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Research Report

Optic nerve transection affects development and use-dependent plasticity in neocortex of the rat: Quantitative acetylcholinesterase imaging

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ABSTRACT

We investigated the effects of neonatal optic nerve transection on cortical acetylcholinesterase (AChE) activity in hooded rats during postnatal development and following behavioral manipulation after weaning. AChE reaction product was quantified on digitized images of histochemically stained sections in layer IV of primary somatic sensory, primary visual and visual association cortex. Rats with optic nerve transection were compared to sham-operated littermates. In all cortical regions of both types of animal, AChE reaction product was increased to peak 2 weeks after birth and decreased thereafter, reaching adult levels at the end of the third postnatal week. During postnatal development, reaction product in primary visual cortex was lower in rats deprived of retinal input than in sham-operated littermates and the area delineated by reaction product was smaller. However, optic nerve transection did not modify the time course of postnatal development or statistically significantly diminish adult levels of AChE activity. Behavioral manipulations after weaning statistically significantly increased enzyme activity in sham-operated rats in all cortical areas examined. Compared with cage rearing, training in a discrimination task with food reward had a greater impact than environmental enrichment. By contrast, in the rats with optic nerve transection enrichment and training resulted in statistically significantly increased AChE activity only in lateral visual association cortex. Our findings provide evidence for intra- and supramodal influences of the neonatal removal of retinal input on neural activity- and use-dependent modifications of cortical AChE activity. The laminar distribution of the AChE reaction product suggests that the observed changes in AChE activity were mainly related to cholinergic basal forebrain afferents. These afferents may facilitate the stabilization of transient connections between the somatic sensory and the visual pathway.

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Abbreviations: AChE, acetylcholinesterase; ANOVA, analysis of variance; BFA, basal forebrain afferents; CHAT, choline acetyltransferase; CO, cytochrome oxidase; LP, lateral posterior nucleus of the thalamus; NMDA, N-methyl-D-aspartate; OD, optical density; ONT, optic nerve transection; P, postnatal day (the day of birth is designated P 0); S1 cortex, primary somatic sensory cortex; TCA, thalamocortical afference; V1 cortex, primary visual cortex

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1. Introduction

Brain plasticity and compensation for the loss of peripheral sensory input have long been recognized as important aspects of brain function (Forel, 1907). Positron emission tomography (Sadato et al., 1996; Büchel et al., 1998) and functional magnetic resonance imaging (Melzer et al., 2001; Burton et al., 2002) have shown that when people with severe visual disabilities read Braille with their fingertips areas in occipital cortex are activated that process visual information in sighted people. These findings constitute a remarkable example of the brain's capacity to reorganize without identifying the neural basis of the occipital activation. In the present study, we examined whether modulating inputs could play a role in the crossmodal reorganization of neocortex in rats deprived of retinal input by optic nerve transection (ONT) on the day of birth (P 0).

As one candidate for modulating inputs, cholinergic innervation has been recognized to profoundly influence the development of neural connections in the peripheral and the central nervous system (Changeux and Danchin, 1976; Rasmusson, 2000). Cholinergic innervation appears to be essential for long-term potentiation mediated by NMDA receptors in the developing primary visual (V1) cortex (Kirkwood et al., 1995, 1999) and is known to facilitate neural responses in primary somatic sensory (S1) cortex (Dykes, 1997). Activity-induced changes of neural receptive fields do not occur when cholinergic mechanisms are compromised in S1 (Dykes and Lamour, 1988; Jacobs and Juliano, 1995; Sachdev et al., 1998; Verdier and Dykes, 2001), V1 (Bear and Singer, 1986) and primary auditory cortex (Kilgard and Merzenich, 1998). Conversely, such changes are facilitated by the intra-cortical administration of acetylcholine (Ego-Stengel et al., 2001).

Based on these findings, we used rats with neonatal ONT and sham-operated littermates to measure the density of the acetylcholinesterase (AChE) reaction product copper thiocholine on sections through flattened cortex stained with a modification of Koelle's method (Geneser-Jensen and Blackstad, 1971) as an indicator of AChE activity (Storm-Mathisen, 1970). We examined how the density and the areal extent of the reaction product evolves during postnatal development in S1 and V1 cortex as well as three regions in visual association cortex. Furthermore, we examined whether the effects of ONT on cortical AChE activity could be countermanded by behavioral manipulations after weaning. That is, we compared local densities and expanse of copper thiocholine in deprived and sham-operated rats reared in standard cages, exposed to enriched environments or trained in a behavioral discrimination task. In the task, the rats were rewarded for correct choices with food maintaining them in a higher state of arousal than in the enriched environment where the animals were left to explore without any particular focus or in the standard cages without any cues. We included S1 cortex in our analysis, because AChE activity in this region may reflect differences in whisker use between rats with ONT and their sham-operated littermates.

2. Results

Table 1 lists the number of rats examined in each experimental group and the number of cases used for quantification of AChE reaction product.

2.1. *The effectiveness of optic nerve transection*

After the rats were euthanized and perfused, the brains were removed from the skull for histochemistry and the exposed optic nerves were examined under a surgical microscope. In the rats with ONT, the epineuria of the optic nerves did not contain nerve fibers to any visible extent at ≥P 11. The nerves had shrunk to half of their normal diameter and appeared dark gray as though they were devoid of myelin. The lateral geniculate nucleus could not be used to examine the effects of the deafferentation on the nuclei of termination. The diencephali were damaged occasionally when the cortical hemispheres were separated to produce preparations of flattened cortex. However, the superior colliculus was not harmed by the removal of the hemispheres and transverse sections stained for both cytochrome oxidase (CO) and AChE activity were fully suitable to ascertain the effectiveness of ONT in each case. Fig. 1 shows typical results at P 11. Consistent with the findings of others (CO: Sukekawa, 1987; Zhang et al., 1996; AChE: Hess, 1960), the superficial gray layer was distinctly less stained and shrunk to about half in thickness in the animal with ONT compared with its sham-operated littermate.

2.2. *S1 cortex*

2.2.1. *Appearance*

Cytoarchitectonic units called 'barrels' (Woolsey and Van der Loos, 1970) represent the whiskers on the snout topographically

Table 1 – Number of animals subjected to optic nerve transection (Deprived) and sham-operations (Sham-operated) grouped by their subsequent treatment and quantitative assessment

Type:	Deprived						Sham-operated					
Age (weeks)	Standard		Enriched		Trained		Standard		Enriched		Trained	
	total	OD[a]	total	OD	total	OD	total	OD	total	OD	total	OD
1	5	3					5	3				
~2	7	4					7	6				
3	5	4					5	4				
≥8	8	5	7	5	7	4	8	6	7	5	7	4

[a] Number of rats used for optical density measurements.

in layer IV of S1 cortex. In rats, the barrels corresponding to the long mystacial whiskers are composed of perikarya-dense centers separated by perikarya-sparse septa (Rice, 1985). In CO preparations, the centers stain dark whereas septa stain light (Remple et al., 2003). Fig. 2 depicts the changes in AChE and CO reaction product across flattened cortex in sham-operated rats (left 3 columns) and littermates with ONT (right 3 columns) between the first and the third postnatal week. Already 1 week after birth (P 6) the reaction products of both enzymes were prominently increased in barrel centers and the topographic representation of the whiskers on the snout was strikingly evident. AChE reaction product diminished greatly in the barrel centers over the following 2 weeks in both types of animal. However, elevated AChE reaction product persisted over the septa and an inverse pattern of staining emerged. In contrast, barrel centers remained densely stained with CO reaction product, and this complementarity was unchanged even at 2 years of age regardless of ONT.

Examples of the laminar distribution of the reaction products of both enzymes across the thickness of S1 cortex are shown in Fig. 3. As in preparations of flattened cortex, in coronal sections the barrels stand out as puffs of reduced AChE reaction product (Fig. 3B; white arrow) that co-localize with puffs of increased CO reaction product (Fig. 3D; white arrow). The puffs appear flame-shaped with greatest width in layer IV narrowing into layers II and III and are separated by radial bands of denser AChE reaction product that overlap with the septa low in CO reaction product. Narrow radial bands of increased AChE reaction product also segment layer V and are distinct in the sham-operated animal (Fig. 3A; white arrowheads). Densitometry across the cortical thickness showed that AChE reaction product was distributed in a three-pronged profile with peaks in layer I, deep layer IV, and deep layer V (Figs. 3A, B and profile; asterisks). ONT did not alter the cortical depths of the peaks (Fig. 3B, profile), but diminished their magnitude (in Fig. 3 by on average 13%). The segmentation in layer V faded and a narrow band of denser reaction product was noticeable at the border with layer IV (Fig. 3B and profile; black arrowheads). CO reaction product also peaked in layers I, IV and V, except the peak in layer IV was situated more superficially and appeared unaffected by ONT (Figs. 3C, D and profile).

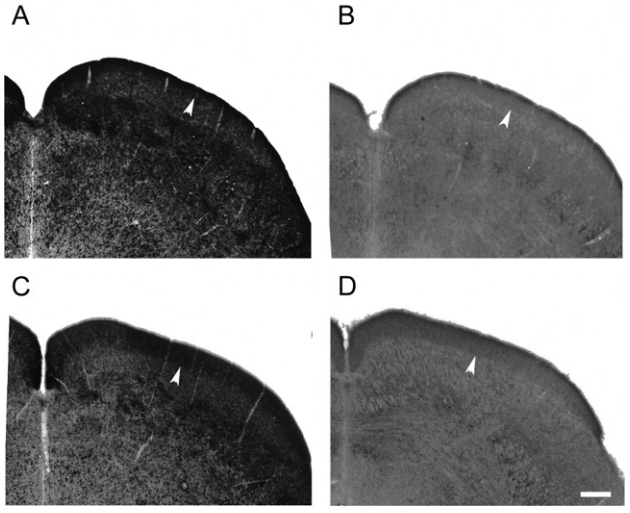

Fig. 1 – Optic nerve transection affects the superior colliculus. Micrographs of adjacent 40-μm-thick transverse sections through the mesencephalon stained for AChE (A, C) or CO (B, D) reaction product from a rat deprived of retinal input by optic nerve transection on P 0 (A, B) and a sham-operated littermate (C, D) both euthanized on P 11. Retinotectal afferents terminate in the superficial gray layer (arrowheads). In deprived rats, this layer was half as thick as in sham-operated littermates (dorsal is up and lateral is right; the calibration bar is 500 μm).

2.2.2. *Barrel size*

The size of the region in S1 cortex chosen for AChE densitometry on sections through flattened hemispheres was used to assess barrel growth. This region comprised the barrels representing the three caudal arcs of the three middle rows of whiskers and the straddlers between them (Fig. 6).[1]

ONT did not affect the postnatal growth of these barrels statistically significantly (Fig. 7A). One week and 2 weeks after birth the least squares means of the examined barrel area were within ±5% in deprived and sham-operated rats and they were only 8% larger in the sham-operated rats at the end of the third postnatal week, though the barrels had grown on average 1.7-fold since the first week. After weaning, neither environmental enrichment (Fig. 4) nor training in a discrimination task (Fig. 5) produced visible differences in barrel size compared to rats reared in standard cages. Training produced the greatest measured increases, i.e. 7 and 13% in deprived and sham-operated rats, respectively (Fig. 7A). However, these increases did not attain statistical significance.

2.2.3. *AChE activity levels in layer IV of S1 cortex*

We limited the quantitative assessment of AChE activity to cortical layer IV because of the presence of morphological landmarks and the prominence of AChE reaction product. Moreover, this layer is the main recipient of sensory input and we anticipated that our manipulations would affect its AChE activity. In both sham-operated rats and littermates deprived of retinal input, AChE reaction product increased 1.4-fold within 2 weeks after birth and decreased 1.6-fold during the third postnatal week (Fig. 8A). The increase did not reach statistical significance in the deprived rats, but was statistically significant in the sham-operated rats ($P=0.03$). In contrast, the decrease was statistically significant in both deprived and sham-operated rats at $P=0.0003$ and $P=0.003$, respectively. Though the increase and decrease occurred concomitantly in both types of animal, it is noteworthy that the concentration of AChE reaction product was lower in the sham-operated than in the deprived animals at the beginning of the 2-week period and higher in the end (Fig. 8A).

[1] The long caudal whiskers on the snout are arrayed in five rows that Woolsey and Van der Loos (1970) named A (dorsal) to E (ventral) and numbered in rising order beginning with 1 at the caudal end. Like-numbered whiskers form an arc. In addition to the whiskers in the rows, four whiskers named α to δ straddle the rows caudally. The barrels in S1 cortex are named in topographic correspondence.

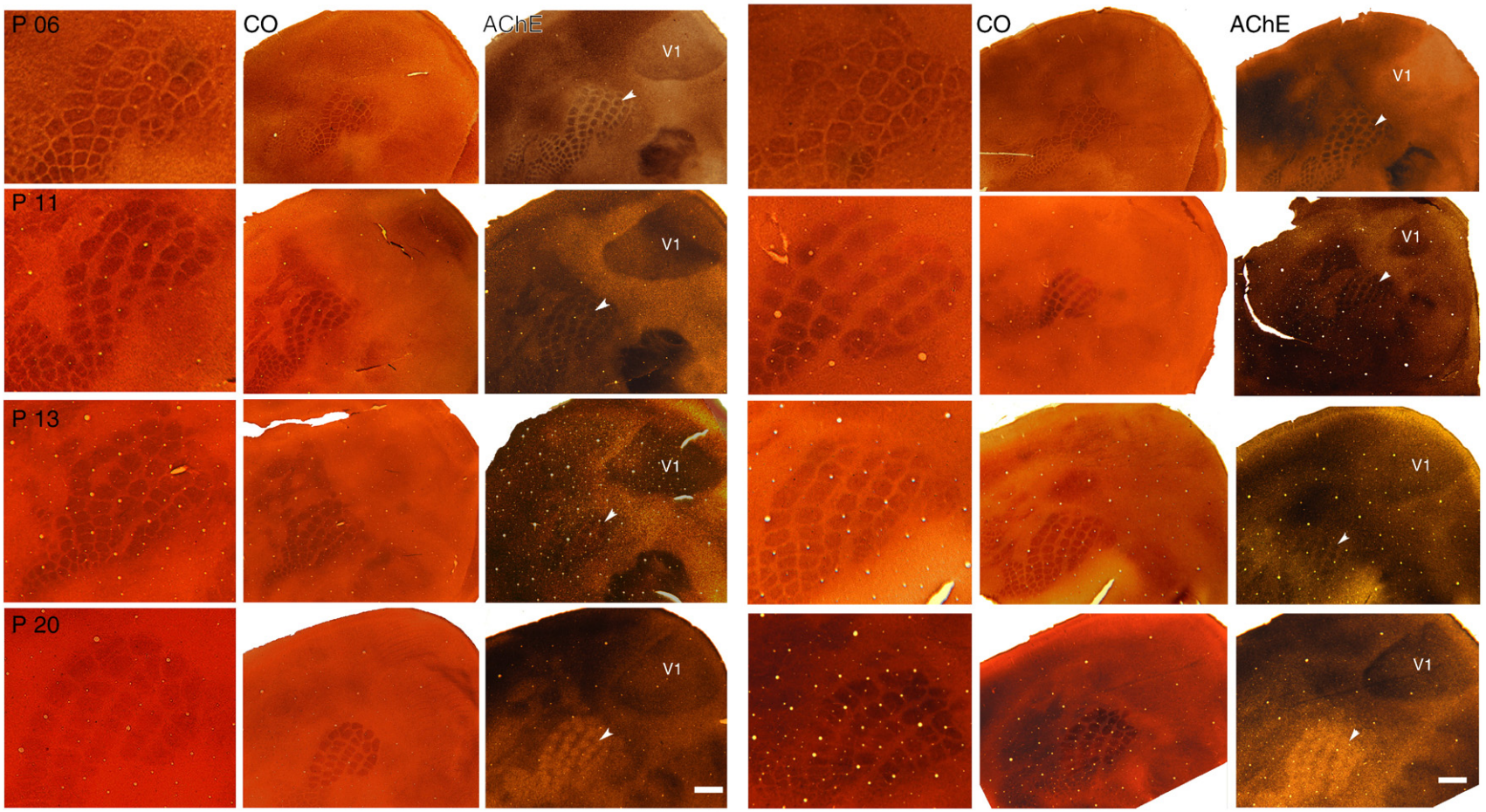

Fig. 2 – Cortical CO and AChE activity during postnatal development. Micrographs of 40-mm-thick sections cut parallel to the pia through flattened cortex of sham-operated rats (columns 1–3 from left to right) and littermates deprived of retinal input by optic nerve transection (columns 4–6) on P 0 at P 6, P 11, P 13 and P 20. The first and the fourth column depict enlargements of the barrel field from the sections stained for cytochrome oxidase activity shown in the second and the fifth column (CO). The patches dense in reaction product are the centers of barrels in layer IV of primary somatic sensory cortex separated by lightly stained septa. The third and the sixth column show adjacent sections stained for acetylcholinesterase activity (AChE). The arrowheads point at the barrel that represents whisker B1. Reaction product prominently labeled barrel centers from P 6 to P 13, but was drastically diminished at P 20, rendering the septa salient. Primary visual cortex (V1) was densely stained in both sham-operated and deprived rats at all ages (medial is up and rostral is to the left; the calibration bars represent 500 mm in columns 1 and 4 and 1000 mm in the other columns).

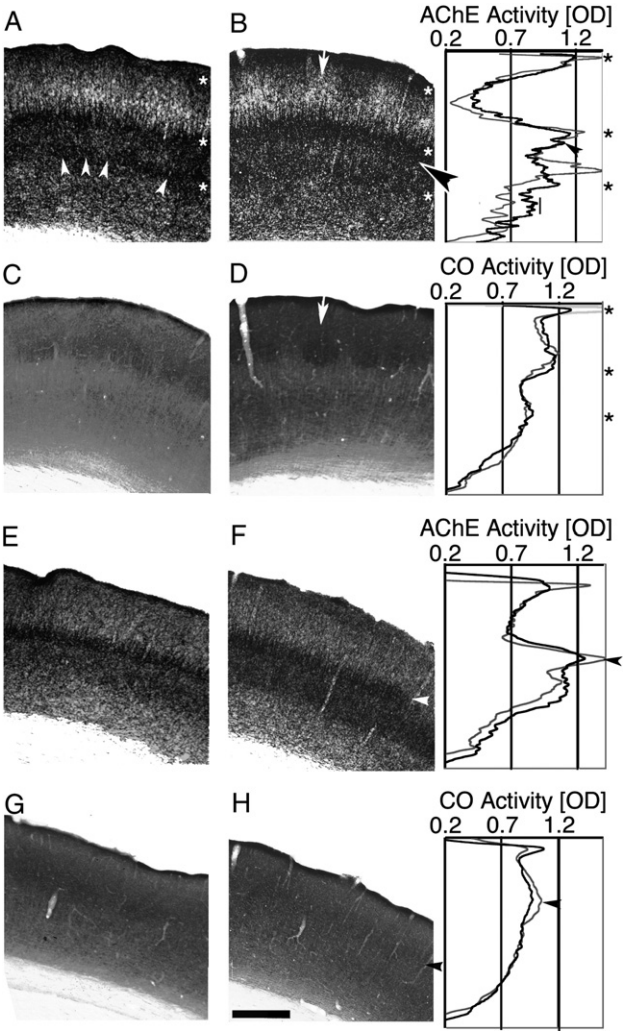

Fig. 3 – Laminar distribution of AChE and CO activity in S1 and V1 cortex. Micrographs from adjacent 40-μm-thick coronal sections stained for AChE (A, B, E, F) or CO activity (C, D, G, H) from S1 (A–D) and V1 cortex (E–H) of a sham-operated adult animal (A, C, E, G) and a littermate deprived of retinal input by optic nerve transection on P 0 (B, D, F, H). Laminar OD profiles of reaction product are shown in the right column (sham-operated: gray; deprived: black). In S1 cortex, the profiles were sampled from septum to septum through the barrel labeled by the arrow. The asterisks indicate the location of the peaks of AChE activity in layers I, IV and V. In V1 cortex, the profiles were sampled along the entire depicted cortex and arrowheads indicate the location of the peaks of AChE (F) and CO (H) activity in layer IV (pia is up; the calibration bar is 500 μm).

Manipulation of behavior after weaning affected AChE activity in S1 cortex of both types of animal in opposed directions (Table 2). Standard cage rearing decreased reaction product in sham-operated rats (Table 2A) and increased reaction product in the deprived rats (Table 2B) by 22%. Moreover, enrichment and training increased AChE reaction product in sham-operated rats statistically significantly by 47% ($P=0.03$) and 45% ($P=0.0005$), respectively, compared with standard cage rearing. By contrast in the deprived rats, reaction product was reduced with enrichment (15%) and training (23%). Consequently, the differences in AChE reaction product between deprived and sham-operated rats attained statistical significance after enrichment and training at $P=0.02$ and $P=0.01$, respectively.

2.3. *V1 cortex*

2.3.1. *Appearance*

In sections through flattened cortex stained for CO activity, V1 cortex can be recognized as a wedge-shaped area of increased reaction product in layer IV situated caudal and medial to

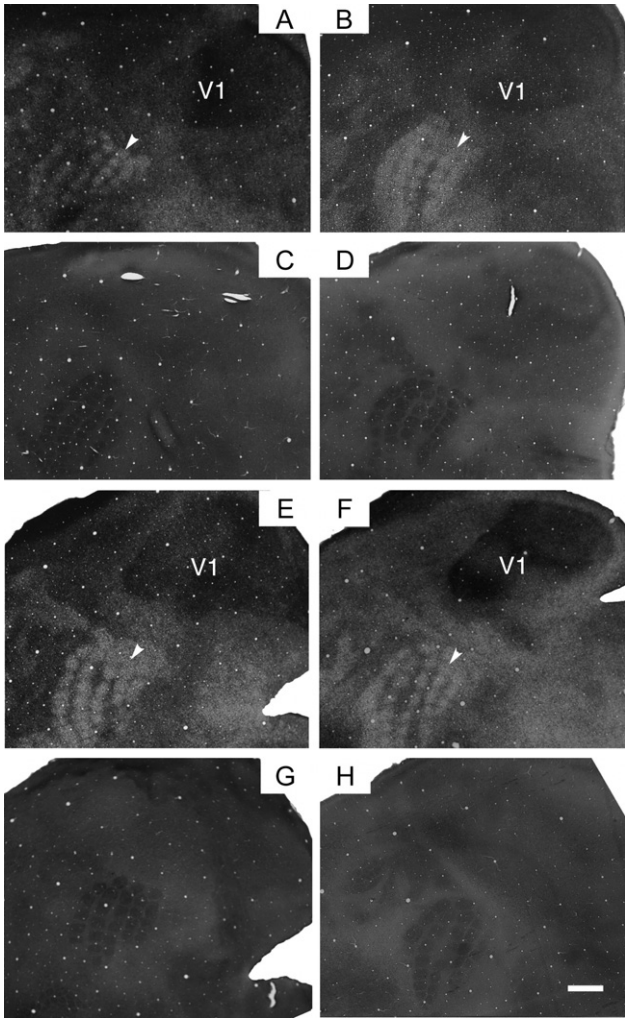

Fig. 4 – Environmental enrichment. Micrographs from adjacent sections stained for AChE (A, B, E, F) or CO activity (C, D, G, H) through flattened cortex from sham-operated rats (A–D) and rats deprived of retinal input (E H). The rats were exposed to an enriched environment (A, C, E, G) or reared in standard cages (B, D, F, H) after weaning. As in weanlings (P 20; Fig. 2), barrel centers are distinct as negative (AChE) or positive (CO) impressions (arrowheads point at barrel B1) and V1 cortex (V1) is marked prominently by elevated AChE reaction product. Enrichment increased AChE reaction product in S1 cortex of the sham-operated animals (A) compared with cage rearing (B) (medial is up and rostral is left; the calibration bar is 1000 μm).

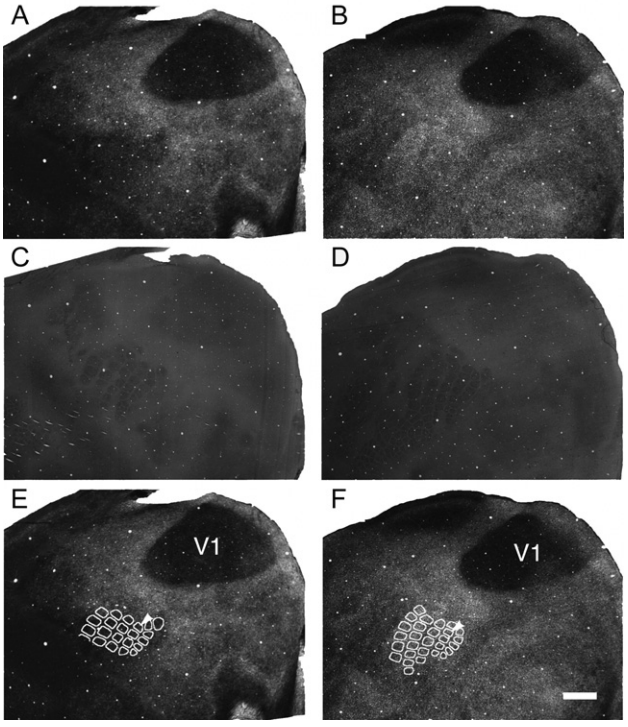

Fig. 5 – Behavioral training. Micrographs from adjacent sections stained for AChE (A, B, E, F) and CO (C, D) activity from a sham-operated rat (A, C, E) and a rat deprived of retinal input (B, D, F). After weaning, the animals were trained to distinguish between 2 objects with food rewards. The bottom row micrographs (E, F) depict the same AChE sections shown in the top row (A, B) overlaid with the outlines of the barrels seen in the CO sections (C, D). The arrowheads point at barrel B1. AChE reaction product in the barrels appears lower in the deprived animal. Brightness has been increased rostral and lateral of the barrels to enhance contrast. AChE reaction product in V1 cortex (V1) was elevated and remarkably uniform in both types of animal whereas CO activity was low (medial is up and rostral is left; the calibration bar is 1000 μm).

barrel cortex (Remple et al., 2003). In the present study, dense AChE reaction product delineated V1 cortex in both sham-operated and deprived rats already 1 week after birth (Fig. 2). The area's wedge shape became readily distinguishable ≥P 11. In comparison, CO reaction product was low in our preparations and the outline of the area was distinct only on occasion (Fig. 2). Across the cortical thickness, AChE reaction product was densest in layer IV with smaller peaks in layers I and V (Figs. 3E, F and profile), closely matching the laminar distribution of the acetylcholine-synthesizing enzyme choline acetyl transferase (CHAT; see Fig. 12 in Eckenstein and Baughman, 1987). CO reaction product peaked in layers I and IV (Figs. 3G, H and profile). ONT resulted in lower levels of reaction product for both enzymes at peak without altering the eminence of layer IV staining (Figs. 3F, H, and profiles; arrowheads).

2.3.2. *Size of V1 cortex*

The AChE-stained wedge in layer IV representing V1 cortex grew statistically significantly 1.4-fold from 4.9 mm^2 to 6. 9 mm^2 between the first and the third postnatal week in sham-operated rats (Fig. 7B; $P=0.004$) and 1.5-fold from 4. 2 mm^2 to 6. 1 mm^2 in the rats deprived of retinal input with ONT (Fig. 7B; $P= 0.006$). One week after birth, the difference between sham-operated and deprived rats did not reach statistical significance. However, statistical significance was attained 2 weeks after birth ($P=0.002$) when this region was 29% smaller in the deprived than in the sham-operated rats. Taken together, growth was slower in the deprived rats and fell short of that in the sham-operated rats. V1 cortex never caught up in size, remaining 15% (median) smaller across all ages examined, and the difference between deprived and sham-operated rats was statistically significant ($P=0.001$; one-tailed, paired *t*-test). After weaning, V1 cortex maintained its marked appearance in AChE preparations (Figs. 4 and 5). Compared with standard cage rearing, the greatest increase in size, i.e. 16%, was found in both types of animal after training but did not reach statistical significance (Fig. 7B). In contrast, V1 cortex of deprived rats was reduced by 12% after enrichment, and the difference to sham-operated rats was statistically significant ($P\leq0.03$).

2.3.3. *AChE activity in layer IV of V1 cortex*

As in S1 cortex, AChE activity in layer IV of V1 cortex peaked 2 weeks after birth. During the second postnatal week, AChE reaction product increased 58% in the rats deprived of retinal input and 73% in the sham-operated littermates and then dropped 16% and 20%, respectively, during the third postnatal week (Fig. 8E). The increases were greater than in S1 cortex and statistically significant in both types of animal ($P\leq0.03$). The decreases were smaller and did not attain

Table 2 – Changes in AChE activity in deprived (A) and Sham-operated (B) rats produced by behavioral manipulations after weaning

Region	Cage-reared	Percent change		Rank
		Enriched	Trained	
(A) Deprived				
S1[a]	22	−13	−23	1
V1	3	1	−3	3
Anterior	2	12	−3	3
Lateral	−8	22	16	2
Medial	1	7	0	5
Median	2	7	−3	
(B) Sham-operated				
S1[a]	−22	47	45	1
V1	−9	11	24	4
Anterior	−11	18	21	3
Lateral	−5	4	10	5
Medial	−17	22	36	2
Median	−11	18	24	

Changes are presented as mean percent differences using AChE activity at P 20 (cage-reared) or AChE activity of cage-reared adults (enriched and trained) as reference.
The medians gauge the effect of the behavioral manipulation and the ranks the degree to which each cortical region was affected.
[a] Statistically significantly different from AChE activity in all other regions at $P<0.002$ in pairwise comparisons with Bonferroni correction.

statistical significance. In sham-operated rats reared in standard cages after weaning, AChE reaction product was 9% lower than in the weanlings (Table 2B). However, reaction product increased 11% and 24% with enrichment and training, respectively. The latter increase was statistically significant ($P=0.0006$). By contrast, behavioral manipulations had no significant effect on AChE activity in V1 cortex of deprived rats and the difference in enzyme activity between the two types of animal attained statistical significant with training ($P=0.02$).

2.4. *AChE activity in visual association cortex*

AChE reaction product was measured in layer IV of the three regions of visual association cortex adjacent to V1 cortex (area 17 of Krieg, 1946) depicted in Fig. 6: an anterior region situated in transitional cortex between V1 and S1 cortex (area 7 of Krieg, 1946), a medial region in rostral area 18 (Krieg, 1946) and a lateral region in rostral area 18a (Krieg, 1946). Since the boundaries of these regions were not as histologically defined as V1 cortex in our preparations, the sizes of the areas of measurement were only included in the statistical analyses of global effects, and no attempts were made to compare differences between pairs of regions. Furthermore, none of the differences in AChE reaction product in the three regions attained statistical significance during postnatal development. In contrast, behavioral manipulations resulted in statistically significant alterations and the noteworthy differences are detailed below.

2.4.1. *Anterior visual association cortex*

The changes in AChE reaction product in anterior visual cortex (Fig. 8B) appeared as an amalgam of the changes observed in the primary sensory areas (S1: Fig. 8A; V1: Fig. 8E). One week after birth, AChE reaction product was at the level found in the barrels. This level was maintained in rats deprived of retinal input, but rose to a peak in their sham-operated littermates 2 weeks after birth. In both types of animal, AChE reaction product decreased during the third postnatal week to levels observed in V1 cortex. Consistent with the changes in AChE activity in V1 cortex, standard cage rearing had negligible effects on the deprived rats (Table 2A) and diminished AChE reaction product in their sham-operated littermates 11% (Table 2B). Compared with standard cage rearing, enrichment increased reaction product 12% and 18% in deprived and sham-operated rats, respectively. Moreover, in the latter training increased reaction product 21% compared with cage rearing. This difference was statistically significant ($P=0.002$) also when compared with trained rats deprived of retinal input ($P=0.05$) in which a small reduction in AChE reaction product (3%) was observed. The pronounced increase in the sham-operated rats and the small diminution in the deprived rats matched the changes in AChE activity found in V1 and S1 cortex (Fig. 7).

2.4.2. *Medial visual association cortex*

In medial visual cortex, the changes in AChE reaction product in both types of animal matched those observed in V1 cortex with a peak 2 weeks after birth (Fig. 8D). Behavioral manipulations after weaning had no marked effect on reaction product

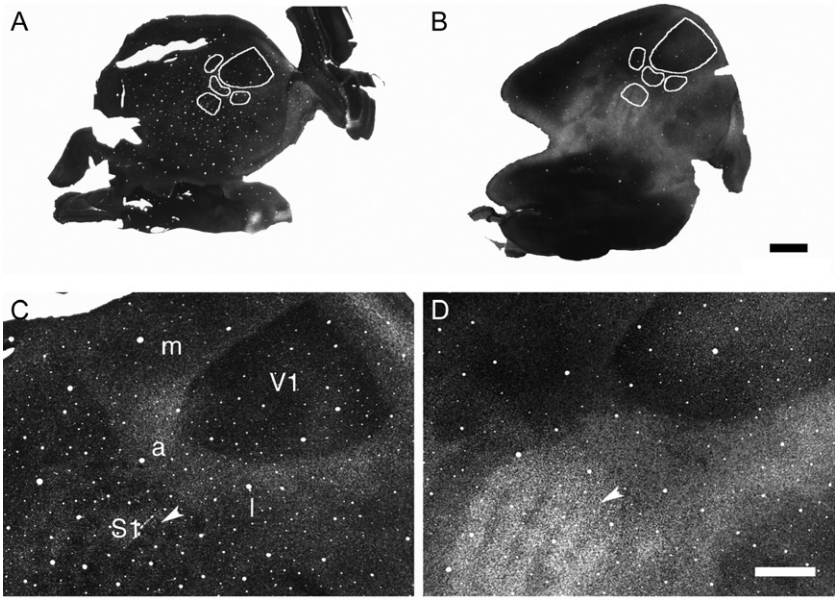

Fig. 6 – Maps of the cortical regions used for densitometry of AChE reaction product. Typical preparations used for densitometry are shown from rats at P 13 (A, C) and P 20 (B, D). ODs were measured in layer IV of 5 regions. The region in S1 cortex (S1) comprised barrels B1–3, C1–3, D1–3, β and γ. In V1 cortex, measurements were taken from the entire wedge-shaped area of increased reaction product (V1). In visual association cortex, 3 regions were chosen anterior (a), medial (m) and lateral (l) of V1 cortex. The 5 regions are identified on the same preparations at low (A, B; outlines) and high magnification (C, D; letters). At ≥P 20, barrel centers stained light, the boundaries were fuzzy, and the region of measurement was determined on adjacent sections stained for CO. The arrowheads point at barrel B1 [orientation: medial is up and rostral is left; calibration bars are 2000 μm (top) and 1000 μm (bottom)].

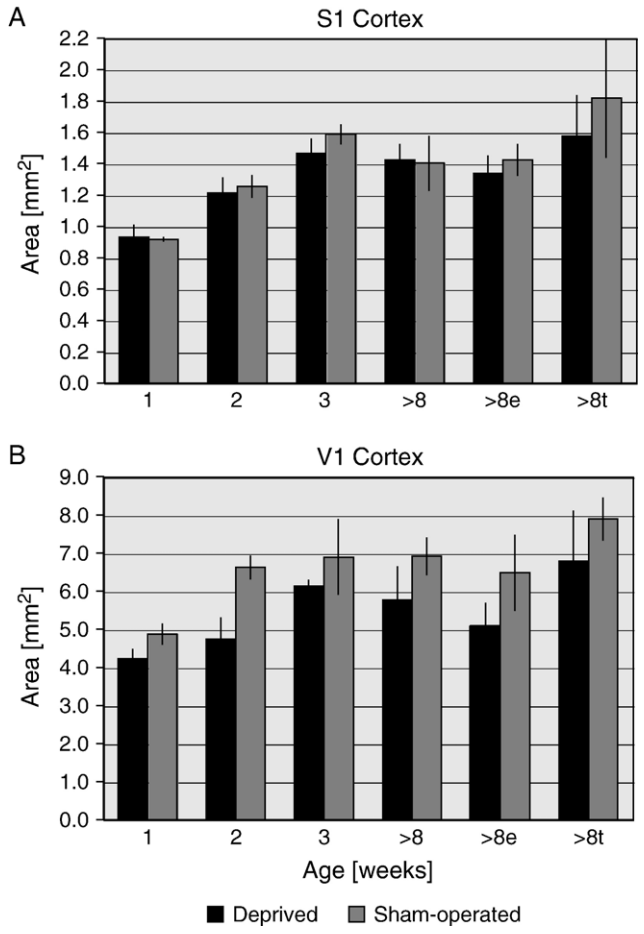

Fig. 7 – Areal extent of AChE activity in S1 and V1 cortex. The sizes of the regions measured in S1 (A) and V1 (B) cortex are shown for rats deprived of retinal input (Deprived) and sham-operated rats (Sham-operated) during postnatal development (1, 2 and 3 weeks) and after rearing in standard cages (>8), enrichment (>8e) and training (>8t) (columns—means; bars—standard errors of the mean). Whereas barrel size developed equally in both types of animal, V1 cortex was always smaller in deprived rats. Though both primary sensory areas were prominently enlarged in the trained rats, the enlargement did not attain statistical significance.

in the rats deprived of retinal input (Table 2A). In sham-operated rats, cage rearing led to 17% less reaction product than in weanlings. Enrichment and training increased reaction product by 22% and 36% compared with cage-rearing (Table 2B). Because of the low levels of enzyme activity with cage rearing, the difference with training attained the greatest statistical significance of all pair-wise comparisons ($P=0.0001$) and reached statistical significance also in comparison with trained deprived rats ($P=0.02$). These were the largest differences related to behavioral manipulations in any visual area.

2.4.3. *Lateral visual association cortex*

In sham-operated rats (Fig. 8C), AChE reaction product in lateral visual association cortex changed in accord with the change observed in V1 cortex (Fig. 8E), though the differences were less pronounced. The increase during the second week after birth was 17% and the decrease during the third week was 14%. In the rats deprived of retinal input, reaction product did not alter within the first 2 weeks after birth remaining 10% below the peak reached in sham-operated rats. During the third postnatal week, reaction product diminished with similar increment as in the sham-operated rats. Rearing in standard cages decreased reaction product in deprived and sham-operated rats 8% and 5%, respectively. However, compared with cage rearing enrichment and training increased reaction product in the deprived rats by 22% and 16%, respectively (Table 2A), and these differences were statistically significant ($P<0.05$). In sham-operated rats, enrichment and training increased reaction product in increments of ~5% (Table 2B), resulting in a statistically significant difference only between trained and cage-reared animals ($P=0.009$).

2.5. *Global comparison of cortical AChE activity levels*

Some trends were common among the five cortical regions examined. One week after birth, AChE reaction product was greater in the pups deprived of retinal input than in their sham-operated littermates, except in V1 cortex. However, the median AChE reaction product across all ages was 12% lower in deprived rats than in sham-operated littermates and this difference was statistically significant ($P=0.04$; one-tailed, paired *t*-test). Regardless of presence or absence of retinal input, AChE reaction product in the five cortical regions examined peaked 2 weeks after birth, falling to comparable levels in the third postnatal week. In sham-operated rats, standard cage rearing resulted in a median global diminution of reaction product by 11% compared with weanlings whereas in the deprived rats such decrease was found only in lateral visual association cortex. Behavioral manipulations after weaning offset the decrease in sham-operated animals, elevating reaction product in the five cortical regions. The greatest increases occurred with training. In contrast, the behavioral manipulations had substantially smaller effects on the deprived rats. Only one region, i.e. lateral visual association cortex, showed statistically significantly increased AChE reaction product. Therefore, the presence of retinal input was crucial for the behavioral manipulation-induced increases in AChE activity in three of four regions examined in visual cortex. It is noteworthy, however, that even in the cortical region most affected by the removal of retinal input, i.e. V1 cortex, AChE reaction product persisted into adulthood at the level observed in weanlings.

3. Discussion

Evidence from a host of studies correlates cortical AChE activity with the strength of extrinsic and intrinsic cholinergic innervation (Storm-Mathisen, 1970; Mesulam et al., 1983, 1991; Eckenstein and Baughman, 1987; Kristt, 1987; Tsai et al., 2002). After methodological considerations, we discuss the implications of our findings for the possible role of cholinergic input in cortical plasticity during postnatal development and its modification by environmental enrichment and behavioral training.

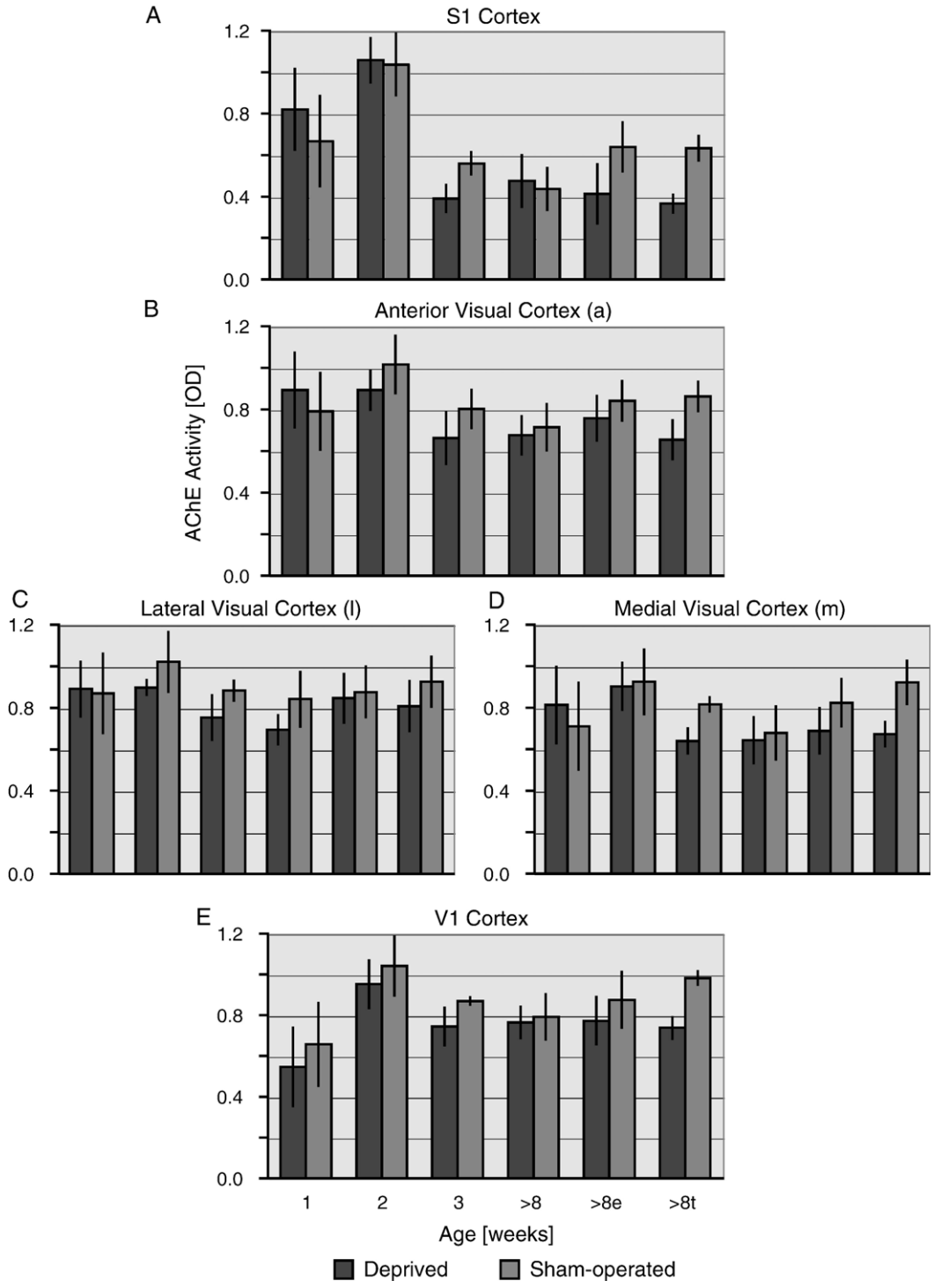

Fig. 8 – Local AChE activity in cortical layer IV. ODs of AChE reaction product are plotted for the 5 cortical regions examined (A–E) (columns—means; bars—standard errors of the mean). The abscissa is labeled as in Fig. 7. The regions are identified in Fig. 6. Note the peak of enzyme activity 2 weeks after birth and the differential effects of behavioral manipulations.

3.1. *Methodological considerations*

Local cortical AChE activity was determined by measuring the mean optical density (OD) of copper thiocholine over morphologically defined regions in digitized images of brain tissue sections stained with the method of Geneser-Jensen and Blackstad (1971). We only used preparations in which the barrels representing the three caudal arcs of whiskers in rows B, C and D in S1 cortex were complete and the reaction product over the wedge of V1 cortex was densest to ensure that the

measurements were sampled from layer IV, though this selection diminished the sample sizes (Table 1). To reduce inter-subject variability, histochemical reactions were carried out on sections from matched pairs of deprived and sham-operated littermates, and only ODs from such pairs were used in paired *t*-tests. ODs were averaged over whole regions of interest regardless of inhomogeneities in reaction product. Hence even if reaction product diffused, the result would reflect a robust average for the entire region. In addition, the ODs measured in brain tissue were calibrated against a staircase of standard ODs reducing apparatus-based measurement error.

Although our densitometry of AChE reaction product provides an indirect measurement of the strength of cholinergic innervation, this method was chosen because the relationship between reaction product and enzyme activity has been shown to be linear (Andreasen et al., 1989). The low goodness of fit between model and measured enzyme activity in our analyses of variance suggests that the variance of the measurements was not fully accounted for by the assessed variables (see Supplement). Variables that were uncontrolled in our procedure include differences in staining intensity among batches and differences in the quality of staining among sections. Tissue sections from all groups of animals could not be processed in the same batch. Yet, the reproducibility of our method was sufficient to detect statistically significant regional differences in AChE activity attributable to optic nerve transection and behavioral manipulations.

3.2. *Postnatal development of cortical AChE activity*

3.2.1. *Growth in size of S1 and V1 cortex*

During the first 3 postnatal weeks, the barrels in S1 cortex of rats deprived of retinal input by ONT grew at similar rate as the barrels in sham-operated rats. We observed no difference in size between the two types of animal in disagreement with observations in mice in which absence of retinal input led to barrel enlargement (Bronchti et al., 1992; Rauschecker et al., 1992). The differing findings may be related to the smaller size of the murine neocortex such that shrinkage of one sensory representation may more directly affect another modality. By contrast, V1 cortex was consistently smaller in the deprived rats measuring 6.1 mm^2 at 3 weeks of age as opposed to 6.9 mm^2 in the sham-operated littermates. These measurements are in good agreement with 5.6 mm^2 observed in microphthalmic rats by Sugita and Otani (1985) and 7. 1 mm^2 reported for sighted hooded rats by Espinoza and Thomas (1983).

3.2.2. *AChE activity levels in S1 and V1 cortex*

The peak of AChE activity measured in both primary sensory areas 2 weeks after birth is consistent with qualitative descriptions by others of rodent S1 (Kristt, 1979; Sendemir et al., 1996) and V1 cortex (Robertson et al., 1987, 1988; Hanes et al., 1992). The peak appears to be the result of transient enzyme expression within the fields of termination of thalamocortical afferents (TCAs) that originate in the thalamic relays of the primary ascending pathways, i.e. the ventral posterior medial nucleus in the somatic sensory pathway (Kristt, 1979) and the lateral geniculate nucleus in the visual pathway (Robertson et al., 1988). However, the TCAs themselves are not cholinergic (Robertson et al., 1988). Rather, the cholinergic input of basal forebrain afferents (BFAs) appears to provide the main source of acetylcholine (Mesulam et al., 1991; Kimm et al., 1995; Tsai et al., 2002). Cholinergic BFAs originate from a subpopulation of neurons in the nucleus basalis of Maynert/Ch4 (Mesulam et al., 1983; Bear et al., 1985; Kristt et al., 1985; Robertson et al., 1998; Siciliano et al., 1999; Gritti et al., 2003). More than half of the CHAT activity in adult rat neocortex has been attributed to these afferents (Kristt, 1987).

In rat S1 cortex, barrels develop during the first postnatal week (Rice and Van der Loos, 1977), and the critical period for the plasticity of barrel morphology concludes with the emergence of the barrels (Van der Loos and Woolsey, 1973; Jeanmonod et al., 1981; Melzer et al., 1993). Yet, in the present study AChE activity reached the highest level only 1 week after the critical period for barrel development ended. During this time, intracortical (Miller et al., 2001; Bender et al., 2003) and cortico-cortical (Ivy and Killackey, 1982) connections continue to evolve. A dependence of this process on inputs from the sensory periphery has been demonstrated (McCasland et al., 1992), and neural responsiveness to whisker stimulation is particularly great (Melzer et al., 1994). These and the present findings suggest that acetylcholine is not necessary for gross barrel development, but may play a role in the fine-tuning of cortical circuitry by sensory experience. In accord, the stable AChE activity observed in V1 cortex of the rats deprived by ONT after weaning may indicate that this region retained a degree of plasticity beyond this point in time. This interpretation is consistent with the findings that AChE activity in V1 cortex matures well into the second month after neonatal enucleation (Hanes et al., 1992; Kimm et al., 1995) and ocular dominance domains remain sensitive to monocular deprivation (Fagiolini et al., 1994). The origin of the cholinergic input related to the remaining AChE activity could be intrinsic, since cholinergic bipolar interneurons have been found uniformly distributed in all cortical layers using CHAT immunohistochemistry (Eckenstein and Baughman, 1987). In addition, multipolar cells scatter across the cortical thickness that stain for AChE, but not CHAT, and are thus not cholinergic. However, the laminar profiles of AChE activity observed in the present study suggest BFAs as the crucial source. This contention is supported by the observation in barrel cortex that AChE activity is profoundly depleted at P 6 after BFA lesions in neonates (Fig. 3; Ricceri et al., 2002). Only small amounts of reaction product remain in barrel centers.

3.2.3. *Mechanisms underlying the effects of ONT on V1 cortex*

It is important to emphasize that in the rats with ONT V1 cortex developed without neural activity related to retinal input. In the presence of this input, ocular dominance domains are known to evolve through the strengthening of synapses that transmit synchronized activity from the same eye (Goodman and Shatz, 1993). Cholinergic (Greuel et al., 1988; Kirkwood et al., 1999; Siciliano et al., 1999), as well as adrenergic (Greuel et al., 1988; Kirkwood et al., 1999; Siciliano et al., 1999) and serotonergic inputs (Gu and Singer, 1995) have been shown to facilitate synapse strengthening, possibly by altering the threshold for long-term potentiation (Bear et al., 1987). Nerve growth factor released by cells with muscarinic

acetylcholine receptors may play an instrumental role in this process (Pesavento et al., 2000). M1 and M2 muscarinic acetylcholine receptors are most likely candidates (Levey, 1996). The laminar distribution of AChE activity we found in V1 cortex closely matches that of muscarinic M2 receptors observed in rats (Levey et al., 1991) and primates (Tigges et al., 1997). Non-invasive imaging of M2 receptors (Podruchny et al., 2003) may, therefore, provide a tool to assess the strength of cholinergic innervation in occipital cortex of people with congenital visual disabilities as an indicator of the potential for plasticity.

3.2.4. *Mechanisms underlying the effects of ONT on S1 cortex*

As in V1 cortex, the laminar profile of AChE reaction product in S1 barrel cortex matched the layer-specific distribution of terminations of cholinergic BFAs (Kristt, 1979; Sachdev et al., 1998; Zhu and Waite, 1998). In analogy with V1 cortex, BFAs are known to influence S1 cortex maturation (Zhu and Waite, 1998; Ricceri et al., 2002) by facilitating the strengthening of synapses of synchronously active sensory inputs (Erzurumlu and Kind, 2001) in concert with serotonergic and adrenergic modulators (Osterheld-Haas et al., 1994; Welker et al., 1996). In the present study, the absence of retinal input affected AChE activity in S1 cortex. Though AChE activity in the deprived rats peaked at similar magnitude and at the same time as in sham-operated littermates, i.e. 2 weeks after birth, the activity of the enzyme was higher before that time and lower after, suggesting that the cholinergic innervation of S1 cortex was modified. Retinal input may have affected the development of cholinergic BFAs directly via corticofugal connections with nucleus basalis (Saper, 1984) or indirectly via the basal ganglia (Grove et al., 1986; Faull et al., 1986).

3.3. *Effects of behavioral manipulation on AChE activity in S1 and V1 cortex*

Increases in neural dendritic lengths, branches, spines and synapses in neocortex have been related to behavioral challenges (Volkmar and Greenough, 1972; Klintsova and Greenough, 1999). Compared with rearing weanlings in standard cages, environmental enrichment and training resulted in statistically significant differences in AChE reaction product between deprived and sham-operated rats, providing further evidence that use-dependent sensory input can influence cortical AChE activity. In the object discrimination task, deprived and sham-operated rats were trained to perform with equal success and timing (Mineo et al., 2002). Hence, differences in cortical AChE activity between the two types of animal are unlikely related to differences in performance. Rather, they may reflect differences in whisker use (Berg et al., 2005). The rats were housed under standard care conditions for months after each behavioral manipulation and yet the effects were persistent.

We observed an enhancement of AChE activity in S1 and V1 cortex after enrichment and training, but only in sham-operated rats. Rats with ONT showed negligible change in enzyme activity in V1 cortex and decreased enzyme activity in S1 cortex. Therefore, the enhancement of AChE activity required the presence of retinal input. Bartoletti et al. (2004) demonstrated that dark rearing may extend the critical period for ocular dominance plasticity. However, the critical period was drawn to a close when the animals were dark-reared in an enriched environment. Our finding that AChE activity in V1 cortex was not altered when rats with ONT were exposed to an enriched environment suggests that retinal activity may be necessary in conjunction with the sensory stimulation added by enrichment.

Training boosted enzyme activity in V1 cortex of sham-operated rats more effectively than enrichment. This difference may be anticipated, since the object discrimination task contained visual cues in addition to the tactile experience. Moreover, the animals were more aroused than in the enriched environment in anticipation of the food reward, complemented by an up-regulation of the cholinergic system. This interpretation is supported by the observation that rats with neonatal BFA lesions spent less time exploring spatially re-arranged objects than controls without lesions (Ricceri et al., 2002). Furthermore, cholinergic input is known to facilitate whisker-evoked neural responses in the somatic sensory and motor pathways (Berg et al., 2005; Masri et al., 2006) commensurate with the state of arousal, influencing the rats' ability to control coordinated whisker movement (Berg et al., 2005), maintain attention and use whiskers for tactile discrimination (Dunnett et al., 1991). In accord, Sachdev et al. (2000) observed that removal of cholinergic BFAs blocks neural activity-dependent plasticity in S1 barrel cortex of adult rats (whisker-pairing plasticity). In that study the deficit could be overcome by rigorous training on the gap-crossing behavioral task.

3.4. *AChE activity in visual association cortex*

Using single- and multi-unit recordings, Espinoza and Thomas (1983) mapped six retinotopic regions in rat visual association cortex. In mice, three complete retinotopic representations were ascertained with the same technique (Wagor et al., 1980) and four maps have been identified with optical imaging (Schuett et al., 2002; Kalatsky and Stryker, 2003). The medial and the lateral region chosen for quantification of AChE activity co-localize with areas AM (anteromedial) and AL (anterolateral) of Espinoza and Thomas (1983). The two areas are reciprocally connected with V1 cortex. The medial area projects to the lateral area, and both areas connect reciprocally with S1 cortex (Sanderson et al., 1991; Coogan and Burkhalter, 1993). Espinoza and Thomas (1983) did not find visual responses in our anterior region though it receives input from V1 cortex (Coogan and Burkhalter, 1993). We chose this region because Wagor et al. (1980) recorded neural responses to visual as well as whisker stimulation at that location in mice. Our anterior, medial and lateral region in visual association cortex correspond to area V2 (lower visual field), Vm and V3 of Wagor et al. (1980), respectively. Medial and lateral visual association cortex receives a strong projection from the posterior thalamic nucleus (Sanderson et al., 1991) and the three regions receive input from separate aspects of the lateral posterior thalamic nucleus (LP) in the rat (Hughes, 1977). Based on these connectional differences, we anticipated local differences in AChE activity between the rats deprived of retinal input and their sham-operated littermates.

In the sham-operated animals, the three regions had developmental AChE activity profiles resembling that of V1 cortex. AChE activity increased with enrichment and training. The steepest increases were found in medial visual association cortex. By contrast, in the deprived rats the developmental AChE profile of the three regions resembled that of S1 cortex. In particular, AChE activity was elevated to equal or greater levels than in sham-operated littermates already 1 week after birth. The similarity of the developmental profile with S1 may be the result of the degeneration of TCAs to V1 cortex and the strengthening of somatic sensory inputs to visual association cortex. For example, LP afferents have been shown to reorganize in blinded rats (Négyessy et al., 2000) with the densest projection terminating in bimodal anterior visual association cortex (Wagor et al., 1980; Toldi et al., 1996).

In addition to TCAs, cortico-cortical connections, notably collaterals of callosally projecting neurons, may provide novel input to the deafferented visual cortex (Olavarria et al., 1987; Dehay et al., 1989; Chalupa and Killackey, 1989; LaMantia and Rakic, 1990; Innocenti, 1995). Moreover, somatic sensory trigeminotectal and tectothalamic connections may reorganize (Rhoades et al., 1981; Mooney and Rhoades, 1983). Primary sensory areas can process subcortically re-routed sensory inputs effectively (Sur et al., 1990). The sustained facilitation provided by preserved cholinergic BFAs may extend the survival of deprived TCAs until they receive, or are replaced by, inputs of novel sensory modality (Bhide and Frost, 1992; Rauschecker, 1995). This extension would allow the stabilization of normally transitory connections with cortical areas of other sensory modalities (Clarke and Innocenti, 1990).

Lateral visual association cortex was the only cortical region in the rats deprived of retinal input where manipulation of behavior augmented AChE activity statistically significantly. Further exploration is needed to attribute a functional role to this area in rats. It is intriguing to note in conclusion that analogous regions in lateral occipital cortex were prominently activated when people with severe visual disabilities read Braille (Melzer et al., 2001).

4. Experimental procedures

4.1. *Animals*

Eighty male and female Long–Evans rats were used in this study. The animals were obtained from an in-house breeding colony where they were raised in standard cages in a 12 : 12 h light/dark cycle with access to food and water ad libitum. Near term, pregnant females were checked for offspring daily. All methods were approved by the Vanderbilt University Animal Use and Care Committee and were in accordance with NIH-approved procedures.

4.2. *Optic nerve transection*

On the day of birth, two pups were removed from each litter and anesthetized with intraperitoneal injections of pentobarbital (5 mg /kg b.w.; Nembutal®; Abbott Laboratories, North Chicago, IL) and hypothermia. On both sides, a small incision was made posterior to the lateral canthus of the eye and gentle blunt dissection was used to gain access to the posterior half of the orb. Each eyeball was rotated to expose and transect the optic nerve. Any bleeding was controlled with gelfoam. After the transection, the eyeballs were repositioned and the incisions were closed and sealed with antiseptic liquid bandage (new-skin; Medtech Laboratories Inc., Cody, WY). Five percent lidocaine (Xylocaine) ointment was applied topically around the wounds' edges to alleviate pain. The pups were warmed to 37 °C and returned to the dams. Two additional pups/litter were subjected to sham operations. The procedure was the same, except the optic nerves were not exposed or cut and the eyeballs were not manipulated to ensure that the eyes were fully functional. Pups with optic nerve transection (ONT) and their sham-operated littermates were euthanized in pairs by intraperitoneal injections of pentobarbital (80 mg /kg b.w.) 1 week [postnatal day (P) 6] 2 weeks (P 11–13) and 3 weeks (P 20) after birth and the brains were processed for histochemistry to examine the postnatal development of cortical levels of AChE reaction product.

4.3. *Manipulation of behavioral activity*

Additional groups of animals were prepared to examine the effects of behavioral manipulations on cortical AChE activity. Pups with ONT and sham-operated littermates were weaned at 3 weeks of age. The weanlings were housed separate from the dams and subjected to the behavioral manipulations described below. Then, the animals were kept under standard care conditions until they were euthanized for histochemistry. The median age at euthanasia was 32 weeks. The oldest rats were ~2 years old, roughly covering the life span of Long–Evans rats.

4.3.1. *Standard rearing*

Eight weanlings with ONT and their sham-operated littermates were maintained in standard plastic cages (two rats/cage) with chow and water available ad libitum until they were killed in pairs 2–25 months after birth (median age: 8 months).

4.3.2. *Environmental enrichment*

Seven weanlings with ONT and their sham-operated littermates were held in large cages with 'enriched' environments (Rema and Ebner, 1999) during the 12-h dark cycle every day. These cages contained three stories connected with ladders. A feed hopper was installed on the highest level. Plastic toddler toys were scattered throughout. During the light cycles, the rats were housed in standard plastic cages. After 3 weeks and ~250 h of enrichment, they were maintained under standard care conditions until they were killed at 2–10 months of age (median age: 6 months).

4.3.3. *Behavioral training*

Seven weanlings with ONT and their sham-operated littermates were allowed to explore a confined space filled with plastic toddler toys for 20 min during the light cycle 5 days/week for 3 to 4 weeks. Then they were trained and tested for 1 h/day, 5 days/week, to distinguish between a yellow plastic duck and a purple plastic egg that were placed at random on a pedestal at the far end of a 0.6-m-long runway. The animals had to run the whole length of the runway, contact the object

with the whiskers and push over the egg to receive a food reward. The discrimination tests were conducted over a period of 4 months (Mineo et al., 2002). During this period, the animals were placed on a reduced diet. In total, each animal spent roughly 90 h in training and testing. They remained housed under standard care conditions until they were killed at 8–13 months of age (median age: 10 months).

4.4. *Histology*

After euthanasia with pentobarbital, the rats were perfused transcardially with 0. 1 M phosphate-buffered saline followed by 2% paraformaldehyde/0.1 M phosphate-buffered saline. The brains and whiskerpads were removed and post-fixed in 2% paraformaldehyde/0.1 M phosphate-buffered saline at 5 °C for 12 h. The whiskerpads were transferred into 0.1 M phosphate-buffered saline and stored at 5 °C for the examination of the innervation of whisker follicles (Rice et al., 1997) in a separate study. The brains were immersed in 30% sucrose/0.1 M phosphate-buffered saline and stored at 5 °C until sectioning. Forty micron-thick sections were cut on a sliding microtome at −20 °C. The brains of one rat with ONT maintained under standard care conditions and its sham-operated littermate were sectioned coronally to affirm the laminar distribution of enzyme reaction product. In all other cases, the cortical hemispheres were separated from the diencephalon and flattened before sectioning. Alternate series of sections were cut parallel to the surface along the rostrocaudal axis of the cortical hemispheres. The remainder of the brain was sectioned transversely. Sections from rats with ONT and sham-operated littermates were processed for histochemistry in pairs. One series of each brain was stained for cytochrome oxidase (CO) activity with diaminobenzidine (Wong-Riley, 1979). The other series was stained for AChE activity with copper thiocholine using Koelle's method modified by Geneser-Jensen and Blackstad (1971). The efficacy of ONT was assessed in two ways. First, the optic nerve was inspected macroscopically. Second, we examined on transverse sections through the midbrain whether the superficial visual gray layer in the superior colliculus differed in thickness and enzyme activity from that of sham-operated littermates.

4.5. *Photomicroscopy*

Histological preparations were inspected with a Nikon Eclipse E800M microscope (Nikon Inc., Melville, NY 11747). Images were acquired with a SPOT 2 Cooled Color Digital Camera (Diagnostic Instruments, Inc., Sterling Heights, MI 48310) and processed with Adobe Photoshop 7 (Adobe Systems Inc., San Jose, CA 95110) and Quark Express 6 (Quark, Inc., Denver, CO 80203).

4.6. *Quantitative imaging*

On digitized images of sections stained for AChE or CO activity, we outlined regions of interest for the measurement of enzyme activity aided by morphological landmarks and the distribution of reaction product. Only sections that did not sustain any damage during tissue processing were used. The images were acquired with a Panasonic WV-CD 50 camera (Panasonic, Secaucus, NJ 07094) mounted on a Micro-Nikkor 55 mm f/2.8 lens (Nikon Inc., Melville, NY 11747), a stabilized light box (Full Spectrum Solutions, Jackson, MI 49201) and NIH *Image* 1.62 (Wayne Rasband, NIMH, Bethesda, MD 20903).

For each region of interest, the pixel-weighted optical density (OD) of AChE reaction product was measured on one section per animal. Measurements could be obtained from 3 to 6 animals per group (Table 1). The number of pixels of the areas of measurement was used to determine their size. ODs were calibrated with a standard gray scale (EK1522267; Eastman Kodak Co; Rochester, NY 14650) and pixel size was calibrated with a calibration grid of 250 μm × 250 μm squares (MicroBrightField, Inc., Colchester, VT 05446). The region comprising barrels B1–3, C1–3, D1–3, β and γ was chosen to represent AChE activity in S1 cortex. We did not differentiate between barrels and septa, because the reaction product spread considerably compared with septum width, resulting in substantial cross-areal contamination of ODs. In addition to S1 cortex, ODs were measured in V1 cortex and three regions in visual association cortex. To assess the laminar distribution of AChE and CO reaction product in S1 and V1 cortex, profiles of ODs were scanned across the cortical thickness on coronal sections.

To test the statistical significance of the effects of ONT and behavioral manipulations on local AChE activity, general linear models were employed for parametric multivariate analyses of variance (ANOVA) of extent and magnitude of reaction product and pair-wise comparisons of means (SAS Institute, Cary, NC 27513). Since AChE reaction product in pups with ONT and sham-operated littermates did not differ at P 11 and P 13, the data of the two ages were pooled. Detailed descriptions of the statistical analyses and their results are provided in Appendix. Differences reaching confidence levels of $P \leq 0.05$ were considered statistically significant.

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Appendix A. Statistical analyses

General linear models were employed for parametric multivariate analyses of variance (SAS Institute, Cary, NC 27513). In order to test whether presence or absence of retinal input and behavioral manipulations affected AChE activity, deprived rats and their sham-operated littermates were compared for differences in local level of enzyme reaction product and size of the area of measurement. First, the measurements obtained during postnatal development and after behavioral manipulations were pooled for the analysis of variance (ANOVA). Then,

the two sets of observations were analyzed separately. As effects, cortical region, retinal input, behavioral manipulations and their possible interactions were included in the model. For each effect, a null hypothesis was tested separately against its interactions as error term, and the effect was considered significant only when this test indicated a confidence level of $P \leq 0.05$. Inter-regional comparisons included a Bonferroni correction when multiple observations from one animal were used. In the ANOVA of postnatal development, we tested whether region, retinal input, the animals' age and the interactions among the three effects influenced AChE activity and measured area statistically significantly. In the ANOVA of behavioral manipulations, we assessed whether enzyme activity and expanse were affected by region, retinal input, behavioral manipulations and their interactions. Age was included as a covariate. In all ANOVAs, missing values were compensated using type IV mean squares as error terms and pair-wise comparisons were carried out on least squares means.

A.1. *Cross-study ANOVA*

The model employed in the ANOVA of the pooled data fitted changes in measured area most closely ($R^2=0.89$) resulting in profound overall statistical significance of the test ($F=53.57$; $P=0.0001$). Cortical region ($F=199.33$; $P=0.0001$), presence or absence of retinal input ($F=5.07$; $P=0.03$), age ($F=4.04$; 0.02) and their interactions ($F=1.62$; $P=0.03$) were statistically significant. Increases in areal extent were statistically significant between 1 week of age and all other ages examined ($P \leq 0.05$). Compared with areal extent, magnitude of AChE activity fitted the model less ($R^2=0.33$) indicating that the effects examined did not fully account for the measured differences in reaction product. Despite this caveat, the observed changes in enzyme activity remained statistically significant ($F=2.76$; $P=0.0001$). Cortical region ($F=3.1$; $P=0.03$), retinal input ($F=3.89$; $P=0.05$) and age ($F=10.74$; $P=0.0001$), but not their interactions ($F=0.88$; $P=0.65$), were statistically significant. AChE activity was highest at 2 weeks of age compared with the other ages ($P \leq 0.0009$) and S1 cortex exhibited the greatest statistically significant changes compared with the other regions ($P \leq 0.0005$ with Bonferroni correction).

A.2. *Postnatal development ANOVA*

As in the ANOVA of the pooled data, the model fitted changes in area during postnatal development most closely ($R^2=0.95$) resulting in profound overall statistical significance of the test ($F=63.13$; $P=0.0001$). Cortical region ($F=139.87$; $P=0.0001$) and age ($F=7.83$; 0.003) attained statistical significance, presence or absence of retinal input did not ($F=3.65$; $P=0.07$), and the interactions among region, age and retinal input were statistically significant ($F=2.73$; $P=0.0005$). Hence, the influence of retinal input on changes in areal extent during postnatal development could not be assessed independently from region and age. The cortical regions examined grew 33% from the first to the second postnatal week and 14% from the second to the third postnatal week (squares means). The first increase was statistically significant ($P=0.0007$). The model fitted the magnitude of AChE activity only at $R^2=0.33$, and the observed differences in AChE activity were not statistically significant in the comprehensive assessment ($F=1.50$; $P=0.08$). However, among the effects tested age attained statistical significance ($F=17.08$; $P=0.0001$). The peak of AChE activity 2 weeks after birth was statistically significant compared with enzyme activity 1 week after birth ($P=0.0005$) and 3 weeks after birth ($P=0.0001$).

A.3. *Behavioral manipulations ANOVA*

As in the analysis of postnatal growth, the model fitted measured area more closely ($R^2=0.89$) than magnitude of enzyme activity resulting in greater statistical significance in the comprehensive test ($F=29.54$; $P=0.0001$). Cortical region ($F=219.71$; $P=0.0001$) and retinal input ($F=4.46$; $P=0.05$) were statistically significant influences, behavioral manipulations were not ($F=1.33$; $P=0.29$), and the effects did not interact ($F=0.96$; $P=0.52$). In general, absence of retinal input reduced the examined regions by ~30%. Training increased their size ~16%, but did not reach statistical significance compared with cage rearing or enrichment. The model fitted the changes in AChE activity after behavioral manipulations as good as for postnatal development ($R^2=0.39$). However, enzyme activity differed statistically significantly in the comprehensive assessment ($F=2.43$; $P=0.0004$), and the influences of cortical region ($F=28.97$; $P=0.0001$), retinal input ($F=22.76$; $P=0.0001$) and behavioral manipulation ($F=19.59$; $P=0.0001$) on enzyme activity, but not their interactions ($F=0.35$; $P=1.0$), were statistically significant. Global cortical AChE activity was statistically significantly decreased ~14% in the animals deprived of retinal input ($P=0.0001$) and increased ~40% by training ($P=0.0001$ and $P=0.0002$ compared with cage rearing and enrichment, respectively).

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