Prenatal and Postnatal Development of GABA-Accumulating Cells in the Occipital Neocortex of Rat

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ABSTRACT The development of the $^3$H-GABA-accumulating cells in the neocortex has been followed by light microscopical autoradiography, and after sectioning of the original autoradiograms, by electron microscopy. The validity of the methods used are discussed. The study has been limited to the primary visual cortex and its precursors of rat, from embryonic day (E) 15 to adult.

GABA-accumulating cells were found from E 16 onwards in the occipital cortex, which is one to two days after cells arrive in the pallial anlage and one day before the first synapses have been found. Until E 18, the prevalent positions of labeled cells were in lamina I and below the cortical plate. Later, labeled cells also occurred as strands within the cortical plate. During the perinatal period, more and more GABA-accumulating neurons and glial cells began to differentiate and show a characteristic distribution at the periphery of unlabeled cell clusters. From postnatal day 11, no apparent change in density or position of labeled neurons took place.

At prenatal stages, two main types of labeled cells were found: 1) Comparatively large cells with rounded nuclei and rough endoplasmic reticulum consisting of narrow, electron-lucent cisternae. These cells were tentatively identified as pre-neurons. 2) Smaller, polymorphous cells with irregular nuclei and rough endoplasmic reticulum with wide cisternae filled with a dense matrix. These cells are probably precursors of glial cells. Both labeled neurons and glial cells were identified at postnatal stages. In young and adult rats, only neurons to be characterized as neornocortical neurons were labeled. Synapses were not found on the perikarya of labeled cells until E 21. Also, in postnatal preparations, labeled neurons showed few axo-somatic synapses.

These data were correlated with other events of the structural and functional development of the neocortex. The delay between the appearance of GABA accumulating cells and synaptogenesis indicates that apart from being an inhibitory neurotransmitter, GABA might play a specific morphogenetic role in synaptogenesis. This could even be its primary function during early developmental stages.

The role of $\gamma$-aminobutyric acid (GABA) as an inhibitory synaptic transmitter is still under discussion. However, in the central nervous system, GABA exerts inhibitory effects on many neurons which are very similar to at least one type of synaptic inhibition (Krnjević, 76). In the adult cerebral cortex, there is growing evidence for a number of stellate cells to act as inhibitory neurons, to produce and to release GABA (see Curtis and Felix, 71; Fuxe et al., 71; Rihak, 78). On the other hand, glycine does not seem to be involved in neocortical inhibition Kelly and Krnjević, 69).

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Extracellular GABA from exogenous sources or GABA released from afferent terminals is accumulated by a high affinity uptake mechanism (Eilott and van Gelder, '78; Iversen and Neaf, '79), which has been localized in certain neurons as well as in glial cells (Henn and Hamberger, '71). However, the major part of the accumulated GABA is released from neurons rather than glial cells (Hannerstad and Lyeth, '76).

The hypothesis that only those neurons using GABA as a transmitter show a high affinity uptake of GABA (Neaf and Iversen, '69) has led to autoradiographic mapping studies and identification of [3H]-GABA accumulating cells. Hökfelt and Lundström ('72) confirmed in the cerebellum that the uptake is more or less restricted to neurons known to act as inhibiting cells.

In the cerebral cortex, labeled cell bodies were first identified as stellate neurons, mainly localized in laminae I, II, III and, although not specifically mentioned, in II, I, as well (Hökfelt and Lundström, '72). Recently, GABA-accumulating neurons were found in all cortical laminae as well as in the subcortical white matter (Broman and Wolof, '79), which fitted the distribution of neurons labeled immunohistochemically for a high content of the GABA producing enzyme glutamate decarboxylase (Ribak, '78). All these results indicate that there is a definite fraction of neurons in the cerebral cortex which can be identified by the enzyme for GABA production as well as by high affinity uptake of GABA, and might use GABA as a transmitter.

The development of GABA uptake has been biochemically investigated in rodents (Kelly et al., '74; Johnathon and Davies, '74; Coyle and Emms, '74) and chick embryos (Bandy and Purdy, '77). Although the material and age vary in all these studies, an uptake mechanism for GABA was found to develop early—i.e., preor perinatally. This suggests that GABA uptake might be used to locate cells with this property, even before birth. The present report on the development of [3H]-GABA accumulating cells in the occipital cortex of rats has two aims: 1) to locate such cells within the inter- and laminar gradients of differentiation (II, III) as early as possible; and 2) to characterize these cells cytologically and if possible to identify them as glial cells or various types of neurons.

### MATERIALS AND METHODS

The development of [3H]-GABA uptake was studied in Sprague Dawley rats from the 16th day of gestation to adulthood (Table 1). Brains of each age were assigned to the day sperm was first found in the vaginal smear.

#### TABLE 1

<table>
<thead>
<tr>
<th>Age</th>
<th>Number of animals</th>
<th>GABA application</th>
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<tr>
<td>16</td>
<td>4</td>
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<td>Immersion</td>
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<td>26</td>
<td>4</td>
<td>S</td>
<td>Occipital cortex</td>
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<td>37</td>
<td>4</td>
<td>S</td>
<td>Presumptive visual cortex</td>
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<td>22</td>
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<td>P</td>
<td>2</td>
<td>S</td>
<td>Presumptive primary visual cortex</td>
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<td>4</td>
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<tr>
<td>Adult</td>
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* S = sacrifice, I = injection.

#### Preparation of the material

To obtain the embryos, the mother was anesthetized with ether and the fetuses were delivered by Caesarean section. The fetuses were additionally anesthetized with sodium pentobarbital after the skull was opened and the meninges removed with a hooked needle. Postnatal (P) animals were anesthetized with sodium pentobarbital and a hole was then either cut (for animals younger than P10) or drilled in the occipital part of the skull. Animals to be treated later than P25 were inscribed above the visual cortex some days before the experiments, to allow the resulting edemas to subside.

[3H]-GABA ([4-carboxy-1-14C]-GABA, specific activity 3.7 × 10⁶ cpm/μg) was obtained from The Radiochemical Centre, Amersham. The solution was evaporated with nitrogen after before the experiment. The substance was then redissolved in 3-4 μl artificial cerebrospinal fluid to give an amount of 20-40 μCi (≈ 0.75 nM, 2.5 × 10⁶ cpm/μg) per superfusion or injection.

In embryonic and postnatal stages up to P24, GABA was superfused. Single postnatal stages and all adult animals were injected with 1-3 μl of [3H]-GABA (Table 1) by pressure in small steps, through 20-30 μm wide glass microcapillaries.

Tissue was fixed in 2.5% glutaraldehyde plus 3.7% formaldehyde in cacodylate buffer, at pH 7.2. The survival time from superfusion or injection to fixation was 2-5 min. The heads of young embryos were immersed in the fixative. From E18 onwards, cardiac perfusion was applied. Tissue blocks containing presumptive, immature or adult visual cortex (Table 1) were embedded in Epon.

#### Autoradiography

For autoradiography (ARG) the 3-4 μm Epon sections, mounted on glass slides, were dipped in K3 emulsion (Ilford) according to the method of Rogers (72). They were exposed 10-12 days at 4°, developed in Kodak D 19 b and poststained according to Richardson et al. ('70).

To reveal the light microscopical (LM) structure of heavily labeled cells, the autoradiograms (ARGs) were photographed and the silver grains were then dissolved (Rogers, '73). In most cases, resectioning and electronic microscopy were necessary for the cyto
tological characterization and identification of labeled cells. This was achieved by two different methods: 1) LM ARGs were carefully photographed and subsequently analyzed using computer-assisted image analysis; and 2) preembedding method of Kaplan and Hinds ('77); 2) to identify single labeled cells within cell clusters. It was advantageous to show the silver grains and the underlying structure at the same time. The rough-thinned moss containing the autoradiogram was cut off the block, turned 90°, glued on to another Epon cylinder, and resectioned for transmission electron microscopy.

The emulsion layer contained aggregated silver grains opposite GABA accumulating structures (e.g., Fig. 4). The main advantage of this method over conventional electron microc
cosmic SEM/ARGs were that the grains do not cover the labeled structures and that topographical ARG-information and EM-resolution of labeled structures could be achieved after 10-12 days exposure.

Television image analysis (Quantimet 720, Cambridge Instruments) was used for counting silver grains. This produced an objective means for the definition of background, assumed background, and unlabeled to strongly labeled cells (Fig. 1).

#### General comments on the methods and characteristics of the [3H]-GABA uptake

A variety of structures take up [3H]-GABA: Cells of the meninges (except for orthocortical and macrourchial), pons and pons of the cerebellar cells of blood vessels, some glial cells and neurons, as well as some components of the neocortex (Hökfelt and Lundström, '71; Makara et al., '76; Chrenowall and Wolof, '78a).

In areas with edema (e.g., injection tracks, places where the brain surface had been torn) the main uptake seems to take place in synapses and glial processes. Hence, it is important to avoid edema to obtain specific labeling of neuronal and glial perikarya.

Additional experiments were performed using 1-aminooxy-acetic acid (AOAA) to block the GABA metabolizing enzyme, 2- and 3-aminooxy-acetic acid (AOAA), to block the GABA uptake, and 3-chloroleucine, to block the transport system. These experiments showed that the effects of concentration gradients of GABA dominate the picture in all ARGs.
Concentrations in the range of $10^{-4} - 10^{-6} \text{M}$ have been used for incubation of slices and homogenates (e.g., Iverson and Neal, '68). Somewhat higher concentrations, $10^{-3} - 10^{-1} \text{M}$, have been used for injections (e.g., Hökfelt and Lundblad, '73). The concentration of GABA follows a gradient around injection sites (Fig. 2). A restricted region around the track (1) is often edematous and shows a heavy diffuse labeling. Only few nonlabeled cell bodies stand out against this dense background. In superfusion experiments (Fig. 2), this type of labeling (region 1) appears when and where the tissue has been torn or pulled. The applied solution is much diluted by cerebrospinal fluid and spreads over a fairly large area, producing heavy uptake in the meninges and I.I., even in the absence of edema. A low concentration ($10^{-4} - 10^{-5} \text{M}$ range) will reach deeper levels. L.I. (region 2) also shows a high, diffuse activity, not only after superfusion, but also when brain slices are incubated in (H)-GABA (Hökfelt and Lundblad, '71) and our unmodified results). This indicates that the uptake capacity for GABA is high in I.I. probably for other reasons than the high concentration resulting from the diffusion gradient. Region 3 (Fig. 2) comprises the majority of GABA-accumulating neurones and glial cells. This is the site of the region is dependent on the depth of the injection or on the level of the brain reached by diffusion. In region 4, glial cells and their processes are predominantly labeled. The diffuse labeling of the neuronal elements in all regions is higher than the unspecific background of ARS as seen outside the tissue and in the lumen of blood vessels. Thus, we consider a cell to be specifically labeled when its grain density is higher than in the surrounding tissue (Fig. 1). The number of GABA-accumulating cells is certainly underestimated by this definition, especially in preparations or regions with highly labeled neurons, where lightly labeled cells cannot be discriminated from background (Fig. 1). For several reasons (Kelly and Dick, '76; Iverson and Neal, '68) most of our superfusions and injections were terminated within five minutes of the application.

The local distribution and the intensity of labeling varied in most of our experiments. Possible explanations are:

1. Tissue damage preventing uptake and accumulation (Hökfelt and Lundblad, '71).
2. Different levels of GABA transmission in different parts of the cortex (P. Mandel, pers. comm.); different pre- and post-synaptic conditions influencing the binding of GABA to the tissue during fixation; and
3. Variable concentrations of GABA reaching different cells. Highly labeled, medium, and lightly labeled neurones can be found close to each other. Similarly, labeled and unlabeled glial cells occur in the same lamina. This indicates that the premedial states of the cells might influence the GABA accumulation and that we cannot exclude the possibility that in some cells, the capability of GABA uptake was suppressed at the time of the experiment (cf. Hökfelt and Lundblad, '71). Therefore, only positive results have been evaluated and individual, nonlabeled cells could not be positively identified as lacking the high affinity uptake of GABA.

RESULTS

The differentiation of GABA-accumulating cells (GABA-C) is described within the context of a simplified version of cortical development. Table 2 contains a time-table of the "birth-date" of the neurones in each lamina. Deviations from the results of berry and Rogers (1971) might depend on differences in the area selected. Additionally, the data are related to the differentiation and syraptogenesis in area 17 of its precursors to which this study is limited. For simplicity, we will restrict the description to the appearance and cytological characterization of GABA-C (GABA) in two phases of development: 1) the embryonic period, which comprises the proliferation and migration of cells, and 2) the postnatal period which is characterized by rapid differentiation in all layers of the cortex. The perinatal period will be underrepresented, because no changes of importance seem to occur, and the interindividual variations were found to be high. In a second part, the GABA-C will be cytologically described.

Embryonic stages

The earliest stages investigated is E 15, at which GABA-labeled cells did not yet exist in the neopallium, but appeared occasionally in the subventricular zone (SV) and the marginal zone (MZ) of the occipital paleocortex (area 51 b after Krieg, '40). In the neopallium, there was only some diffuse labeling in the subependymal and paleopallial area, and strands of processes were labeled even in the ventricular zone.

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1 The term MZ denotes the precursors of the total pallium. The paleopallial zone is denoted by the stippled part. P, I.I., and subventricular zones (SV) by the appearance of the cortical plate (SV, Monti-Pallia, '71). Hökfelt et al., '78; Robinson et al., '77; Stadler and Stadler, '61; von der Mark, '63). The term "pallial" designates the pallium and its precursors. The term "subcortical" is used instead of "subventricular" (Stadler and Stadler, '71).
The amount of GABA-C was increased at E 16, especially in the lateral neocortical positions. The number of GABA-C decreased towards the more medial parts of the occipital and the neocortex, following the general lateral-medial gradient of differentiation. Most often, these earliest GABA-C are situated in two levels, i.e., in L I and/or below the cortical plate (Fig. 6A). A few cells were labeled at the lower border of the CP (Fig. 6B) as well as in the intermediate zone (L II) and in the SV. In medial parts, where the CP has not yet formed or appeared as cell clusters, the labeling was limited to the neuropil.

Labeled cells are more numerous at E 17, but are distributed in essentially the same pattern as on E 16 (Fig. 3). The number of GABA-C has further risen on E 18, especially in the more medial positions where they prevail in L I, just above the CP (Fig. 6C) and in the multipolar part of the CP. At this stage, a number of GABA-C occur for the first time within the bipolar part of the CP (Figs. 3, 6D).

Numerous cells are labeled in the CP at E 21. In overview pictures (Fig. 5), it is still possible to trace the higher number of GABA-C on top of the CP and in the multipolar part of the CP, although the bipolar CP contains isolated strands and islands of labeled cells. Three dimensionally, this distribution pattern corresponds to a meshwork of GABA-C that remains recognizable in postnatal stages.

Postnatal stages

During early postnatal stages (P 1–3), the hetero-medial gradient has vanished. Labeled cells and GABA-accumulating neurons are scattered throughout the cortex and the presumptive white matter, and form a pattern which essentially persists till adulthood (Fig. 3).

Labeled neurons are diluted by the expansion of the neuropil (prominent around P 10–30). The GABA-accumulating neurons appear in clusters between zones, with densely packed unlabeled neurons and zones with

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**Fig. 3. Drawings of autoradiograms where labeled cells or neurons are marked; nonlabeled cells are excluded. The time of GABA application is given on the left side. The shaded region in E 21, showing no labeling, has not been reached by the diffusing GABA.**
more advanced development of neuropil. A preliminary quantitative analysis of the distribution pattern of labeled versus unlabeled neurons also shows that labeled neurons are not randomly distributed. Details will be published elsewhere.

A major change takes place in L I. Only a small fraction of the preneurons is labeled (Fig. 4a, 6a, 8a, b) at early embryonic time, whereas almost all neurons take up GABA at postnatal time (Fig. 10).

Cytological characterization of embryonic GABA-accumulating cells

At E 16, two types of labeled cells could be distinguished light-microscopically: 1) cells with large or medium sized nuclei, either horizontally or vertically oriented, and 2) small, dark, and polymorphous cells with no preferential orientation. The latter occurred in all parts of L I and in regions below the cortical plate. Many appeared also along the marginal surface and in a satellite position to unlabeled neurons, indicating that many labeled cells were glial cells.

Two cells of the first type in L I have been resectored for EM analysis (Figs. 4, 5). One cell (Fig. 4a) has a large, oval, smooth and vertically oriented nucleus. The nuclear membrane does not show any foldings or indentations. The chromatin is finely distributed, but forms small aggregates along the nuclear membrane and at the centrally situated nucleolus. The thin rim of cytoplasm surrounding the nucleus contains mitochondria, free ribosomes, and a few cisterns of rough endoplasmic reticulum (ER), which are narrow and show electron-lucent contents (cf. Fig. 6b).

The other cell (Fig. 5) has a horizontally oriented nucleus, with large foldings filled with cytoplasm containing mitochondria. In this cell, the cytoplasm and organelles are concentrated at the two poles. The proximal parts of the process containing mitochondria in these poles contain smooth ER and microtubuli. No synaptic contacts were found on the serially sectioned perikarya and proximal processes. The ultrastructure of the cytoplasm of these cells is very similar to that of the cells of the CP.

The cells of the second type have smaller nuclei (diameter half that of the first type in which the chromatin is condensed in rather large clumps). Cells of a third type, the largest of L I, have a big nucleus and one or two major processes which reach the marginal surface. Their ER consist of wide cisterns with a dense matrix (cf. Fig. 9a). These cells are always unlabeled. The ultrastructure of two cells from the multipolar part of the CP selected for resectoring and EM analysis closely resembled the two L I cells described at E 16.

In contrast to earlier stages, the LM-ARGs for E 18 were prepared from tangential (horizontal) sections through the hemisphere. Some ARGs of L I were resectored parallel to the initial plane of sectioning (method 1). Comparatively big, round or oval cells with a nuclelus with condensed chromatin and a central nucleolus (Fig. 7a, d) are labeled. A second type of labeled cell (Fig. 7a, b) is characterized by a large polygonal nucleus and a cytoplasm with Golgi apparatus, rough ER, and many free ribosomes. These cells appear similar to marginal glial cells, which, however, were not labeled. The big, unlabeled cells of L I described above still exist at this stage of development.

A frontal section through the multipolar part of the CP (Fig. 7e, 9c, d) shows a labeled horizontal cell, which was resectored. The nucleus is indented, with little to no aggregation of chromatin. The thin rim of cytoplasm contains few organelles, but is rich in free ribosomes. The majority of the CP cells being radially oriented were not labeled.

From E 19-20, no major changes in the cytological characteristics of labeled cells were found. A wide variety of labeled show GABA uptake at E 21 (Fig. 8a). Despite the variability in position and orientation, the typical features of labeled cells allowed most to be assigned either to glial cells or preneurons. Labeled hor-

Fig. 4. Autoradiograms of GABA-accumulating cells labeled at E 16 in L I of the medial occipital cortex. a: Labeled cell with a large nucleus in L I (arrow) and a labeled cell of the small, dark, polymorphous type (arrow). b: Labeled cell with a long, thin process containing aggregates of nuclei. c: An unlabeled cell with the same morphology as the labeled cell in a. d: An unlabeled cell with a small, round nucleus. e: A labeled cell with a large, oval nucleus. f: A labeled cell with a small, round nucleus. g: A labeled cell with a large, oval nucleus. h: A labeled cell with a small, round nucleus. i: A labeled cell with a large, oval nucleus. j: A labeled cell with a small, round nucleus. k: A labeled cell with a large, oval nucleus. l: A labeled cell with a small, round nucleus. m: A labeled cell with a large, oval nucleus. n: A labeled cell with a small, round nucleus. o: A labeled cell with a large, oval nucleus. p: A labeled cell with a small, round nucleus. q: A labeled cell with a large, oval nucleus. r: A labeled cell with a small, round nucleus. s: A labeled cell with a large, oval nucleus. t: A labeled cell with a small, round nucleus. u: A labeled cell with a large, oval nucleus. v: A labeled cell with a small, round nucleus. w: A labeled cell with a large, oval nucleus. x: A labeled cell with a small, round nucleus. y: A labeled cell with a large, oval nucleus. z: A labeled cell with a small, round nucleus.
Fig. 5. Autoradiograms of a horizontally oriented cell labeled on E18 in L1 of the medial occipital cortex. a: Light microscopy. b: Lamina I; CP = cortical plate; SV = subventricular zone. Bar = 10 μm. b-d: Single EM sections cut at a series from the right to the left of the cell in (a). b: A horizontal process containing microtubuli, polyribosomes, and membrane-bound organelles. Bar = 0.5 μm. c: A section from the middle of the cell showing the nucleus with finely condensed chromatin and only a thin rim of cytoplasm which is devoid of organelles. Bar = 1 μm. d: Silver grains in the ependymal layer. d: A section towards the pole of the cell with the end of the nucleus (n) and abundant cytoplasm containing mitochondria, microtubuli, polyribosomes, narrow ER (narrow) and a large Golgi zone (G). Bar = 1 μm.

Fig. 6. GABA-immunostaining cells above and below the cortical plate. a-b: Cells labeled on E18. c: labeled cell in L1 and diffuse immunolabeling. d: A labeled horizontally oriented cell at the bottom of the CP and a group of labeled polymorphonuclear cells in L1. e: Cells labeled on E18. f: Three labeled cells in L1. The eel of the upper COP are transversally oriented. d: A labeled horizontal cell at the bottom of the CP. At the time, labeled cells begin to appear within the CP (arrow). Abbreviations: L = Lamina I; CP = cortical plate; L2 = intermediate zone; Bar, applicable to all figures = 10 μm.
Fig. 7. GABA-accumulating cells labeled at E 18. a, b. Labeled cell in L 1, tangentially sectioned (a) and obliquely sectioned (b). E = silver grains. Note the irregular shape of the nucleus and the dense accumulation of organelles (arrows). c, d. Labeled cell in L 1, stained parallel to the section. The nucleus is oval and contains a central nucleolus (N). No indentations of the nuclear membrane were found. e, f. Labeled cells at the bottom of CP (cortex region). The arrow indicates the direction of staining of the cell above in (a, b). g, h. The nuclear membrane of the resected cell shows numerous deep indentations. The cytoplasm contains few organelles. Arrow: subplasmalemmal density. E = silver grains in the emulsion. Bar in a, x = 10 μm; b, c, f = 1 μm.

Fig. 8. GABA-accumulating cells labeled at E 21. a, L 1 and upper part of CP showing numerous labeled cells. At arrow, labeled with silver grains which is resected in (b). One cell from L 1 (not visible) has been resected. (Details from Fig. 6-7). b. EM resecting of the cell at the arrow in (a). A cell processes at electron-dense profile oriented toward the pia. The process contains numerous microsomes, narrow profiles of ER, and mitochondria. Nucleus and membrane densities (arrows) are directed toward neighboring cells, sometimes accompanied by small dense bodies. Several dense bodies are resected in the neighboring cells (similar to intermediate junctions). Bar = 0 μm. c, d. Enlargement of subplasmalemmal densities. Note vesicles in the neighborhood of density (1), and corresponding aggregation of filaments in the neighboring cell (2). Bar = 1 μm.
sensorial cells occur (Fig. 9b) in the upper part of the CP.

The perikaryon and nuclei of labeled cells often appear bigger and lighter than those of the surrounding unlabeled cells (Fig. 9a). At deeper levels, labeled and unlabeled neurons of seemingly the same developmental age are more closely packed together (Fig. 9c). Some vertically oriented GABA-C appear for the first time in the bipolar plate. Some of these cells show conspicuous ascending processes (Fig. 8a, b, and 9c) resembling apical dendrites. The processes contained numerous mitochondria, axon initial segment, and rough ER of the narrow type. The presynaptic nerve in Figure 8b did not have a descending process in the series of thin sections available from the LM-ARG. In our resections there were no obvious differences in cytoplasmic characteristics between labeled and unlabeled vertical neurons in the CP.

Surprisingly, no synapses with typical structure (e.g., postsynaptic densities, aggregated presynaptic vesicles) were found on the perikarya of labeled cells until E 21 (Fig. 9f, g), although a number occurred in the neuropil of 1. 1. However, these were mainly located on peripheral dendrites, which in the resections could not be followed back to a labeled perikaryon. It is not possible to ascertain if these were innervated or whether axosomatic synapses are missing until E 21. Densities like those seen in Figure 8c can be seen on labeled and unlabeled cells and might in some cases represent immature synapses.

Identification of postnatal GABA-C

Since it is often possible to discriminate between young neurons and glial cells at LM level, few reconstructions were performed between E 21 and P 15. In other cases, EM did not eliminate ambiguous cases or improve discrimination. The young neuron has a large oval, light nucleus without a nucleolus, whereas the glial cell appears smaller, polymorphous, and has a darker nucleus.

From P 15, some of L 1 has been serially reconstructed (Fig. 10). In this series of sections, a large neuron is heavily labeled, a small neuron and a pericyme is lightly labeled and an astrocyte is unlabeled (Fig. 10a). The labeled neuron has a cytoplasm with narrow, empty rough ER, mitochondria, a Golgi apparatus and polysomes (Fig. 10b). Two synapses were found on the part of the perikaryon included in the 3 μm section of the LM ARG (Fig. 10c). The cytoplasm of the lightly labeled neuron did not differ from that which was heavily labeled and no synapses were found in the sections analyzed.

From P 22, one large polymorphous, horizontally oriented labeled neuron of L VI was reconstructed. Labeled neurons occur fairly frequently in the lowest part of L VI (Fig. 11a, d, e). Deep injections of GABA show that labeled glial cells are prevalent in the subcortical white matter, whereas labeled neurons are isolated and rare (Fig. 11). Although a nonlabeled neuron should stand out against the relatively high labeling of this level (cf. the deep L VI in Fig. 11a and also Fig. 1), none were observed. No synapses were found on the perikaryon and dendrites of reconstructed neurons in the WM.

Using the criteria of Bradford et al. (77) to describe neuronal cell types (size and shape of the perikaryon, orientation, and position), we found that the material from young and adult animals contained labeled cells of all types with the possible exception of "cells without axons," which we could not identify positively in our ARGs. Within the young (P 15, P 22) and adult L II-L VI, only neurons to be characterized as nonpyramidal neurons, i.e., neurons lacking an apical dendrite terminating in L I (Chronwall and Wolff '78b), accumulate GABA. In Figure 12, a variety of labeled neurons are shown. They appear bipolar or polymorphous and the neuron in Figure 12D might be described as an upside down pyramidal neuron. Most of these are comparatively small, although large ones (Fig. 12D appear as well. In all of the preparations examined, none of the defined pyramidal neurons, such as seen in Figure 12, appear labeled.
DISCUSSION

Two methods are available to locate cells which could release GABA: 1) the immunohistochemical localization of glutamate decarboxylase (GAD), the GABA producing enzyme (deLange and et al., '74, and 2) the autoradiographic localization of sites of GABA accumulation (Hökfelt and Lundahl, '70, '73). There is some evidence (see Introduction) that GABA-ergic neurons accumulate GABA. However, the results of these two methods appear to differ in that GAD antibodies exclusively label neurons, while [3H]-GABA is taken up by neurons and certain glial cells (apart from other non-neuronal cells; see Materials and Methods). On the other hand, glial cells seem to have a smaller synthetic capacity for GABA (Haber and Kuroyanagi, '72) but may release GABA, which has been mostly accumulated from surrounding extracellular sources (Bowery and Brown, '72). According to the present results, glia and glial precursors seem to accumulate GABA in very early stages of development, even before a reasonable number of synapses have been formed. Therefore, it might be expected that the two methods produce some extent complementary results, especially in early developmental stages and with respect to glial cells.

Despite the spatial limitations of the various methods of GABA-application (see Fig. 2, and general comments on the methods), the fact that even neighboring cells differ in their uptake capability indicates that internal cellular factors are decisive for their labeling by [3H]-GABA. Neither the strongly nor the lightly labeled cells showed typical or even preferential positions, orientations, or fine structure. If this depends on the amount of membranes available for GABA-uptake (Iversen and Kelly, '72), these variations have to concern neurons as well as glial cells, since both cell types show variable labeling. Also, since the intensity of labeling is not due to a special cell type, unknown, perhaps transitory, states of the cells might cause the difference. Perhaps the recent "history" of uptake and/or release of GABA might influence the actual capacity for GABA uptake at the time of the experiment.

The types of cells we have described as accumulating GABA for adult neocortex of rat in a previous paper (Chronwall and Wolff, '76a) and for young rats in this paper correspond with the types shown by Hökfelt and Lundahl ('72) by the same method, at least in the superficial layers of the neocortex. There was very good agreement between the recent immunohistochemical study of GAD-rich cells by Ribak ('78) and ARG methods of identifying those neurons which were systematically not labelled, such as typical pyramidal neurons (Chronwall and Wolff, '76a, '79), which are known to contain no GAD (Ribak, '78).

The capacity for GABA uptake appears in many cells very early, i.e., about one day after they stop migrating. The first GABA+C can be labeled in the pallidal area before the first synapses have been found (temporal cortex: Kretz et al., '76; occipital cortex: Wolff, '76, '78). This confirms Lashner's ('74) findings in rat cerebellum in vitro that GABA uptake is a function of cell differentiation and does not represent a property which appears after neuronal interactions or synaptogenesis. It might therefore be asked whether the early GABA-uptake is different from that of adult cortex and whether it represents a transitory property of certain cells which appears and vanishes during various stages of differentiation. It can not be denied that the capacity to take up GABA characterizes a certain differentiation stage, as also has been suggested for the acetylcholine system (Fulton and Marschall, '71). For example, during embryonic stages, many labeled cells are found in the bipolar part of the CP. In the so-called "pyramidal plate" (Marin-Padilla, '72) some labeled cells in fact possess what could be interpreted as an apical dendrite (Fig. 8a). Since in adult brain, pyramidal neurons were not found labeled either by GABA uptake (Chronwall and Wolff, '76a) or GAD-immunohistochimistry (Ribak, '78), their GABA accumulating capacity might be transient. There are, however, other possibilities. Cell death occurring in many parts of the developing CNS (see Oppenheim et al., '78; Rager, '78) eliminates isolated cells from all layers of the neocortex of rats during pre- and postnatal stages (L.R. Wolff and H. Büttner, unpublished results). Radially oriented GABA+C might then be selectively eliminated. The portion of GABA+C (laminar and in the periphery of clusters) is similar throughout development, indicating that at least the majority of them (although not necessarily the same cells) maintain this capacity in the same positions and are not eliminated. The arrangement of these GABA+C and the fine structure of their "apical" processes are not typical for glial cells. The most likely possibility is, however, that these early GABA+C are precursors of vertical fastigial neurons, such as double bouquet neurons, which seem to be GABA-ergic neurons (Ribak, '78; Chronwall and Wolff, '76a).

It is not at all settled to what extent it is possible to discriminate between early glial
precursors and progenitors. Cytological descriptions of glioblasts, glial precursors, and young astrocytes have used the following histological markers:GFAP, S-100, and one of the various class III basic fibroblast growth factor, which are known for their specificity. This chapter has not been described for glioblasts, glioblasts, and young astrocytes (Wolff and Rickman, '77), and (e) nuclei which tend to be irregular in shape. All of these features appear very early in certain cells (Rickman and Wolff, '78). The present reaction of autoradiograms demonstrates that from R 16 until adulthood, certain GABA-C might be classified either as precursors or as glioblasts and gli precursors. Thus, the first GABA accumulating glial cells appear somewhat earlier or at the latest, together with the first astrocyte-like neurons in lamina I and the intermediate zone (König et al., '70; Wolff, '78). The glial uptake has been thought to stagnate from the extracellular space the GABA released from neurons (König and Schwarzh, '79). A more comprehensive function for gli cell has been postulated by Schrier and Thompson ('78). Gli cells have been shown to increase the accumulated GABA with an electrical stimulation and degradation by increased local concentrations of potassium (Bowry and Brown, '72; Jansen and Kelly, '75). During development, innervation of gli cells by collateral growths in which it was not found in adults (see Oppenheim et al., '78), including the oculomotor nuclei of rats on R 8. Wolff et al., '76). Since it turned out that the same type of glial cell the innervated one accumulates GABA, the gli cell release might be modulated by axons which, at least in the early developing stages of the neocortex, belong to the same neurons (Thomas, '77, '79). There is some evidence for an onset of inhibitory function of the 18 neurons after 1-2 days in vitro (Cranin and Bornstein, '74). In vivo experiments on rat show the possibility of GABA being involved in the regulation of the brain. In particular, the study of the inhibitory neurons is progressing. Corresponding results are found in kittens, where IPSPS are present during the 1st postnatal period (Purvis, '76).

Thus, several factors control the idea that the earliest GABA-C exert an inhibitory action, which is transmitted by interneuronal synapses early in the developing cortex.

All these facts raise the question as to whether GABA plays a specific role in the undifferentiated nervous system before inhibition develops. In this regard, the question arises whether a possible role for GABA was suggested in a very general manner as that of an "embryonic hormone"? Recent experiments in the adult superior cervical ganglion of rat suggest a dual role of GABA (Wolff et al., '78a, '78b), b), as a synaptic transmitter of inhibition and as a regulator of the number of potential and real postnatal synaptic sites of neurons (Wolff, '79). During ontogenesis, the latter function seems to be the primary one, which triggers the synaptogenesis.

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LITERATURE CITED


Corticopontine Visual Projections in Macaque Monkeys

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ABSTRACT

These experiments were designed to study the projections to the pons from visual and visual association cortex of monkeys by degeneration staining and horseradish peroxidase (HRP) methods. When lesions were made in these cortical visual areas, degenerated fibers were found in the rostral dorsal-lateral area of the pontine nuclei. When HRP was injected among visually responsive cells in this region of the pons, layer V cortical pyramidal cells were labeled. These labeled cells were concentrated most heavily on both banks of the superior temporal and intraparietal fissures, and on the rostral bank of the parieto-occipital fissure. The effector targets and receptive field properties of these cortical regions are consistent with their possible role in visual guidance of movement.

The cerebellar cortex of mammals receives a visual input over much of its extent. Flashes of light evoke a gross potential in the posterior vermis (Glover and Snowball, '84) and hemispheres (Fujita and Fujitani, '84) of cats. Visual inputs are relayed to the rabbit flocu-

lus by both mossy (Maekawa and Takeuchi, '73, '76) and climbing fibers (Maekawa and Simp-

sen, '72, '75). In the monkey, the firing rate of

some Purkinje cells in the anterior and poste-

rior lobes of the cerebellum may be modi-

fied by visual stimuli (Drummond, '75). Thus, every region of the cerebellar cortex may receive a visual input in one or another species of mammal.

The largest source of mossy fibers to the cerebellar cortex is cells of the pontine nuclei. It is likely that mossy fiber visual input to the cerebellum is relayed in large part by way of the pons. Sections of the middle cerebellar peduncle, which contains the axons of pontine cells, abolishes cerebellar responses to stimulation of the cerebral cortex, while section of the peduncle does not (Jansen, '71). What structures relay visual information to the pons, and where in the pons do visual fibers terminate? To date, the visual cortico-

cortico-cerebellar pathways have been described in some detail. The visual cortex (Brodal, '72a; Glickstein et al., '72; Sandnes et al., '78), the superior colliculus (Kawamura and Brodal, '73), and the ventral lateral geniculate nucleus (Graybiel, '74) project to the pons and are interconnected with the pons. Later, this was further shown to be corticopontine projection. In the case of rats, it has been shown to be a parallel projection to the pontine nuclei. The response properties of cells in two of these visual regions of the pons have been described (Baker et al., '76; Mower et al., '79). There is a growing body of evidence on the nature of pontocerebellar connections in cats from the study of retrogade transport of horseradish peroxidase (HRP) (e.g., Hoddevik '75; Brodal and Walberg, '77; Brodal and Hoddevik, '78; Burns et al., '78).

Far less is known about visual input to the cerebellum in the monkey. Sunderland ('40) and later Nyby and Jansen ('51) studied the corticopontine projection using Marchi methods. Nyby and Jansen concluded that the principal source of cortical visual projections to the pons was from visual association areas, outside of the primary striate cortex. These visual association areas appeared to project principally to the lateral part of the peduncular nucleus, the lateral pontine nucleus, and the adjacent part of the dorsolateral nucleus, throughout the rostral two-thirds of the pons. Nyby and Jansen also found a projection to the lateral caudal region of the pons following a lesion which included a portion of both banks of the superior temporal sulcus (their case 17), a region which now is known to receive