SYNOPSIS
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1. AIMS:

The first aim of this research was to study electron microscopically the normal ontogenetic changes of numerical densities of synapses of limbic cerebral cortex, and to examine the effect of chronic caloric undernutrition on that ontogeny. Two cortical regions of the limbic system were proposed to be studied: i) the cingulate cortex, and ii) the hippocampus (CA1)-dentate region. The molecular layer was the target of study in the two regions. Incidentally the maturity status of the synapses was also proposed to be assessed to the extent possible by differentiating the synapses into the positive, negative and flat types, in a part of the data sample. Secondly, the study aimed also to find out whether deviations caused by undernutrition could be altered or prevented by nutritional rehabilitation.

An additional aim was to examine whether the chronic caloric undernutrition alters catecholaminergic axon varicosities (en passant synapses) in frontal cortex (CNS), and in iris which contains sympathetic postganglionic noradrenergic innervation (PNS).

2. METHODS:

The study was conducted on Wistar rats. Undernutrition was produced by restricting the amount of diet to approximately half of that consumed by
the normal control subjects of the same age as described earlier (Mascarenhas et al, 1984). That, resulted in 45-55% deficit in the body weights of the undernourished group. The offsprings born to such undernourished parents were used in this study. Hence, they had chronic caloric undernutrition through all stages of development (gestational, post-natal, and post-weaning ages). The subjects of rehabilitated group were provided with normal diet - ad libitum from weaning age (21 days) onwards till 150 days of age.

Synapses were visualized by treating the tissues with ethanolic-phosphotoxtingstic acid (E-PTA) and examining under electron microscope, as described by Aghajanian and Bloom (1967). The method of Jones and Dyson (1981) and Dyson and Jones (1984) was used to categorise the synapses into negative, positive and flat, sub-types. The negative synapses have the presynaptic density dipping into the post synaptic side, in contrast to the opposite pattern in the positive type, and the flat ones have parallel pre and post synaptic elements. The synaptic counts were made through the viewing chamber of the electron microscope, in twenty randomly selected fields for the cingulate cortex, and in ten randomly selected fields for hippocampus, for each animal. For each age, data from 5-6 animals were collected. For categorization of sub-types of synapses, negatives were exposed at x 7,200 magnification for cingulate cortex and at x 10,000 magnification for the hippocampus, and examined subsequently. The thickness of molecular layer was assessed in toluidine blue stained semithin sections of cingulate cortex.
For quantitative study of the intervaricosity distances of sympathetic post-ganglionic nor-adrenergic axons (PNS), the iris preparation was processed according to formaldehyde histofluorescence method (FIF) of Falck and Hillarp (1962). For the study of catecholaminergic axons of the frontal cortex (CNS) the cortical tissue was processed according to the glyoxylic acid histofluorescence method of Lindvall (1973) and Bloom and Battenberg (1976). The fluorescent axons with varicosities were traced on graphic tablet with the aid of camera lucida and the intervaricosity distance measured using the HP-85 computer. Iris was studied in the control and undernourished rats of 4-5 months age. Frontal cortex was studied in age groups of 5, 15, 21 and 50-60 days.

3. RESULTS AND DISCUSSION:

a) **Alterations of body and brain weights:** Chronic caloric undernutrition produced significant reduction in both body and brain weights, and post-weaning rehabilitation with ad-libitum food produced a substantial catch up, but not a complete recovery.

b) **Serum protein levels:** In order to check whether the chronic caloric undernutritional regimen adopted here has caused any incidental protein deficiency or not, the serum protein levels were test checked in a group of animals (150-180 days old) using Lowry's method (1951). The data revealed no significant difference of serum protein levels of the undernourished group from the control
group, thereby ruling out a protein deficiency state being added to the chronic caloric undernutrition.

c) Alteration of thickness of molecular layer of cingulate cortex: During the ontogeny of normal animals, there was a progressive increase in the thickness of the molecular layer of the cingulate cortex till 21 days of age, a subsequent decrease as observed at day 45, and again an increase by day 150.

In the undernourished animals, the thickness of molecular layer was significantly less till day 21 of age, but was significantly higher than in control at 45 and 150 days of age. The undernourished group showed a slower and gradual increase (without pruning) in the thickness, with age from day 10 to day 150. No recovery with rehabilitation was seen.

d) Alteration of numerical density of synapses of cingulate cortex: In the cingulate cortex of the normal group, the synaptic density in molecular layer significantly increased with increasing age from 10 to 45 days of age and later decreased significantly as observed at 150 days of age. The increase was 57% between 10 and 15 days, 89% between 15 and 21 days, and 30% between 21 and 45 days. This was followed by a significant decrease at 150 days by 56%. Hence, there was a phase of rapid increase in the synaptic acquisition followed by a phase of synapse elimination in the
normal course of development.

The cortex of the undernourished group also showed an increase in the synaptic numbers with increasing age until 45 days of age, but the number at a given age was less than that of control group. In contrast to normal group, there was no phase of elimination of synapses in the undernourished group after 45 days of age. Instead, the increase in synaptic density continued after 45 days of age in the undernourished. As a result, the synaptic density in the undernourished group was higher than in control group at later age (e.g. 150 days). Rehabilitation significantly increased the number of synapses as compared with the age matched undernourished.

The data revealed, that at any given age the flat sub-type of synapses contributed to the bulk of the synaptic number, and they followed the trend of ontogenetic changes described above for the total number of synapses in the control and experimental groups. Positive type synapses were less in the undernourished group at all ages, and rehabilitation produced substantial recovery of them.

e) Changes in synapses of hippocampus - dentate molecular layer:
In the molecular layer of dorsal hippocampus (CA-1) - dentate region of normal group, the synaptic number was seen to progressively increase with age from day 15 to 150 days significantly. There was no phase of elimination of synapses
noticed in this cortex, contrary to what was seen in the cingulate cortex. The synaptic number increased by 49% from 15 to 21 days, and by 69% between 21 and 45 days, followed by an increase by 63% by 150 days.

In the undernourished group, the synaptic number was higher than in age matched control at 15 days of age, but at other ages, it was less than control.

Rehabilitation caused no significant recovery from the deviations caused by the early insult of undernutrition in the hippocampus-dentate molecular region. This is in contrast to what was observed in cingulate cortex.

In this region also, at any given age the number of flat type of synapses was more than others and contributed to the trend of changes described above for the total number of synapses. Positive type synapses increased with age in both the control and undernourished groups, while the negative type synapses were less in control and significantly more in the undernourished. Rehabilitation caused a significant increase in the positive type of synapses as compared to the number seen in the undernourished.

The results indicated that the hippocampus-dentate region was more vulnerable than cingulate cortex to chronic caloric undernutrition,
and that rehabilitation provided (subsequent to early undernutrition) failed to produce a substantial recovery.

f) **Inter-varicosity distance on catecholaminergic axons:** Study of intervaricosity distances of sympathetic post ganglionic axon fibers of iris indicated that the noradrenergic synapses of the peripheral nervous system (PNS) are not altered significantly by chronic caloric undernutrition.

Study of ontogeny of central nervous system catecholaminergic axons in the frontal cortex of normal rat showed that, the intervaricosity distance decreased (i.e., increase of density of synapses) with age from postnatal day 5 to day 21 and later increased by day 50/60. This pattern was not altered in the undernourished group, except that the distance was usually less than that in age matched control and this being statistically significant at day 5 of postnatal age (i.e., more synapses than in control).

From the above data, one can infer that the catecholaminergic innervation as measured by the intervaricosity distance may not be affected by the present type of regimen of chronic caloric undernutrition in the PNS but not so in the CNS. This study however, does not reveal an occurance of any change in the level of the neurotransmitters in the varicosities in the undernourished group.
4. GENERAL OVERVIEWS AND CONCLUSION:

The present study on developing rat brain revealed:

i) in the normal group the postnatal ontogeny of events of acquisition and elimination of synapses in the limbic cortical molecular layers, and alterations of densities of varicosities of catecholaminergic axons in the frontal cortex;

ii) how moderate-to-severe chronic caloric undernutrition might alter the normal sequelae of these cortical developmental processes and,

iii) how far rehabilitation with normal diet provided after weaning might prevent or help to recover from effects of early undernutrition.

The data on numerical density of synapses showed that the two types of limbic cortices (cingulate and hippocampus) have different ontogenetic sequelae, and that both the types of cortices were affected by chronic caloric undernutrition with some differences. The deviations produced by the early undernutrition could be altered or prevented to a considerable extent by subsequent rehabilitation in the cingulate cortex, but not in the hippocampus, thereby showing that the hippocampus reacted differently to the undernutrition during development, or a revelation of a differential vulnerability