Deficits in synapse-to-neuron ratio due to early undernutrition show evidence of catch-up in later life[1]

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Summary. 30-day-old rats undernourished from birth are known to have large deficits in the synapse-to-neuron ratio in certain brain regions. It has not been possible to demonstrate any statistically significant deficits in this ratio in animals undernourished from birth to 30 days but then provided with an ad libitum amount of food till 6 months of age.

The developing brain is particularly vulnerable to environmental adversity. For instance, undernutrition of the infant during the 'brain growth spurt', a period when the brain is going through its rapid phase of growth, causes a series of permanent deficits and distortions of brain structure and composition[3]. For instance, in 1972 Cragg[4] reported that rats killed at the end of a period of undernutrition during early life had a deficit of about 30–40% in the synapse-to-neuron ratio (a ratio which may be regarded as a measure of neuronal connectivity[5]) in both the visual and frontal cortices. We have recently obtained data which confirm and extend these findings[6]. In our experiments 30-day-old rats undernourished from birth had a 31% deficit in the synapse-to-neuron ratio in the granular layer of the cerebellum and a 37% deficit in the frontal cortex. The question therefore arose whether such deficits persist into adult life following maximal nutritional rehabilitation. There are no previously published reports on the synapse-to-neuron ratio in animals which have been nutritionally rehabilitated after a period of undernutrition during early life. The present report concerns such an investigation. We have not been able to show a permanent deficit in the synapse-to-neuron ratio in rats which were undernourished for 30 days from birth and then provided with an unlimited amount of food until 6 months of age.

Material and methods. The rats used in this study were of the black and white hooded Lister strain. At birth pups from well fed mothers were fostered to dams which had been either well fed or undernourished throughout pregnancy. Pups born to the latter group of mothers were discarded. Litter sizes were standardized to 8, containing 4–6 males. The food intake of the undernourished mothers was reduced to about half that eaten by control mothers fed ad libitum. At 30 days of age, control and undernourished male rats were caged singly and placed on an ad libitum diet. At 6 months of age 6 rats which were not siblings were killed by perfusion with 2.5% phosphate-buffered glutaraldehyde (pH 7.3). Pieces of visual, frontal and cerebellar cortex were taken in a standardized fashion, washed in buffer, post-fixed in 1% osmium tetroxide, dehydrated and embedded in araldite.

Quantitative stereological procedures[7,8] at the light and electron microscopical levels were used to estimate the numerical density (number per unit volume) of neurons and synapses. These estimates were based on counts and measurements of neuronal nuclei and synaptic membrane thickenings respectively. It was assumed that each neuron possessed a single spherical nucleus, and each synapse a single disc-shaped membrane thickening[9]. The stereological procedures adopted[6] allowed correction for the effects of section thickness and size-frequency distribution of the 'synaptic-discs' and neuronal nuclei on the estimates of numerical density. Initially, the values of the various parameters investigated were calculated for each rat. Care was taken to ensure that sample sizes for each animal were enough to give 95% confidence limits of the mean densities better than $\pm 10\%$[10]. These mean values were then pooled to give the means $\pm$SE for a given group. Differences between groups were examined using Student's t-test. A more detailed account of the method is given elsewhere[6].

Results and discussion. After the period of rehabilitation the previously undernourished rats showed mean deficits of 20% in body weight, 14% in 'forebrain' (brain minus cerebellum and olfactory lobes) weight, and 23% in cerebel-

Table 1. Mean$\pm$SE body and brain weights for 6-month-old control and previously undernourished rats

	Control (C)	Previously undernourished (PU)	Difference (%)
Body weight (g)	450.1$\pm$8.7	360.0$\pm$14.4	−20*
Forebrain weight (g)	1.69$\pm$0.05	1.46$\pm$0.03	−14**
Cerebellar weight (mg)	300$\pm$10	230$\pm$10	−23**

Data from 6 C and 6 PU rats. * $p<0.02$; ** $p<0.01$.

Table 2. Neuronal and synaptic data for 6-month-old previously undernourished rats and their controls

	Control (Mean$\pm$SE)	Previously undernourished (Mean$\pm$SE)	Difference ($\pm$SE)	Difference (%)
Visual cortex				
No. of rats.	4	6		
Nv_n ($\times 10^3$/mm^3)	71.50$\pm$6.48	81.66$\pm$5.90	10.16$\pm$8.76	+14.2*
Nv_s ($\times 10^9$/mm^3)	0.73$\pm$0.09	0.71$\pm$0.03	0.02$\pm$0.07	− 2.7*
S/N ($\times 10^3$)	10.29$\pm$0.69	8.99$\pm$0.78	1.30$\pm$1.04	−12.6*
Frontal cortex				
No. of rats	6	6		
Nv_n ($\times 10^3$/mm^3)	55.50$\pm$1.97	61.20$\pm$3.61	5.70$\pm$4.11	+10.3*
Nv_s ($\times 10^9$/mm^3)	0.75$\pm$0.04	0.72$\pm$0.03	0.30$\pm$0.05	− 4.0*
S/N ($\times 10^3$)	13.54$\pm$0.97	11.80$\pm$0.69	1.74$\pm$1.19	−12.9*
Granular layer of cerebellum				
No. of rats	6	6		
Nv_n ($\times 10^6$/mm^3)	1.68$\pm$0.06	1.88$\pm$0.07	0.20$\pm$0.10	+12.0*
Nv_s ($\times 10^9$/mm^3)	1.15$\pm$0.06	1.16$\pm$0.07	0.01$\pm$0.10	+ 1.0*
S/N	688$\pm$38	627$\pm$56	61$\pm$68	− 8.9*

Nv_n, numerical density of neurons; Nv_s, numerical density of synapses; S/N, synapse to neuron ratio. * Not significant.

lar weight compared with controls (table 1). Similar deficits in body and brain weights in previously undernourished rats have been described previously[11].

There were no statistically significant differences in the numerical densities of neurons, synapses, nor in the synapse-to-neuron ratios between control and previously undernourished rats in any of the brain regions studied (table 2). Our measurements do not preclude the possible existence of persisting deficits in the synapse-to-neuron ratio, which although less than the current limits of experimental error, could nevertheless be of biological importance.

Turning to a comparison of the present data with that in our previous paper[6], a much larger number of synapses per neuron appears to have been developed in the frontal cortex by 30 days of age[6] than persists into adult life, when only about half the number formerly present are found.

The present experiments do not give any information on the total number of neurons or synapses in the brain regions studied. It may be possible to have significant deficits in the total number of these components and yet have similar synapse-to-neuron ratios. Although it would be desirable to obtain estimates of the total nerve cell number (and/or total synapse number) in whole brains or whole brain regions, dependable and simple histological methods for doing so do not as yet exist, due to the brain's extreme structural heterogeneity.

In conclusion, early undernutrition produces a well documented series of distortions of brain structure and function, including large deficits in the number of synapses per neuron in certain layers of the frontal, visual and cerebellar cortex. Many of these effects are permanent; however the present experiments show that the deficits in the synapse-to-neuron ratio are at least partially reversible. Whether this was primarily due to the nutritional rehabilitation, or whether it would still have occurred in the presence of continuing undernutrition remains uncertain but it may well be that a return to normal numbers may demand the provision of an optimum nutritional environment, as occurred in these experiments. The functional consequences of the initial deficit and the subsequent catch-up must, however remain an open question.

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Effects of intracellular or extracellular application of tetraethylammonium on the action potential in cultured chick embryonic heart muscle cell

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Summary. The effects of tetraethylammonium (TEA) on the action potential in cultured chick embryonic heart muscle cells were investigated. The onset of prolongation of the action potential occurred within 10 min following intracellular iontophoretic application of TEA, but after more than 50 min following extracellular application. These facts suggest that the major site of action of TEA is on the inner surface of the membrane in these cells.

It is well known that tetraethylammonium (TEA) prolongs the action potential in various excitable cells by decreasing the potassium outward current (squid giant axon[1], Ranvier node[2], barnacle muscle[3] and smooth muscle[4]). In squid giant axon, TEA prolongs the action potential when it is injected into the axon, whereas external application has no effect. In the Ranvier node of the myelinated nerve, however, the site of action of TEA is both on the inside and on the outside of the membrane. In myocardial cells, Ochi and Nishie have successfully applied TEA to the inside of the membrane from a cut-end of ventricular muscle and have shown that the site of action of TEA is on the inside of the membrane[5]. Considering the difference in the site of action of TEA in different cells, we examined the effect of TEA applied internally or externally in cultured chick embryonic heart muscle cells.

Material and methods. The tissue culture techniques used in the present experiments were similar to those reported previously by many workers[6,7]. In brief, hearts of 5–7-day-old chick embryos were dispersed by 0.2% trypsin in a Ca-Mg free solution. The incubation medium consisted of Eagle's solution containing 10% embryo extract and 10% horse serum. The dispersed cells usually assembled into monolayer groups, the rosette-like aggregates of cells growing to 200–300 μm in diameter in the course of 5–10 days. During the experiment, the culture medium was replaced by a bathing solution having an ionic composition of (in mM): 149.3 Na^+, 2.7 K^+, 1.8 Ca^{++}, 1.0 Mg^{++}, 145.3 Cl^-, 11.9 HCO_3^-, and 0.42 $H_2PO_4^-$. The pH of the ungassed solution was 7.9 and was constant in each experiment lasting a few hours. The bathing solution was stagnant and less than 2 mm deep. A hot water perfusion bath was used to maintain the temperature of the culture dish (Corning tissue culture dish) at 36 °C. These conditions were essentially the same as those reported by Sperelakis and Lehmkuhl[8]. Under these conditions, muscle cells contracted rhythmically, and the rate of firing and also the shape of the action potentials remained unchanged during several hours. Transmembrane potentials were recorded through a conventional glass microelectrode with a resistance of