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Development of Neurotransmitter Parameters in Lateral Geniculate Body, Superior Colliculus and Visual Cortex of the Albino Rat

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The postnatal development of some neurotransmitter parameters was measured in lateral geniculate body, superior colliculus and visual cortex of the rat. The following parameters were studied: (i) high-affinity uptake of L-glutamate or D-aspartate as markers for glutamergic neurons; (ii) high-affinity uptake of GABA, which reflects both glial and neuronal uptake of GABA; (iii) HA β-alanine uptake as a marker for accumulation of GABA in glial structures; (iv) activity of glutamic acid decarboxylase which reflects GABAergic neurons; and (v) activity of choline acetyltransferase as a cholinergic marker. K_m and V_{max} were determined for high-affinity uptake of glutamate and GABA in newborn and adult animals. The possible glial influence on the uptake during development is discussed.

In lateral geniculate body and visual cortex the HA glutamate uptake showed increasing activity from birth to adulthood, whereas in superior colliculus, the uptake was higher at birth, reaching a small significant peak after 12 days of age, and was then reduced to adult level. K_m showed no such change between neonatal and adult animals. At birth, high-affinity GABA-uptake was similar to the adult level in superior colliculus and lateral geniculate body. In visual cortex, the uptake of GABA was 50% of adults. However, on day 15, the GABA uptake showed 2 to 3-fold higher activity in all regions when compared to adult level. K_m for GABA uptake in neonatals and adults differed only in lateral geniculate body. High affinity uptake of β-alanine was 50–80% lower in adults than in newborn rats. Glutamate decarboxylase activity, however, increased continuously in all 3 regions examined. This was true also for choline acetyltransferase.

INTRODUCTION

In several lower mammals the main visual centers are immature at birth, and develop gradually from birth onwards. The lateral geniculate body, superior colliculus and visual cortex in rats, rabbits and cats will hence undergo postnatal changes mainly by formation of synaptic contact zones[10,11,21,24,25,32,40,43]. This development is expected to be correlated with the development of retinal function, and to the response to light in subcortical visual centers[20].

In rabbits, the visual corticofugal fibers have already innervated lateral geniculate body and superior colliculus at birth[20,21]. The fibers to lateral geniculate body originate in layer VI, while those to superior colliculus originate in layer V of visual cortex in the rat[1]. Nevertheless, these projections are probably not yet irreversibly established, since a certain amount of plasticity is retained in the corticotectal fibers of the rat up to postnatal day 20[36]. Moreover, the distribution of synaptic input from visual cortex to superior colliculus seems to alter markedly in the upper 3 laminae in both rabbits and rats[21,32] between postnatal weeks 1 and 2, but does not seem to be the case in lateral geniculate body.

We have therefore studied the development of several neurotransmitter parameters in the 3 visual centers of the rat to see if they correlate with the postnatal anatomical changes. In particular, we have been interested in following the development of the corticofugal projections from visual cortex to lateral geniculate body and superior colliculus[18,31,37], where we have previously found evidence for glutamate as a neurotransmitter. In the cat, D-aspartate is retrogradely transported to visual cortex from lateral geniculate body but not from supe-

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rior colliculus[1]. However, the high-affinity uptake of L-glutamate is markedly reduced in lateral geniculate body and superior colliculus after visual cortex ablation in the adult rat[33]; hence we have studied this parameter as a marker for corticofugal input. As the uptake is only partially reduced in superior colliculus after visual cortex ablation, probably due to other glutamergic structures in superior colliculus, we have studied the maturation of the glutamergic input to superior colliculus after performing visual cortex ablation on different days after birth. In addition we have studied the development of GABAergic and cholinergic structures in the 3 regions. The development of GABAergic structures was followed by measuring the activity of glutamate decarboxylase (GAD)[15]. This was complemented by measurement of GABA uptake, as well as uptake of β-alanine which is a marker for glial uptake[42]. Finally, the activity of choline acetyltransferase (ChAT) was employed as a marker for the development of cholinergic fibers.

Altogether, the parameters measured reflect the maturation and the integration of glutamergic, GABAergic and cholinergic structures in the main visual centers of the rat.

MATERIALS AND METHODS

Materials

[1-^{14}C]acetyl-CoA, [3-^{3}H]β-alanine, [2,3-^{3}H(N)]-GABA, L-[3-^{3}H]glutamic acid and D-[2,3-^{3}H]aspartate were obtained from New England Nuclear, Boston. [1-^{14}C]glutamic acid, and [U-^{14}C]glutamic acid were obtained from the Radiochemical Centre, Amersham. Membrane, filters, pore size 0.45 μm were purchased from Millipore. White Wistar rats of both sexes were obtained from Møllergaard, Denmark.

Ablation of visual cortex

Young rats were anesthetized with ether (until day 19), while adults were anesthesized with valium/Hypnorm Vet. Visual cortex, as described in ref. 27, was aspirated with the aid of a Pasteur pipette fitted to a water-jet. Rats (9-12, newborn and adults) were operated on 6 or 7 days prior to the high-affinity uptake assay, and of these, 6 satisfactory lesions from macroscopical examination (i.e. complete removal of areas 17, 18 and 18a as well as undisturbed white matter) were selected for the assay.

Preparation of tissue and dissection

The animals were rapidly killed by cervical dislocation and the brain taken out in a cold room (4 °C). The dorsal part of the lateral geniculate body (LGB) was dissected out from 600 μm thick horizontal slices as defined in König and Klippel[29]. In adults the total weight of the dissected tissue was about 6 mg. The superior colliculus was also dissected out from 600 μm slices and the total weight of tissue was about 12 mg in adults. Areas 17, 18 and 18a of occipital cortex, as defined by Krieg[27], were taken as visual cortex. In rats up to 12 days of age, the caudal third of cortex was used as visual cortex.

The tissue was homogenized in 0.32 M sucrose in a Teflon glass homogenizer (10 strokes/900 rpm); the final tissue concentration was approximately 2% (w/v). In some cases the homogenate was centrifuged at 20,000 *g* for 60 min and the pellet resuspended in the original sucrose volume.

High-affinity uptakes

The high-affinity uptakes of L-glutamate, D-aspartate, GABA and β-alanine were performed in a shaking bath at 25 °C in 0.5 ml modified Krebs-phosphate buffer solution (15 mM Tris–HCl, 140 mM NaCl, 5 mM KCl, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 1.2 mM Na_2HPO_4 and 0.2% glucose (w/v)) pH 7.4, freshly oxygenated with 95% O_2: 5% CO_2. Samples, homogenate or resuspended pellet, containing 10–30 μg protein were preincubated for 20 min in a shaking bath. Incubation time was 3 min for L-glutamate, D-aspartate and GABA uptake, and 5 min for β-alanine uptake. Final concentration of [^{3}H]L-Glu or D-Asp was 10^{-7} M. The uptake was terminated by adding 10 ml 0.9% NaCl containing 0.5% bovine serum albumin (w/v), at room temperature, immediately followed by vacuum-filtration through prewashed Millipore filters (fitted in a Millipore Manifold). The filters were washed once with additional saline/BSA solution (5 ml). Blank values were obtained by incubating samples in 0.32 M sucrose on ice. Under these conditions the uptake was linear with tissue concentration in the range examined. Protein measurements were performed according to Lowry et al.[30].

Enzyme assays

Homogenates were treated with 0.25% (v/v) Triton X-100 to release enzyme activity. Choline acetyltransferase was determined as described by Fonnum et al.[13]. GAD was assayed by the CO_2 trapping technique previously described by Fonnum et al.[15].

Determination of K_m and V_{max} for HA glutamate and GABA uptake

The uptake studies were performed with tissue homogenates in the same manner as described above. The substrate concentrations, however, ranged from 0.6–10 μM to 0.1–5 μM for glutamate and GABA uptake, respectively. K_m and V_{max} were determined from linear regression of Lineweaver–Burk plots.

RESULTS

HA glutamate uptake

The uptake was studied in detail in brain homogenate, as well as samples from a resuspended pellet containing synaptosomes. In visual cortex and lateral geniculate body the development of the high-affinity uptake of glutamate increased continuously (Figs. 1 and 2) and reached an adult level of activity on days 15 and 20, respectively. In 2-day-old rats the uptake was about 30% of the adult level.

In contrast, in superior colliculus the uptake of glutamate was 20% higher at birth than in adults (Fig. 4). The activity showed a significant peak on day 12, and then levelled off to reach the adult level of activity from day 20, onwards. The same developmental time-course was also found when D-Asp was employed as substrate (not shown).

Maturation of the glutamergic input from visual cortex to superior colliculus was studied by unilateral aspiration of visual cortex in neonatal rats. We found a marginal decrease in L-Glu uptake when assayed before day 15. However, on days 15–16 we found a dramatic decrease in uptake on the lesioned side. Thereafter, the decrease remained at the level observed in adults, i.e. 30–40%. This was also observed with D-Asp as substrate.

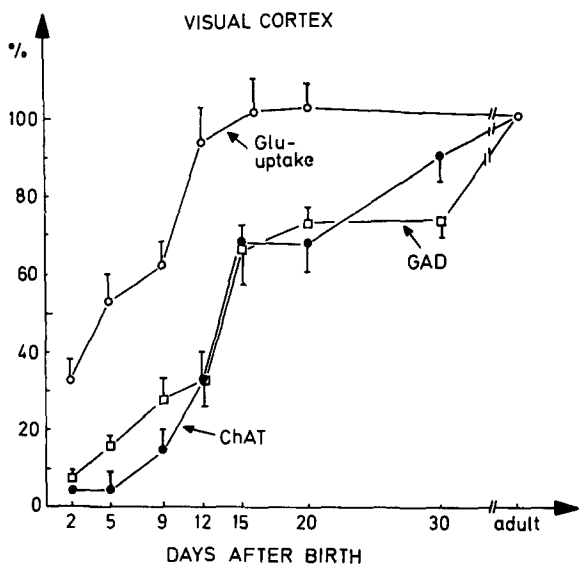

Fig. 1. The postnatal development of high-affinity uptake of L-glutamate (Glu-uptake), glutamic acid decarboxylase (GAD) and choline acetyltransferase (ChAT) in the rat visual cortex. Each point on the curve is expressed as percent of adult level and represents the mean value from 5–7 separate experiments. Adult levels are (mean ± S.D.): high affinity L-glutamate uptake, 86.5 ± 10.5 pmol/mg protein/3 min; GAD, 100.1 ± 5.4 nmol/mg protein/h; ChAT, 52.4 ± 1.2 nmol/mg protein/h.

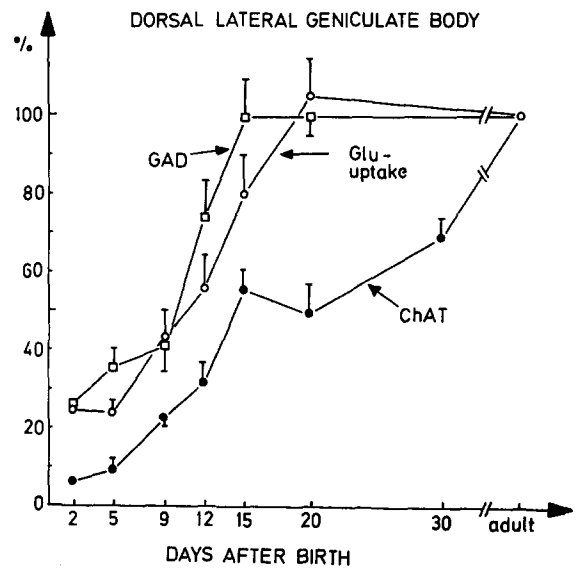

Fig. 2. The postnatal development of high-affinity uptake of L-glutamate (Glu-uptake), glutamic acid decarboxylase (GAD) and choline acetyltransferase (ChAT) in a homogenate from dorsal lateral geniculate body of the rat. Each point on the curve is expressed as percent of adult level and represents the mean value from 5–7 experiments. Adult levels are (mean ± S.D.): high-affinity L-glutamate uptake, 180.7 ± 44.8 pmol/mg protein/3 min; GAD, 79.8 ± 8.45 nmol/mg protein/h; ChAT, 56.1 ± 4.0 nmol/mg protein/h.

TABLE I

K_m and V_{max} determined for high-affinity uptake of L-glutamate in lateral geniculate body (LGB), superior colliculus (SC) and visual cortex (VC)

The values are obtained from Lineweaver–Burk plots, and are based on 5 separate experiments, each using 5 different substrate concentrations. K_m is expressed in μM, V_{max} as cpm/μg protein. S.D. never exceeded 12%.

	K_m			V_{max}		
	LGB	*SC*	*VC*	*LGB*	*SC*	*VC*
Two days old	7.1	5.6	2.6	376	642	337
Adults	5.2	3.5	3.9	3535	310	2312

The percentage decrease in L-Glu was not significantly different when assayed either in crude homogenate or resuspended pellet. In homogenates from adults the uptake on the lesioned side was 67 ± 5 (n = 9) as compared with the unlesioned side, while it was 74 ± 4 (n = 9) when assayed in resuspended pellet.

Apparent K_m and V_{max} values were determined in neonatal and adult rats (Table I). The K_m values did not change significantly during development. The changes in V_{max} found were in principle similar to the changes shown in Figs. 1, 2 and 4. There was, however, a large difference in superior colliculus where the V_{max} in the newborn rat was 207% of adult level (Table I), compared to 120% adult level when measured at 0.1 μM substrate.

ChAT-activity

ChAT, being presumably the best neuronal marker for cholinergic fibers, increased postnatally in all 3 regions (Figs. 1, 2 and 3). The activity on postnatal day 2 was less than 10% of that in adults. Hence, in all cases, ChAT increased more than GAD and high-affinity uptake of glutamate from birth onwards. Between days 15 and 20, ChAT did not increase in either of the 3 structures. Adult level of ChAT activity was not fully reached before 30 days.

GAD-activity, HA uptake of GABA and β-alanine

GAD is localized to GABAergic neurons in CNS, and its activity describes the development of these. During postnatal development of lateral geniculate body, superior colliculus and visual cortex, GAD increased progressively. In lateral geniculate body GAD activity reached adult level on day 15, whereas in visual cortex and superior colliculus the activity

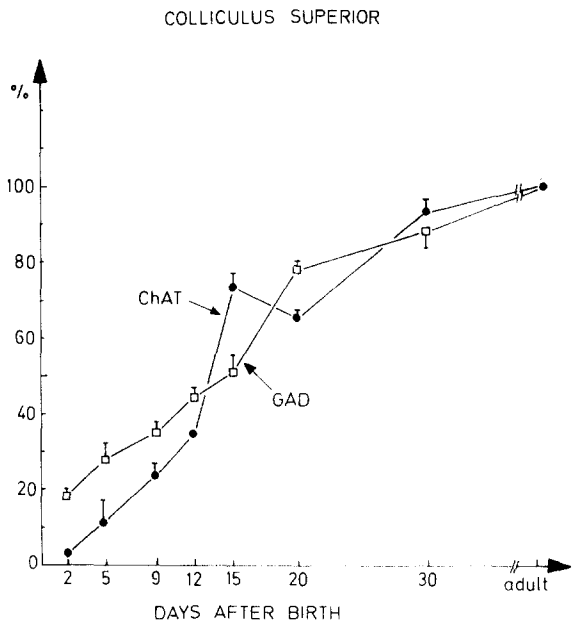

Fig. 3. The postnatal development of glutamic acid decarboxylase (GAD) and choline acetyltransferase (ChAT) in a homogenate from superior colliculus of the rat. Each point on the curve is expressed as percent of adult level and represents the mean value from 5–7 separate experiments. Adult levels were (mean ± S.D.): GAD, 202.6 ± 9.7 nmol/mg protein/h; ChAT, 49.4 ± 7.2 nmol/mg protein/h.

was not fully developed after 30 days.

GABA has been shown to have a high-affinity transport system in both glia and neurons. The uptake was measured on postnatal day 2 and 15, and in adult rats, and it differed from the postnatal changes in GAD (Table II). Two days after birth, the GABA uptake in visual cortex was 50% of the adult level, whereas in superior colliculus and lateral geniculate body the uptake at birth was similar to the adult activity when measured at 0.1 μM. Interestingly, GABA uptake showed a 2- to 3-fold

TABLE II

The high-affinity uptake of GABA (final substrate concentration 10^{-7} M) measured on postnatal days 2 and 15, and in adult rats (2.5 months) in lateral geniculate body, superior colliculus and visual cortex

The results represent mean ± S.D. from 5 separate experiments and are expressed as pmol/mg protein/3 min.

	2 days old	*15 days old*	*Adults*
Superior colliculus	20.1 ± 2.1	58.8 ± 4.7	17.4 ± 0.6
Lateral geniculate body	6.9 ± 2.1	23.7 ± 1.6	7.7 ± 0.9
Visual cortex	8.7 ± 0.7	30.4 ± 3.3	17.8 ± 2.5

TABLE III

K_m and V_{max} determined for high-affinity uptake of GABA in lateral geniculate body (LGB), superior colliculus (SC) and visual cortex (VC)

The values are obtained from Lineweaver–Burk plots, and are based on 5 separate experiments, each using 5 different substrate concentrations. K_m is expressed in μM, V_{max} as cpm/μg protein. S.D. never exceeded 15%.

	K_m			V_{max}		
	LGB	*SC*	*VC*	*LGB*	*SC*	*VC*
Two days old	1.5	2.1	1.4	120	488	33
Adults	5.4	2.4	2.2	295	510	592

TABLE IV

The high-affinity uptake of β-alanine (final substrate concentration 10^{-7} M) measured in 2-day-old and adult (2.5 months) rats in lateral geniculate body, superior colliculus and visual cortex

The results represent mean ± S.D. from 5 separate experiments, and are expressed as pmol/mg protein/3 min.

	Lateral geniculate body	*Superior colliculus*	*Visual cortex*
Two days old	1.03 ± 0.16	1.48 ± 0.20	0.40 ± 0.06
Adults	0.28 ± 0.03	0.32 ± 0.04	0.21 ± 0.03

higher activity in all 3 regions on day 15 and was 171, 308 and 338% when compared to adult levels in visual cortex, lateral geniculate body and superior colliculus, respectively. The K_m measured for GABA uptake differed significantly only in lateral geniculate body when comparing neonatal and adult rats (Table III). Here it increased about 4 times. When based on V_{max} measurements, the values for the 2-day animal was lower than measured at 0.1 μM. Thus the 2-day values were 40%, 100% and 5% of the adult level for lateral geniculate body, superior colliculus and visual cortex respectively.

HA uptake of β-alanine was higher in neonatal animals in the 3 cases (Table IV). The decrease during development was 50, 70 and 80% in visual cortex, lateral geniculate body and superior colliculus, respectively. β-alanine uptake is assumed to reflect GABA uptake into glial cells, and the decreasing β-alanine uptake may reveal relatively lesser importance of such an uptake in adult animals.

DISCUSSION

The present study confirms earlier indications that visual structures are immature in the newborn rat. Interestingly, the corticofugal pathways from visual

cortex to lateral geniculate body and superior colliculus do not appear to follow the same innervation pattern during development. Our findings will be discussed in relation to the morphological description of changes during the postnatal development of visual structures in the rabbit and rat.

Holländer et al.[21] conclude that in rabbits, at the time of birth, the corticofugal projection from visual cortex has already reached the lateral geniculate body, and probably does not give rise to additional cortical fibers postnatally. But few synapses are found in lateral geniculate body in neonatal rats[25]. During development, groups of synapses in lateral geniculate body have been suggested to mature at different stages of development[16,40], and the synaptic contacts formed are believed to be axo-dendritic[3,26]. Ablation of visual cortex in the adult rat leads to a 75% fall in the high-affinity uptake of glutamate[33]. However, when performed in neonatal rats, no such effects could be observed in the lateral geniculate body (unpublished observation). If the uptake is mainly localized to nerve terminals, the results support the notion that these axons have not yet formed synaptic complexes. Hence, the glutamate uptake present at this time cannot be accounted for by cortical fibers or terminals. Glutamate uptake increased progressively after parturition. In the 2-day-old rats, glutamate uptake in lateral geniculate body was only 25% of adult level. This activity probably reflects glutamatergic interneurons with high-affinity uptake mechanisms already developed, glio-somal uptake in our tissue preparation, or axonal uptake. The increase during development could then be due to the maturation of glutamergic nerve terminals from visual cortical input to lateral geniculate body. High-affinity glutamate uptake[14], ChAT and GAD are considered to be neuronal markers. Hence, our findings that all 3 parameters increased from birth onwards, correlates with the increasing number of synapses. A similar postnatal development of GAD activity in lateral geniculate body has also been observed by others[34].

Similarly, increasing postnatal density of synaptic formations has been shown in regions of the cerebral cortex of the rabbit[11,43], the cat[10] and the rat[4,24]. In the rat, at the end of gestation, cortical synapses are sparse and confined to the molecular layer, deep in the cortical plate[39]. Marked postnatal changes are found in all layers of visual cortex in the rat[44,45]. Vrensen et al.[43] state an increase in the ratio (terminal formation/number of neurons), suggesting that development of synaptic contact zones plays the dominating role in maturation of visual cortex, more than further formation of neurons. The glutamate uptake reached adult level of activity already at day 15 in the visual cortex. Obviously, there are also more pronounced regional differences in the development of glutamergic fibers. In striatum, after 4 weeks, the synaptosomal uptake of glutamate is only 80% of adult level, and is therefore not yet fully developed[5,46]. In whole rat, cortex glutamate uptake has been found to develop differently in different subgroups of synaptosomes[19]. Surprisingly, GAD and ChAT were not fully developed after one month, although the number of synapses in visual cortex seem to reach a peak at this period. The increasing number of synapses during development correlate with the increasing number of synaptosomes in the rat during development in the rat brain[8].

It is not possible to predict which neurons are responsible for the accumulation of glutamate in the visual cortex from our development studies. There are several candidates, such as the homolateral cortical association fibers and cortico-cortical projections from one visual cortex to the contralateral one, as described in the rat[37] and the rabbit[21,22]. Interneurons could also be responsible for the uptake activity.

Superior colliculus develops differently from visual cortex and lateral geniculate body. In the rabbit superior colliculus there seems to be a small overshoot of visual cortical input between day 10 and 15 after birth. In the rat superior colliculus neuronal death occurs in the optic layer until day 8 after birth[17]. Interpretation of work by Lund and Lund[32] might suggest a transient increase of excitatory nerve terminals in the superior colliculus of the rat. These results interdigitate with our findings that the high-affinity glutamate uptake shows a small but significant peak on postnatal day 12, and then levels off to reach adult level of activity on day 20. Based on other experiments, 30–40% of the glutamate uptake in the superior colliculus in adult rats can be related to input from visual cortex[33] (Fig. 4). Unilateral ablation of visual cortex on days 6 or 8

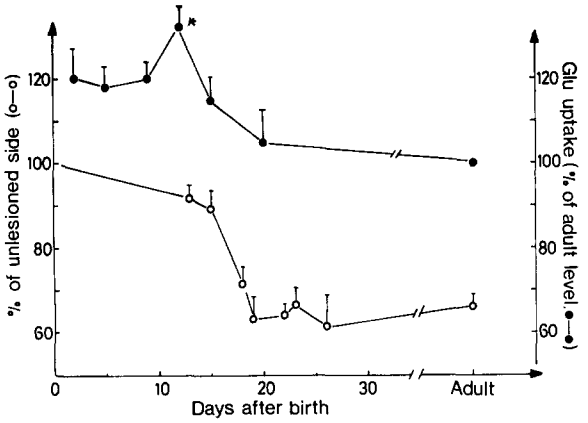

Fig. 4. The postnatal development of high-affinity uptake of L-glutamate (or D-Asp) (●—●), and the effect of unilateral ablation of visual cortex on the same activity (○—○), in superior colliculus. Each point on the upper curve is expressed as percent of adult level, and represents the mean value from 5–7 separate experiments. The adult level was (mean ± S.D.): 37.3 ± 5.4 pmol/mg protein/3 min with [^{3}H]L-Glu as substrate, and 68.4 ± 6.2 pmol/mg protein/3 min with [^{3}H]D-Asp as substrate ($P < 0.04$ vs 9 days and $P < 0.01$ vs 15 days). Each point on the lower curve is expressed as percent activity as compared to the superior colliculus on the unlesioned side, and represents the mean value from 6 animals. Only animals with satisfactory cortical ablation were assayed. Lesions were introduced 6 or 7 days prior to assay.

has only a marginal effect on the uptake of glutamate when assayed 6 or 7 days later. In contrast, when visual cortex was aspirated on subsequent days, we observed a similar decrease as in adults. Assuming that the effect of this type of lesion is near maximally developed after 3 days[33], we can estimate that the corticofugal fibers to superior colliculus matures around day 12. This coincides with the peak of glutamate uptake at this time (Fig. 4). It is also interesting to note the correlation with initiation of retinal function. Injection of kainic acid, which exerts toxic action on cell bodies and dendrites[9], suggests that interneurons account for most of the remaining glutamate uptake in the superior colliculus[13]. Thus, glutamatergic interneurons seem to mature far earlier in superior colliculus of the rat than the corticofugal fibers. The conspicuous high glutamate uptake in newborn rat superior colliculus, could be influenced by glia also. Both ChAT and GAD increased continuously after birth, and we consider this to reflect maturation of cholinergic and GABAergic nerve terminals.

The high-affinity uptake of GABA, in all 3 cases, showed a marked peak on day 15, which is in contrast to GAD. This is in line with other reports for uptake studies performed in sucrose homogenates[8] or resuspended pellets (P_2)[46] from different regions, but contradicts findings from GABA-uptake performed in cortical slices[23]. Nevertheless, HA uptake of β-alanine, which is associated with the glial accumulation of GABA[41,46], was higher at birth than in adult animals in all 3 structures. This is also true for uptake of β-alanine in whole rat brain[40] and striatum, but not frontal cortex[46]. At present our explanation is that glial cells are responsible for the main part of early postnatal changes in high-affinity GABA uptake. In superior colliculus GABA is mainly localized in interneurons, particularly in the upper layers[38], or in GABAergic fibers from substantia nigra to superior colliculus in the rat[6,13]. Both structures will probably also contribute to the GABA uptake and GAD activities during development. In visual cortex of the rat, specific GAD neurons (possibly stellate cells) are found[41], and GABA could be an interneuronal inhibitory transmitter[1,7].

Kinetic measurements of the high-affinity uptake will probably be influenced by the changes in neuronal and glial compartmentations and the endogenous levels of biogenic amino acids. We find it difficult to elaborate on different uptake mechanisms.

In conclusion, the development of neuronal transmitter markers in superior colliculus, visual cortex and lateral geniculate body parallels the morphological maturation of nerve terminals. The present study also suggests that the maturation of glutamate uptake in superior colliculus and lateral geniculate body can in part be accounted for by visual cortical input.

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