ELECTROPHORETIC PATTERNS OF INSOLUBLE PROTEINS IN THE SENSORY CEREBRAL CORTEX OF VISUALLY DEPRIVED AND NORMAL KITTENS

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Abstract. Insoluble proteins were investigated in the visual cortex (area 17) and somatic sensory cortex of one month old kittens. Visual deprivation did not affect the electrophoretic pattern but changed percentage distribution of proteins in both cortices. Some of the alterations seemed to appear only in the visual cortex, which is morphologically and functionally deficient in deprived kittens. Electrophoretic patterns of normal kittens differed from those of normal adult cats.

INTRODUCTION

Recent electrophysiological and morphological investigations showed that early visual experience is critical for the development of the primary visual cortex (area 17) of young kittens. Blakemore and Sluyters (2) and Pettigrew (11) showed that (i) in normal kittens at about 1 mo. of age the specificity of unit responses to some features of visual stimuli (particularly to the orientation of stimulus) is already similar to that of adults, and (ii) in visually deprived kittens the specificity of neurons is strongly reduced. In striking accordance with the above, Cragg (4, 5) found that (i) in normal 1 month old kittens and in adult cats the density of neurons and synapses is similar, and (ii) in deprived kittens the neuronal density is higher (neuronal diameter smaller) and the number of synapses associated with one neuron reduced. It is reasonable to assume that these morphological and functional deficits of deprived kittens' neurons are connected with changes in the biochemical composi-
tion of neuronal membranes. This paper describes the investigation of electrophoretic patterns of insoluble proteins from the visual area 17 in normally reared and deprived kittens. The somatic sensory area was analysed for comparison.

MATERIAL AND METHODS

Eleven kittens reared in large family cages were used. On the 8th day of life (just before eye opening) seven kittens were dressed in double layer linen hoods (Fig. 1) which prevented pattern vision. The remaining four kittens were reared normally. Normal kittens were from one litter and from 28 to 34 days old, and weighed from 350 to 420 g. Deprived kittens were from three different litters and from 28 to 35 days old, and weighed from 400 to 540 g. In addition, two normally reared adult cats were used.

In all animals the brainstem was transected at the pretrigeminal level under ether anesthesia (20)\(^1\). Anatomical verification showed that in all kittens the pretrigeminal transection was complete. After tran-

\(^1\) The isolated cerebrum of the pretrigeminal cat is awake and free from pain. Thus the pretrigeminal transection allowed to remove cortical samples without anesthesia, and moreover, to use these kittens in a different experiment, in which incorporation of labeled amino acids during visual stimulation was investigated (in preparation). This stimulation was directed to the right hemisphere, whereas for the purpose of the present experiment the samples from the left cortex were analysed.
section the kittens' eyes were occluded with bandages soaked in saline, and body temperature was maintained at 38°C. Some physiological features of these preparations and the technique of the pretrigeminal transection in young kittens were described in the previous paper (21).

The animals were sacrificed 2-7 h after the transection. When craniotomy started, the isolated cerebrum of seven kittens was in a good state, as shown by normal cortical EEG activity and ocular reflexes. In four cases (3 deprived kittens and one normal) the craniotomy had to be done earlier than intended because of gradual flattening of the EEG activity, and was started 10 to 20 min after the flattening was noticed. However, these differences did not seem to affect the electrophoretic patterns. The cortical samples were removed with a scalpel. The anterior part of visual area 17 and a part of the first somatic sensory representation (SI) were removed according to criteria available for adult cats. For visual cortex the cytoarchitectonic map of Otsuka and Hassler (10) and the results of our studies (22) and for somatic cortex the functional map of Woolsey (18) were used. The visual samples were taken from the medial aspect of the hemisphere, from A2 to A8 of H-C stereotaxic coordinates (Fig. 2). The removed tissue weighed about 80 mg. The sample of the somatic cortex was of similar size, and was taken from the posterior sigmoid gyrus.

Removed cortices were analysed either immediately or after storing for 10-16 h on dry ice. They were homogenized in ice-cold 0.32 M sucrose with 1 mM EDTA pH 7.4 (about 0.5% w/v of tissue) in glass-teflon homogenizers. Homogenate (6 ml) was centrifuged at 100,000 g for 1.5 h in a Spinco ultracentrifuge, model L. The supernatant fluid was discarded and the pellet was washed with sucrose and centrifuged in the same conditions. The pellet with insoluble proteins was solubilized in 300 μl of the mixture of 5% (w/v) Triton-X-100, 8 M urea, 10% (v/v) β-mercaptoethanol and 0.05 M kalium carbonate (7).

Disc electrophoresis of insoluble proteins was performed using discontinuous buffer system on 10% polyacrylamide according to Lim method (7) in Müller and Biesold modification (9). The solubilized pellet
(90 µl) was put on top on each gel (diameter 5 mm, length 70 mm). During the first phase of electrophoresis (about 30 min) the current was 0.1 mA/mm² of the gel surface, and during the second phase (about 90 min), 0.18 mA/mm². The gels were stained with 0.7% amido-black in 10% acetic acid, destained with the same acid, and densitometry was carried out using Vitatron (MPS, 940, 800). The electrophoretograms were divided arbitrarily into nine zones (Fig. 3), and the amount of protein in each zone was expressed as a percentage of the total content in the gel. Percentage values were transformed using function arcsin $\sqrt{P}$, and two-dimensional analysis of variance was used.

The amount of proteins was determined by Lowry method (8) in Schacterle and Pollach modification (16) with bovine albumin as standard.

Fig. 3. Typical electrophoretic patterns of insoluble proteins of visual cortex of a normal 34 days old kitten and a normal adult cat. For the kitten a densitogram divided into 9 zones, and the scheme of bands on gel are also shown. The horizontal arrow indicates the direction of migration.
RESULTS

Total protein content in the cortex of normal and deprived kittens is shown in Table I. No differences between the groups of kittens and between visual and somatic sensory cortex were observed (two-dimensional analysis of variance).

<table>
<thead>
<tr>
<th>Cortex</th>
<th>Normal group ( n = 4 )</th>
<th>Deprived group ( n = 5 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual</td>
<td>92.1 ± 5.0</td>
<td>85.8 ± 1.6</td>
</tr>
<tr>
<td>Somatic</td>
<td>98.5 ± 3.1</td>
<td>90.5 ± 3.5</td>
</tr>
</tbody>
</table>

In the electrophoretic patterns of insoluble proteins about 25 protein bands were usually detected. A typical pattern for visual cortex of a normal kitten is shown in Fig. 3. Similar patterns were found for the visual cortex of deprived kittens, and for the somatic cortex of both normal and deprived kittens. However, this conclusion may not be valid for zone 9, where the bands were situated very closely and individual variation in the course of electrophoresis made difficult the comparison of different gels.

The results of densitometric measurements are shown in Fig. 4. The analysis of variance did not reveal interactions between groups of animals and types of cortex. \( F \) of interaction was near the level of significance in zone 3, lower than 2 in zone 9, and below 1 in other zones. In deprived kittens the transformed percentage of protein content was higher in zone 4 (\( F_{1,18} = 6.96; \ P < 0.05 \)) and zone 7 (\( F_{1,18} = 8.81; \ P < 0.01 \)), and lower in zone 5 (\( F_{1,18} = 14.43; \ P < 0.01 \)). These differences appeared for both visual and somatic cortex. In zone 3 the value of protein content for the visual cortex of normal kittens seemed to be higher than in the other cortices. The analysis of variance for this zone did not show significant differences. However, \( F \) of interaction was near the level of significance (\( F_{1,18} = 3.49; \ F_{1,18}^* = 4.41; \ F_{1,18}; 0.1 = 3.01; \ P < 0.1 \)).

In both groups of kittens some differences between visual and somatic cortex were observed. The relative amount of proteins in visual

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2 Additionally Duncan test was used. The value for visual cortex of normal kittens was higher than for the deprived kittens at the 0.05 level.
cortex was smaller in zone 2 ($F_{1,18} = 9.6; P < 0.01$) and higher in zone 6 ($F_{1,18} = 10.43; P < 0.01$) than in the somatic cortex.

In Fig. 3, typical electrophoretic patterns for visual cortex of normal kittens and normal adults are compared. Clear-cut differences were found in the fast migrating cathodic bands. The protein bands of zone 2
was hardly visible in kittens, whereas it was heavily stained in adults. In addition in adult cats several distinct bands appeared in zone 1 (well visible on gels but less clearly on the photograph), whereas in kittens these proteins were absent or hardly detectable. Probably in zone 3 the protein amount was also higher in adult cats. These differences seem to be mainly connected with the presence of myelin proteins in the visual cortex of adult cats. Myelination in cats' visual cortex begins in the third week of postnatal life (see 3) and lasts up to the third month (6). In this period a distinct increase of basic proteins of myelin occurs. In adult rats Müller and Biesold (9) detected basic myelin proteins in a zone corresponding to zone 2 of our cats.

**DISCUSSION**

Our main result is that visual deprivation affected the distribution of insoluble proteins in sensory cortex of one month old kittens, as shown by the densitometric analysis of the electrophoretic patterns. In zone 3 the difference seemed to appear only for the visual cortex. Further investigations of this zone are needed to tell whether or not this difference may represent the biochemical concomitant of the before mentioned morphological (4, 5) and/or functional (2, 11) deficits of the inexperienced visual cortex.

An important question arises: what is the reason of the effect of visual deprivation on the somatic sensory cortex. It is reasonable to think that visual deprivation affects many cortical areas. First of all, the development of associative processes between visual cortex and other cortical areas may be impaired. Our recent behavioral data (19) suggest that this impairment is mainly responsible for a dramatic deficit in visual discrimination learning of deprived cats. The role of the remaining sensory inputs may also become different. Interestingly, Ryugo et al. (15) recently found that in rats the bilateral eye enucleation or vibrissae removal increases the number of spines in auditory cortex. It should be also noted that visual deprivation may influence everyday behavior of cats. P. Korda (unpublished observation) found recently in this Laboratory that after the second month of age deprived kittens become very active in their home-cages, and thus they receive much stronger kinesthetic stimulation than normal kittens.

On the other hand, total protein content and electrophoretic patterns were similar in both groups of kittens and for both cortices. For the visual cortex similar data were recently obtained on rats. Both content and patterns of insoluble and soluble proteins were similar in dark-reared and normal rats (12). However, in deprived rats the level
of amino acids was elevated (13), and rapidly labelled neuronal protein fraction was absent (14). Visual deprivation affected also the activity of some enzymes (see 1, 17).

An important question left for further investigations is: what would be the effects of long-lasting visual deprivation on the electrophoretic patterns of insoluble proteins. In this respect the adult visually deprived cats should be compared with one month old deprived kittens and with adult normal cats.

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