EFFECTS OF POSTNATAL INFLUENCES ON CONDITIONING AND SOME FUNCTIONAL AND STRUCTURAL BRAIN PARAMETERS IN RATS

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Abstract. Early postnatal influences were studied in rats in three different ways: (i) different litter size, (ii) feeding with restricted amount of protein, (iii) different level of afferent stimulation. A complex evaluation of spontaneous activity, conditioning, electrophysiological and biochemical analyses was used in these experiments. Medium sized litters showed the greater electrophysiological reactivity (cortical evoked potentials) and the most stable performance in different kinds of conditioning (avoidance) experiments. Animals reared on a low protein diet were subnormal in electrophysiological indices, and had a lower level of biochemical activity for materials involved in neural excitability, electrogenesis, and cellular and subcellular energetic metabolism (phospholipids, proteins, DNA, RNA). In all respects (behavioral, electrophysiological and biochemical tests) animals with partial sensory deprivation in the postnatal period had the lowest indices. The "stimulated" animals on the other hand were superior to both other groups in the tests used for the analysis of higher nervous activity, but electrophysiological and biochemical analyses did not show clear differences between the "stimulated" and control animals. Optimal development of brain functions requires adequate conditions in the early postnatal period. However the "adequacy" of these conditions has still to be determined more precisely.

INTRODUCTION

The importance of early periods of life for the further development of the central nervous system is generally recognized. Two principal groups of early influences may be distinguished — nutritional factors
and afferent influences (in spite of the fact that both are acting in permanent mutual dependence).

Some attention has been given to these problems even in the past century (e.g., 5, 8, 24, 25) but recently they have been studied more systematically (e.g., 1, 2, 6, 7, 9, 11, 21, 27). Our laboratory contributed to these studies by a complex approach, chiefly by means of electrophysiological analysis of the sequelae of early influences (3, 4, 10, 12–20, 23, 24, 26, 28).

The present work involves three different experimental designs: (i) unequal litter size, (ii) rearing on a low protein diet, and (iii) increased or decreased level of afferent stimuli in the early postnatal period.

**METHOD**

Wistar rats of both sexes were used in the experiments

1. Offspring of unequal litters were divided in three groups to make litters of: A, 3–4, B, 7–8 and C, 13–15 pups.

2. Low protein diet (5 cal % of protein, 17 cal % of lipids and 78 cal % of carbohydrates) was administered to lactating females and after weaning at the age of 21 days it was given to the young until the 8th week of life.

3. Increased level of afferentation (Group SB) was achieved by complex stimulation 3 times a day for half an hour (from the 5th to 35th day) with light and sound stimuli (3 sec intervals) combined with somesthetic–kinaesthetic stimulation (rotating cylinder with circumferential velocity 200 mm/min) whereas the animals with a decreased level of afferent stimulation — partially deprived (Group DB) were reared and kept in darkness up to 35th day with minimal stimulation connected with cleaning. All animals in these experiments were offspring of medium sized litters and thus control animals were marked as in the first series — Group B.

Spontaneous exploratory activity was measured in a compartment with automatical registration of horizontal and vertical movements; conditioning was tested by an apparatus with semiautomatic or automatic programming which elaborated the avoidance reaction. Averaged cortical evoked potentials to sound and light stimuli were recorded in animals immobilized with succinylcholine-iodide. Biochemical analysis of the cerebrum was carried out after weighing, homogenization and determination of the dry weight; cholesterol, phospholipids, DNA, RNA, protein cholinesterase activity, sodium and potassium contents, were determined. A summary of the procedures used in experimental groups is given in Table I.
TABLE I
Summary of procedures

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Measurement</th>
<th>Exploratory activity</th>
<th>Avoidance</th>
<th>Evoked potentials</th>
<th>Biochemical analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Different litter size</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Low protein</td>
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<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Level of sensory input</td>
<td></td>
<td>+</td>
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</tbody>
</table>

RESULTS

Different litter size

Animals of Group C (large litters) showed significantly higher exploratory activity (horizontal component) and also a more intense emotional reaction (defecation) to nociceptive stimulation than animals of both Groups A and B. In the three experiments with different schedules of avoidance conditioning, there were practically no significant differences. However, in the second series a break of 2 weeks caused animals of Group A (small litter) to show a statistically significantly lower number of avoidance reactions than animals of either other groups (evaluated in five experimental session before and after the break) (Fig. 1). Extinction of avoidance in the third experiment was significantly slower in Group C than in Groups A and B, which may be related to their higher reaction to nociceptive stimuli (18, Fig. 1).

Fig. 1. Mean values of avoidance reactions (AV) in three groups of rats in different sized litters (A, B, and C) calculated from five sessions just before and after a break in the experiment of 14 days (10 trials per session). Significant differences indicated by arrows (p < 0.05).
Cortical responses to sound (clicks) and light (flashes) at different stimulation rates were characterized in Group B by the shortest latencies and the highest amplitudes (Fig. 2 and 3). Animals from small as well as large litters had longer latencies and lower amplitudes. The latency differences are significant however in some components within stimulation frequencies 0.2/sec and 1/sec only (Table II). The i.v. administration of pentobarbital, accentuates the differences between groups without changing their basic relations.

![Graph](image)

Fig. 2. Mean values of cortical evoked potentials (EPs) in the temporal cortex to auditory stimuli at different frequencies. The same groups of animals as in Fig. 1. Abscissae, latent periods in msec; ordinates, amplitudes (arbitrary scale, true calibration given on right, standard errors of means indicated).

**Low protein diets (LP)**

In this series of experiments only females were used. The experiments were performed at age 15–16 weeks. The exploratory activity of the animals was measured in a single session of 10 min, which revealed that the LP animals were significantly more active in vertical activity ($p < 0.05$) than the controls without significant differences in the horizontal component.
Fig. 3. Mean values of cortical EPs to visual stimuli in the occipital cortex. Other details as in Fig. 2.

TABLE II
Significance of differences between mean latency values (to peak) of components P₁ and N₁ of evoked potentials (EP) to auditory and visual stimuli in appropriate cortical areas (by Student's $t$-test)

<table>
<thead>
<tr>
<th>Stimuli</th>
<th>Comparison of groups</th>
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<tr>
<td>Modality</td>
<td>Frequency</td>
</tr>
<tr>
<td></td>
<td>EP component</td>
</tr>
<tr>
<td>Visual</td>
<td>0.2/sec</td>
</tr>
<tr>
<td></td>
<td>1/sec</td>
</tr>
<tr>
<td>Auditory</td>
<td>0.2/sec</td>
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<td></td>
<td>1/sec</td>
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</table>

The next day, average cortical EPs were recorded and the brains were then biochemically analyzed. The auditory cortex of LP animals were less reactive (longer latencies, lower amplitudes) to clicks (Fig. 4).
The evidence of significant differences only within lower frequency ranges is due to the fact that for the mean values the responses were counted only in animals in which the corresponding component was easily detectable, and thus animals not responding within higher frequency ranges were eliminated in the evaluation of higher stimulus frequencies. The differences in the visual cortex were less evident and the absence of a response to a higher stimulation rate in some LP animals caused apparently better results at higher frequencies in the LP group (Fig. 5). The percentage of animals not responding at higher frequencies to stimuli of either modality was about 50% in the LP group whereas about 10% in control.

There was significant decrease of the total weight and of total contents of DNA, RNA, proteins, phospholipids and cholinesterase activity in the brains of the LP rats (Fig. 6). The comparison of the concentrations calculated from the obtained values showed a decrease of DNA and RNA concentrations and a small increase of cholesterol and protein. The concentration ratios phospholipid/protein, phospholipid/cholesterol and K+/Na+ were significantly lower in the LP group. Thus it may be seen that protein deficiency caused a decrease of the brain cell number and evidently a deterioration of the subcellular energy processes requiring norm-

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**Fig. 4.** Mean values of cortical EPs to auditory stimuli in the temporal cortex in rats on low protein (LP) diet and in control females (N). Significant differences of latencies and amplitudes indicated.
mal phospholipid metabolism. It was interesting that individual animals with inferior electrophysiological parameters, which were unable to follow a higher stimulation rate, were characterized also by low DNA and phospholipid contents, acetylcholinesterase activity and a $K^+/Na^+$ ratio.

**Level of afferent stimulation**

Spontaneous exploratory activity was measured in a semi-darkened room in four sessions (four successive days) at the age of 6 weeks. Rats of the Group SB were most active in the vertical as well as in horizontal components, and did not habituate from day to day. The lowest activity in the DS group was significantly different in the vertical component only (if compared with the controls).

The avoidance reaction was elaborated in semi-darkness until a criterion of three successive avoidances in three successive days was reached. Animals reared in darkness needed 44 trials, control animals, 34 trials. Stimulated animals required 28 trials only (Fig. 7). A similar criterion for extinction of the avoidance reaction was reached in SB animals after 38 trials, in the control Group B after 50 trials, whereas DB rats needed 72 trials. The differences between the Group DB and both other groups were highly significant.
The cortical potentials evoked by acoustic stimuli in the auditory cortex in the Group DB show lower amplitudes and longer latencies, mainly if compared with those in SB animals (Fig. 8). In the visual cortex the optimal parameters of potentials evoked by flashes were recorded in the Group B (Fig. 9).
Biochemical analyses did not reveal significant (Fig. 10) differences between B and SB groups. The DB animals showed significantly lower values of many important components if compared to the control animals (Group B) and in some cases also when compared to SB animals (in which the results were more variable). If these data are related to the DNA content, i.e. to cell number, it may be seen that the cholinesterase activity per cell is significantly higher in the deprived animals than in both other groups (both $p < 0.01$). Their ganglioside contents are also higher than in the Group SB ($p < 0.05$). The phosphatidylserine per cell ratio in Group DB was significantly lower than in the control (B) animals.

**DISCUSSION**

The first series of experiments revealed in Group B (medium sized litters) the best ability to follow external stimuli with an electrical
response in the specific projection areas. In conditioning, animals of this group did not show any signs of a deteriorated reaction as in Group A after a break in the experiments, or in Group C—poorer extinction.

Moreover, animals of this group were better in some indices of differentiation (even if not statistically significant). Thus the animals of medium sized litters which have evidently the most adequate nutrition, as well as an adequate level of stimuli in the nest, showed the best parameters in the studied functions.

In animals reared on a low protein diet we obtained with our electrophysiological and biochemical estimations an indirect evidence—for the time being—that their subnormal development of structures, and of mechanisms involved in excitation and electogenesis is among the most important causes of their generally impaired dynamics of neural function.

The indices of higher nervous activity—elaboration of a conditioned avoidance reaction and its extinction—showed the following sequence of increasing level of performance: partially deprived animals<controls<stimulated animals. However the bioelectrical and biochemical
Fig. 10. Differences of mean values of brain weight and composition in three groups of animals with different levels of afferent stimulation in the early postnatal period. White columns, partially deprived animals (DB); hatched columns, controls (B); black columns, stimulated animals (SB). Two asterisks, differences significant at the 5% level. Other details as in Fig. 6.
analyses showed only clear and significantly decreased values in the deprived group. The stimulated group was less homogenous, and thus we can only suggest that the applied amount of stimulation was optimal for some individuals, whereas for others it exceeded their capacity and was stressful in nature. This view requires, however, further experimental analysis and the really adequate level of stimulation has to be determined.

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*Received 3 July 1972*

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