

Morphometric study on the interhemispheric asymmetries in the wistar rat and the effects of early experience

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Summary. Sixty male Wistar rats were used for this experiment and assigned at random to the control group or to the stimulation method. Control rats show cerebral asymmetry with right bias at the frontal and occipital lobes. In the case of stimulated rats the differences from the occipital zone increase while those from the frontal lobe disappear.

Key words: Hemispheric asymmetries, Early experience, Wistar rat, Morphometry

Introduction

One sector of the efforts to understand the mechanisms underlying functional lateralization and the clinical consequences of its alterations includes research work into interhemispheric morphological asymmetries, both in human beings and in animals. In this line of inquiry, Albert Galaburda's works (Galaburda et al., 1978; Galaburda, 1984), showed important asymmetries in an area known as Tpt, which takes up a considerable portion of the human supratemporal plane and is usually damaged in Wernicke's aphasia. In most of the individuals this area is larger in the left hemisphere.

Numerous published works show that both phenomena - the existence of morphological asymmetries between both brain hemispheres and hemispheric specialization for certain aspects of behaviour - are very common characteristics along phylogenetic scale (Diamond, 1977). With regard to Brain Laterality in the rats chosen as experimental samples, the results found in the bibliography vary among different research teams as for race and sex of the animals used.

Dr. Marion Diamond has been working for more than thirty years on the study of interhemispheric morphological asymmetries in Long-Evans rats, finding

that, in this rat race, males present thicker right hemispheres compared to the left ones in most of the measurements made in all brain areas (Diamond et al., 1975, 1983). This asymmetry pattern is modified by sex, in such a way that males show much more pronounced asymmetries than females, in which a slightly bigger left hemisphere can be noticed (Diamond et al., 1981, 1983).

The other phenomenon studied in this work is early experience, used as a therapy to improve deficient individuals' adaptation to the environment they live in. Two methods widely used in research works are handling and the rearing in enriched environments. (Both of them are extensively described further on). Denenberg's and other authors' investigations have proved that both methods decrease the rat's emotional reactivity and increase their exploratory behaviour (Krech et al., 1960; Denenberg, 1977).

The effect of early experience on the cerebral histology of Long-Evans rats has been comprehensively studied by Dr. Diamond, discovering in all the cases an increase in cortical thickness in those rats subjected to handling and rearing methods in enriched environments; an increase which is more marked in the occipital cortex (Diamond, 1983).

The present research has been centered on the study of the morphological asymmetries existing in the brain hemispheres of our laboratory Wistar rats, attempting to find out whether early experience causes changes in asymmetry patterns or not, provided that there is asymmetry in the control rats.

Materials and methods

Sixty male Wistar rats were used, since, according to Diamond's findings, male brains are more asymmetric than female ones (Diamond et al., 1983). Thirty of them were controls and the other were subjected to early stimulation. On their day of birth, the newborn were sexed and every litter was reduced to eight pups, with a minimum of four males in each litter. All the litters were assigned at random to the stimulation method or to the

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control group (Denenberg et al., 1978).

The rats assigned to the control group were kept undisturbed with their mothers until the twenty-first day; then they were weaned and, after discarding the females, were placed in standard laboratory cages, at the rate of three per cage.

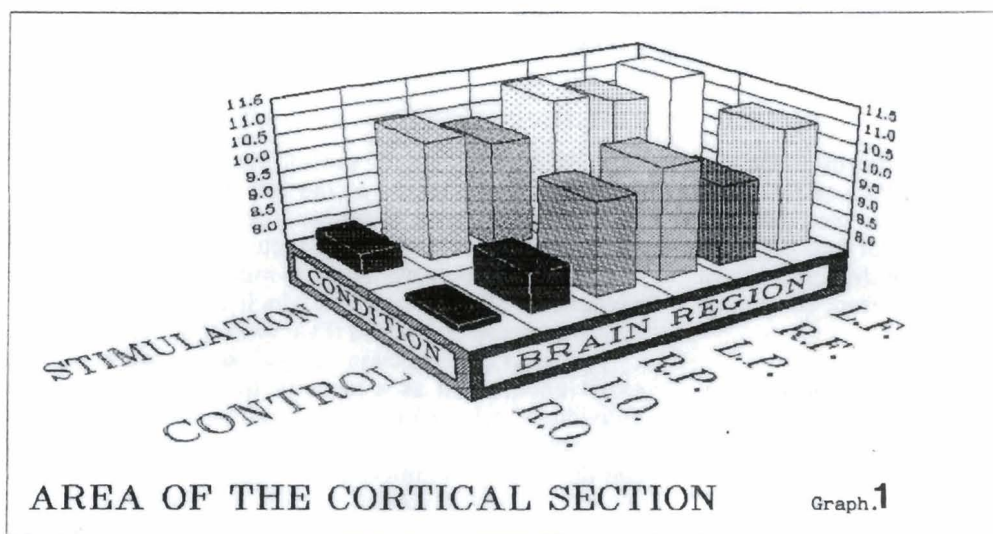
On the contrary, from the first day until their weaning, the rats assigned to the stimulation condition were daily subjected to handling process, as described by Denenberg (Denenberg et al., 1978). This process consists of individually taking the pups out of the maternity cage and putting them for three minutes into a can whose bottom is covered with wood shavings; afterwards they are placed

beginning of the decussation of the posterior commissure.

The cuts were stained by Nissl's method and, subsequently, the respective cortical areas were measured with a Biological Data Program, by delimiting them first and then giving the desired colour (Fig. 1 - a,b,c,d), not knowing whether the preparations belonged to the control group or to the stimulation group. The data obtained by measuring were statistically treated with Statview and Statwork programs.

Results

The percentage-based study of the right-left



Graph 1. Tridimensional graph indicating the effect of the stimulation on the six cerebral regions studied, as well as the asymmetry of the left and right of the brain.

again with their mother.

On the twenty-first day the rats were definitively taken away from their mothers and placed with up to twelve other rats also assigned to stimulation. The special large cage had, at medium height, a platform with a ramp so that the rats could reach it. Every day four different toys, chosen from a pool of twenty-five, were placed inside the cage. (This procedure is known by the name of rearing in enriched environment and is generally carried out by Rosenzweig's team at Berkeley University (Rosenzweig et al., 1972).

Every animal was sacrificed on the 50th day by perfusing through its left ventricle with 10% formal. The histological cuts were made in those areas described by Diamond in 1975 (Diamond et al., 1975).

- In the frontal area four sections were taken at the point immediately anterior to the initial crossing of the fibres of the corpus callosum.

- In the parietal cortex four sections were taken at the beginning of the decussation of the anterior commissure.

- In the occipital cortex four sections were taken at the

beginning of the decussation of the posterior commissure. The differences were significant. The differences were smaller in the parietal cortex: they only amounted to a 4% in favour of the left hemisphere, whereas with Student's t test the significance was also much smaller. Finally, in the occipital area the left-right asymmetry increased again to a 9.9% (Fig. 2).

For the purpose of checking whether this pattern of asymmetry recurred in stimulated rats, first we studied the modifications caused by the rearing in enriched environments upon each cerebral area in the control rats. In this case, stimulation had unequal effects on all cerebral areas. The most striking increase took place in the left occipital area, where it amounted to a 25%, followed by the right frontal lobe, in which there was a 12.6% rise, with a statistical significance in both cases of $p < 0.0001$. In the left parietal area the cortex growth with stimulation amounted to a 6.6% and the differences between the two conditions were also significant ($p < 0.03$). There were significant differences between the control and stimulated groups in the left frontal area ($p < 0.01$) but the growth was smaller, only a 4%. It is in the right parietal and occipital areas where the smallest growths take place (3.7% and 5.19%), without any significant differences being found in either case ($p < 0.07$ and $p < 0.05$). The three dimensional graph sums up the relations existing among all the groups taking into account the six left-right cerebral areas studied (Graph 1).

As one can see, stimulation modified the cerebral asymmetry pattern existing in the control rats in such a

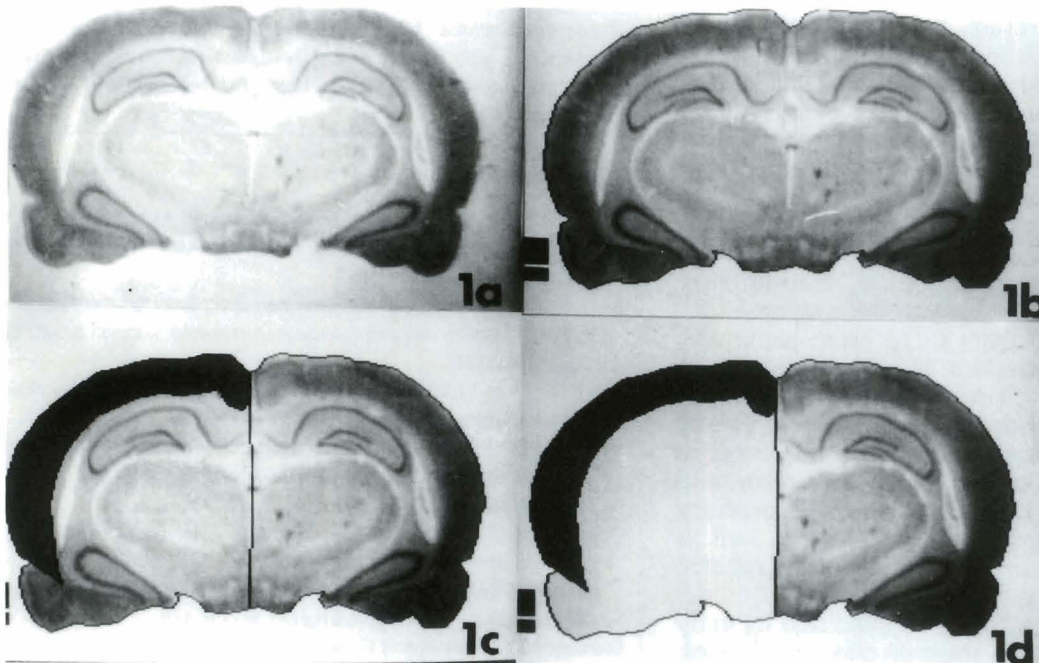


Fig. 1. Occipital cortex. Graphic treatment. The cortical areas are measured with a Biological Data Program, by firstly delimiting them and then giving the desired colour.

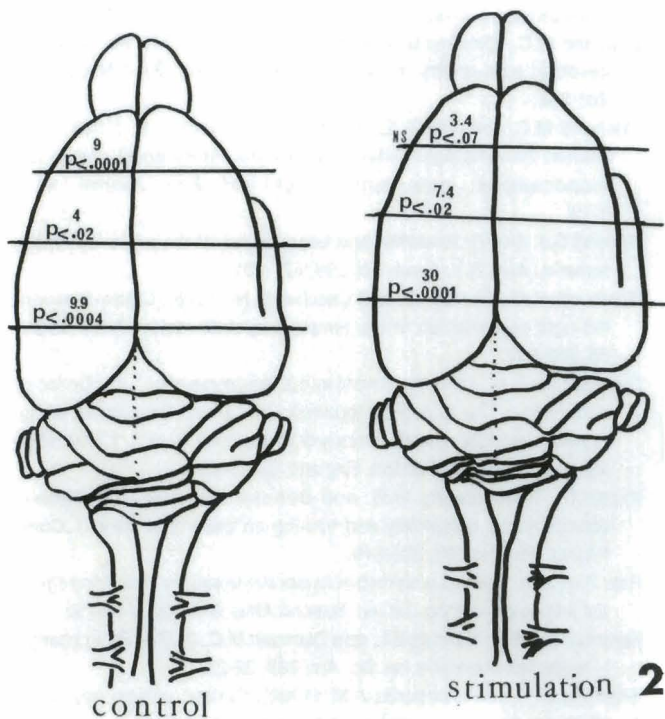


Fig. 2. Cerebral asymmetries in each one of the regions in control rats and stimulated rats.

way than in the frontal area, which showed an evident asymmetry toward the left hemisphere in the control animals, decreased to a 3.4%, without any significant differences being found now. Instead, in the parietal area differences increased. In the control groups the asymmetry amounted to a 4% but, with stimulation, it increased to a 7.4% and the differences became

significant. However, the most striking change continued to be in the occipital area, wherein the difference rate between both hemispheres in the control animals, which was a 9.9%, became nothing less than a 30% in the stimulated rats (Fig. 2).

Discussion

Comparing Diamond's result with her Long-Evans rats with those of the present work, the following differences can be assessed. In the parietal area, the results of both works are similar, whereas in the frontal and occipital zones our asymmetries are slightly larger than those obtained by Diamond (Diamond et al., 1981). But the main difference between both groups of results lies in the asymmetry sign. Whereas in the brains of the male Long-Evans rats measured by Dr. Diamond the predominant hemisphere is the right one, in our laboratory Wistar rats the reverse happens, that is to say, there is a dominance of the left hemisphere in cortical thickness; these dissimilarities are fully justified on account of the different rat race used.

As for morphological asymmetry in Wistar rats, the only work found in the bibliography is owed to Sherman (Sherman et al., 1982). In that work it was reported that, in the animals studied, the right hemisphere was, on the average, thicker than the left one at an important rate, and above all in males. But, although significant, the differences between the average measurements of the two hemispheres were only a 1.1%. In the frontal area, in non-handled animals, a slight asymmetry in favour of the right hemisphere was observed, whereas in the parietal and occipital areas the differences were not significant.

In general, the asymmetry in favour of the right hemisphere was positively correlated to lesser activity in

the open field and, therefore, to a greater emotional reactivity. This is logical when it is remembered that the right hemisphere is more intensely activated than the left one as to the behaviour related to the expression of emotionality. However, since the measurements corresponding to stimulated or non-stimulated animals are not specified, we do not know if the stimulation process during infancy is associated with a decrease in the size of the right hemisphere in relation to the left one. This agrees with the phenomenon of emotionality decrease, which is well proved in the bibliography.

The results obtained from the present paper are, therefore, similar to Sherman's as to the discovery of asymmetries between both hemispheres. They are unlike Sherman's in regard to their intensity and direction. We must look for the reasons for these differences in our rats' different genetic information with relation to that of Sherman's rats. In this case, the primary tendencies of lateralization will be different, as well as the subsequent patterns of interhemispheric morphological asymmetry.

With regard to the modifications generated by early experience on cerebral cortex, the main effects are located, as we have seen, in the left occipital pole, followed by the right frontal lobe. This point coincides with Diamond's first results (Diamond et al., 1964), in which it could be seen that the occipital cortex was the most sensitive zone to early experience, as for rapidity and intensity of modification. These two poles of maximum growth provoked by early experience recall the brain asymmetry pattern propounded by Le May in 1976 (Quoted by Rojo Sierra, 1984), in which the line of maximum cortical development in right-handed humans has an oblique inclination from the left occipital pole to the right frontal one. It is possible, therefore, that early experience brings the cerebral asymmetry pattern characteristic of lower species nearer to the one found by Le May in humans beings.

Consequently, we can affirm that the effects of early experience are asymmetrically distributed between both brain hemispheres. The left hemisphere is the most favoured in the occipital and parietal areas, mainly in the former, whereas the right hemisphere is the one which grows the most in the frontal area.

We can establish a new pattern of cerebral laterality generated by the stimulation effect in our control rats, in the sense that the interhemispheric differences increase in a spectacular way in the occipital area of the control animals, whereas these differences practically disappear in the frontal pole. These phenomena coincide with the results expounded by Denenberg and Diamond (Denenberg, 1981; Diamond, 1983).

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