; ** $P<0.01$ versus fetal/neonatal; ∇, $P<0.05$; ∇∇, $P<0.01$ versus infant/children.

(ANOVA followed by Newman–Keuls)

4.2. Neurotransmitter receptors and brain regional development: a trophic role to play?

A relevant question concerns the functional role of neurotransmitter receptors during the development of the nervous system. In most cases these receptors behave just as a target for their endogenous ligands, the neurotransmitters, playing the same transignalling role that characterize them at the adult stage. Furthermore, considerable amount of evidence suppports an 'additional' role for some neuroreceptors, especially those whose highest densities occur during brain development at a time when properly functioning synapses are not present. In general terms, transiently and early expressed receptors can play a role themselves in brain development, sometimes by producing high levels of second messengers (see Whitaker-Azmitia, 1991). Furthermore, these trophic effects can be region-specific. Among the numerous examples of this behaviour (Bucharles et al., 1999; González, et al., 1988; Haydon and Drapeau, 1995; Whitaker-Azmitia, 1991), 5-HT_{1A} receptors for serotonin may clearly illustrate this issue.

Serotonin (5-HT) is present in fetal central nervous system (CNS) tissue prior to assuming its role as a neurotransmitter in the mature brain. Its ability to regulate, in a complex manner, several aspects of nervous development, including migration, synaptogenesis and neuronal division, is well documented (see Lauder, 1993; Whitaker-Azmitia et al., 1996). Thus, 5-HT has been shown to halt neurite elongation in cultures from snail ganglion neurones (Haydon et al., 1984), facilitate synaptogenesis in explant cultures of rat cerebral cortex (Chubakov et al., 1986) or stimulate mouse neural crest migration (Moiseiwitsch and Lauder, 1995). Furthermore, it has been reported that neonatal depletion of 5-HT reduces the number of dendritic spines in rat hippocampus (Yan et al., 1997a).

A wide number of biochemical and functional studies suggest that 5-HT_{1A} receptors, that could be located on glial cells, are especially involved in this regulatory role of 5-HT. Stimulation of this receptor subtype by specific agonists decreases the branching of neurites in cultured cortical neurones and induces other neurotrophic effects on septal cholinergic neurons (Riad et al., 1994; Sikich et al., 1990). Blockade of 5-HT_{1A} receptors results in a modification of the number of dendritic spines on hippocampus dentate granule cells (Yan et al., 1997b). Because of that, the knowledge of the ontogenetic pattern of this receptor subtype is of

special interest. So, the developmental evolution of 5-HT_{1A} receptors has been studied in rats during the late prenatal and early postnatal period by radiometric procedures. These studies have revealed a pattern of early development for these receptors: a progressive increase in the density in areas such as the hippocampus (CA fields and dentate gyrus), septum and cerebral cortex, reaching adult or even higher levels around the third postnatal week; together with a high and transient expression of both protein and mRNA in the cerebellum (Purkinje cells), which progressively decreases until it is not detectable in postnatal life, probably eliminated in a programmed way in the course of development (Daval et al., 1987; Matthiessen et al., 1992). As previously described, our results in humans (also found in tissue homogenates) show a similar anatomical pattern of development throughout the brain, but already evident at the prenatal stage (Pazos et al., 1992; Olmo et al., 1994, 1996, 1998; present results). These findings constitute strong support of the neurotrophic role of the 5-HT system in mammals (Whitaker-Azmitia et al., 1996).

The high degree of selectivity of the responses mediated by a neurotransmitter explains why different receptor subtypes of the same neurotransmitter can present completely different patterns of development in terms of temporal appearance: so, while 5-HT_{1A} attain peak levels at fetal stages in the human brain, tha pattern of 5-HT_{1B} receptors is mainly postnatal (basal ganglia, cortex) (Olmo et al., 1996). This illustrates the complexity of the functions of serotonin in the developing brain.

4.3. Developing receptors as a site of action of drugs: identifying regions of choice

The fact that neurotransmitter receptors are frequently present in the early stages of development makes it necessary to analyze the possible influence that the action of drugs (or other factors), with affinity for these receptors, could exert on the regulation of the development of the central nervous system. The results of this interaction can be negative, inducing teratogenic phenomena. There might also be a positive influence of these actions, which could lead to a 'developmental' pharmacology. Since the development of the human brain is not complete until the 2nd decade of life (Chugani et al., 1987; Huttenlocher, 1990), the chance of a positive or negative pharmacological intervention is at least feasible. While there are relevant examples of teratogenic effects, the possibility of modifying brain development in a positive way still remains on a theoretical basis. One of the requirements for determining with certainty the possibilities and risks of this influence is the detailed knowledge of how and where the receptor appears during the pre- and postnatal periods in the human species. Taking again the 5-HT system, as an example, infants born to mothers treated with 5-HT_{1A} agonists or antagonists, or with drugs affecting 5-HT dynamics such as antidepressants, could eventually present some alterations due to their influence on brain development. But this could also be used in order to intend a therapeutic 'intervention' in the developing brain. According to the data available on the development of these receptors, the hippocampus, cerebellum or the serotonergic neurons located on the raphe nuclei can be identified as candidate areas to be the subject of such intervention.

The ability of drugs to modify the development of the central nervous system through interaction with receptors requires that those receptors be really functional. In this regard, Lauder et al. (2000) have recently demonstrated that serotonergic drugs do regulate up and down in utero rat 5-HT_{1A} receptors. On the other hand, our preliminary data with [^{35}S]GTP-γ-S autoradiography demonstrate that the stimulation of 5-HT_{1A} receptors is able to activate G proteins in several regions of fetal human brain. These data suggest that 'developing' receptors can really be in a functionally active site (Rodríguez-Puertas et al., 2000).

5. Conclusions

In conclusion, the studies on the visualization of monoamine receptors during the development of the human brain provide important information, which is relevant from two points of view: (1) they contribute to the knowledge of the neurochemical anatomy of the developing brain, supporting the possible existence of functional and pharmacological interactions; and (2) they provide the anatomical basis for the analysis of the trophic role that several endogenous amines play during the development of the human nervous system.

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