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SOCIAL ISOLATION AND ENERGY METABOLISM IN RAT HIPPOCAMPUS

José M. Cimadevilla, Héctor González-Pardo, Laudino López,
Luis M. García-Moreno* y Jorge L. Arias
Universidad de Oviedo y * Universidad Complutense de Madrid

El aislamiento social puede entenderse como una forma de deprivación sensorial. Se sabe que ambientes enriquecidos o complejos estimularmente afectan al SNC tanto a un nivel anatómico como fisiológico. En nuestro estudio, se usaron dos grupos de ratas, uno formado por animales aislados durante 30 días desde el final de la lactancia y un grupo control, que permaneció en grupos de tres animales durante el mismo período. Se analizó en los animales el metabolismo oxidativo cerebral del hipocampo (areas CA1, CA3 y giro dentado) mediante histoquímica para la citocromo c oxidasa (CO). Los resultados muestran un incremento significativo de la actividad CO en todas las regiones estudiadas en el grupo aislado, con diferencias entre las areas hipocampales en ambos grupos. Se discute la sensibilidad de la histoquímica de la CO en el estudio del posible papel activador del estrés en los animales aislados.

Social isolation and energy metabolism in rat hippocampus. Social isolation could be understood as a kind of sensorial deprivation. It is well known that enriched or complex stimular environments affect CNS at both anatomical and physiological levels. In our study, two groups of rats were used: one was comprised of isolated animals from the end of the lactation period during 30 days and a control group of animals housed in groups of three during the same period. Brain hippocampal oxidative metabolism was measured using cytochrome oxidase histochemistry (CO) in CA1, CA3 and dentate gyrus regions. The results show a significant increase of CO activity in all of the regions studied in the isolated group, with differences among the studied hippocampal regions in both groups. In this paper, the possible role of brain arousal caused by stress and the sensitivity of CO histochemistry to detect these changes are discussed.

The question of whether the environment can physically modify the brain has intrigued scientists from the XVII century until the present day. The contention about individual differences between men with different cognitive ability related with differences in cerebral structures has prompted the design of a number of experiments (Rosenzweig, 1979) and whether animals exposed to enriched environments differ with respect to their behaviour, neuroanatomy and neurochemistry from those brought up in an impoverished environment (Bennet et al., 1964; Hubel, Wiesel and Levay, 1997; Parks et al., 1992; Olsson et al., 1994). Animals reared in enriched environments have been demonstrated to learn more quickly in

Correspondencia: Jorge L. Arias
Facultad de Psicología. Plaza Feijoo
33003 Oviedo (Spain)
E-mail: jarias@sci.cpd.uniovi.es

behavioural tests and present, in comparison with animals reared in an impoverished environment, increased brain weight, neural size, dendritic extension, glial proliferation and activity of certain neurotransmitters such as cortical acetylcholine (Parks et al., 1992). Likewise, manipulated animals have been shown to have earlier maturation of adrenocortical response (they secrete less corticosterone) than animals not manipulated in response to certain stress inducing stimuli (Levine 1957). Anxiety or stress is not only generated in animals by the presence of a physically aversive stimulus but also when faced with the prospect of this when the stimulus itself is absent. Social isolation is considered too as a kind of stress. Thus animals under isoleted environment show disorders of physiological parameters related with stress (Gardiner and Bennett, 1977). The reaction of organisms to stress is mediated by hypothalamus-pituitary-adrenal axis (Sutanto and Kloet, 1994). The action of this system is under control of the corticosteroid receptor system. The excess of glucocorticoids or stress hormones which occurs in animals is toxic for neurones, especially those of the hippocampus, a cerebral process closely associated with memory and spatial orientation (Sloviter et al., 1989; Stanford and Salmon, 1993). Changes occur in this cerebral region according to the different environmental conditions in which an animal is reared. Therefore, animals in environmental enrichment programmes show hippocampal changes presenting a thicker hippocampus (Walsh et al, 1992). Metabolic changes also occur with increased levels of nervous growth factors, NGF, (Mohammed et al, 1993) in these animals. With regard to cerebral energetic metabolism, a high energetic consumption has been recorded in areas such as the hippocampus compared to other cerebral regions, with differences recorded in the different areas of the hippocampus (Hevner and Wong-Riley, 1995). Several techniques are used to estimate energy metabolism of a certain brain region. One of the most used nowadays is 2-deoxyglucose incorporation as a marker of neuronal activity. On the other hand, a number of studies have been based on use of the mitochondrial enzyme cytochrome c oxidase (CO; ferrocytochrome c oxygen reductase; E 1.9.3.1), as a histochemical marker, enabling indirect estimation of the neuronal oxidative metabolism, an index of energetic consumption of ATP of any cerebral region (Wong-Riley, 1989). This technique has been used previously by our group to study spatial learning behaviour in the rat (López et al. 1995).

In the present study the CO histochemical technique is applied to a study of the oxidative metabolism of three hippocampal areas (dentate gyrus, CAl and CA3) of rats suffering social isolation for one month, aiming to check out whether with this technique it is possible to reveal changes in brain metabolism after social isolation compared to normal reared animals.

Materials and methods

In this experiment 14 male Wistar rats were used, 7 animals in each experimental group. Animals were maintained in standard conditions of temperature (23±1 ºC), humidity (50%) and light (photoperiod: 12 hours, from 8 to 20 h) with ad libitum food and water. The regulations of the *American Psychological Association* (1985) for the use and care of experimental animals were respected at all times. Rats were separated at 21 post natal days, coinciding with the end of lactation and animals from the isolated group were placed in individual cages of 26 cm x 26 cm x 16 cm. Animals in the control group were distributed into groups of three and placed in cages of 60cm x 39cm x 18cm. In oder to have the same number of animals in each cage, 2 males of the same

age were incorporated together with control animals.

EXPERIMENTAL CONDITIONS

Isolation

After being separated randomly from the litter the animals from this group were maintained in the same room as those of the enriched group. Manipulation of the animals was restricted to once per week to change the sawdust. These conditions were maintained for 30 days.

Control Group

Rats belonging to this group were randomly selected from the males in the litters. These were reared in groups of three animals per cage for 30 days.

After this 30 day period, animals were anaesthetized by inhaling ethylic ether and vascular perfusion via the left ventricle with 0.1 M (pH 7.6) phosphate buffer. Brains were sectioned in the coronal plane and the portion between the optic chiasma and the caudal zone of the hippocampus was selected, in accordance with Paxinos and Watson atlas (1986) and covered with 0.C.T. (® Miles Inc., U.S.A.), frozen with freon-22 and stored at -70 ºC. These selected portions were sectioned in a cryostatic microtome at -20 ºC into sections 20 (m thick. Sections were histochemically stained for CO in accordance with the Wong-Riley method modified by Sukekawa (1991), based on labelling this enzyme with the marker diaminobenzidine.

CO activity was quantified using the method of González-Lima and Cada (1993) modified by González-Pardo et al. (1996). This basically consisted of introducing, together with the sections of nervous tissue, other sections of homogenized rat brain in the same staining bath. Previously, the real CO activity of the homogenate was determined spectrophotometrically according to the method of Wharton and Tzagoloff (1967). It was, therefore, possible to associate the intensity of histochemical staining with the real CO activity measured in specific units (1 specific unit = 1 (mol of oxidized cytochrome c per minute and gramme of tissue in wet weight and at pH 7.0 and 23 ºC). Using different homogenate standards with different CO activities it was possible to draw up a calibration line between the CO units and the relative intensity of histochemical staining measured by optic densitometry. Optic density of the homogenized sections stained for CO was determined by image analysis and densitometry with a computer (IMCO-2, Microm España) connected to an optical microscope. Finally, using this equipment the optical density of the sections of the hippocampal structure were quantified. In order to determine the region to be quantified we used alternate sections to those used for measuring CO activity but stained according to Nissl. The optical density obtained was converted into CO activity by first applying the calibration equation. A total of 4 sections per animal and hippocampal region studied were used.

STATISTICAL ANALYSIS

The data referring to the CO activity of the different hippocampal areas studied in both experimental groups were statistically treated by applying bifactorial analysis of variance (ANOVA) of repeated measures. When any of the factors was statistically significant Tukey's test (DHS) was then applied to the data.

Results

The animal group maintained in the control environment showed decreased CO activity in the hippocampal areas studied (CAl,

CA3 and dentate gyrus) compared to the isolated group (Fig. 1). The statistical tests applied reveal that there were significant differences between both experimental conditions applied ($F_{1,12}$ =40.7; P<0,001), and also between the different areas of the hippocampus ($F_{2,24}$ =18.13; P<0,001). On the other hand, within each experimental group, area CA3 maintained significantly higher levels of metabolic activity with CA1 area ($p<0.05$; Tukey test) and dentate gyrus area ($p<0.05$; Tukey test), showing similar levels of activity CA1 and dentate gyrus regions (Fig. 2).

Discussion

Many studies have focused on the hippocampus and some of these have used CO histochemical labelling (Wong-Riley, 1989). However, this is the first time this technique has been used to determine oxidative metabolism of these hippocampal areas (CA1, CA3 and dentate gyrus) after modification of the animals' environment as described here.

Morphological and biochemical changes have been described after rearing animals in enriched environments (Paylor, et al 1992) compared to animals kept in isolation (Paylor et al, 1992).

The results obtained in our study show a marked difference in CO activity between the isolated group and that maintained in normal conditions, with higher CO activity being recorded in the former. The isolated animals do not develop social contacts like the other group, but the fact that they are isolated would generate a feeling of stress which would result in an increase in short term cerebral activity. In the literature we have found studies which demonstrate an increased response of animals in isolated conditions due to stress (Mohammed et al, 1993). During the period of isolation stress may be manifest by increased encephalic activation translated into an increase in the spontaneous firing rate of neurones. The state of anxiety produced by an increase in cortisol blood levels appears to have a considerable effect on the hippocampus due to its high levels of glucocorticoid receptors (Sapolsky, Krey and McEwen, 1986). The role of this brain structure in neuroendocrine feedback circuit is well known (Meaney et al, 1992). Other authors have described an increased consumption of cerebral glucose in humans measured by TEP (Reiman et al., 1971). It is also known that the excess of glucocorticoids in the blood of animals suffering stress increase glucose consumption in the organism due to a decreased sensitivity in insulin receptors (Rizza et al, 1982; Mandarino and Gerich, 1992). Moreover,

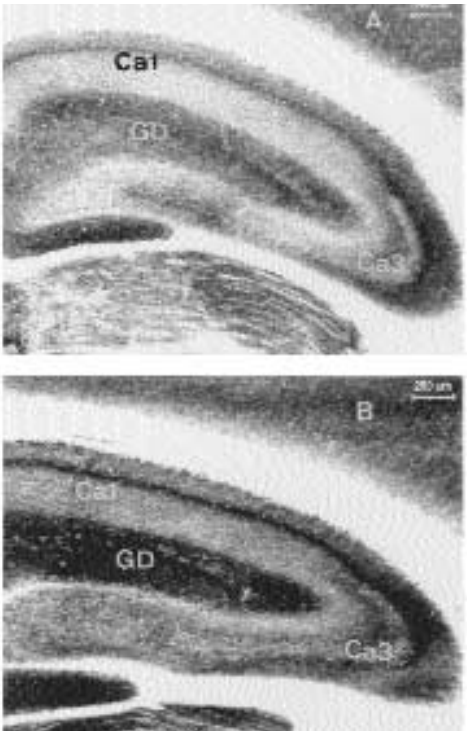

Figure 1. Photomicrographs showing coronal sections of hippocampus stained histochemically for cythochrome oxidase. Dark areas are more active. A. Control group. B. Isolated group.

the association between the rate of spontaneous neuronal firing and CO activity (Wong-Riley, 1989) is also known. This phenomenon brings about increased ATP consumption by the post-synaptic neurone, necessary for the reestablishment of the resting potential after stimulation and thus increased CO activity. It is, therefore, not altogether unexpected that this group presents higher CO activity. In fact, other authors have shown that increased cerebral activity in response to electric shock in rats manifests as increased CO activity in regions of the limbic system, to which the hippocampus belongs (Nobrega et al., 1993).

On the other hand, with respect to CO activity in the different regions studied, the difference in activity demonstrated by other authors in areas CA1 and CA3 (Hevner and Wong Riley, 1995), is confirmed by this study. One possible explanation for the decreased metabolic capacity of area CA1 is the greater sensitivity of its neurons to the external aggressions to which this hippocampal region is frequently exposed (Dell´Anna et al, 1991; Mennel and Müller, 1994).

Finally, this histochemical technique is clearly valuable in studying the changes which takes place in the brain as a result of different treatments, conditions or illnesses, a clear example of which are the changes in CO activity found in individuals with Alzheimer's disease (Simonian and Hyman, 1994; Kish et al. 1992).

In summary, changes in the metabolic activity of the hippocampus after maintaining rats in two opposing experimental conditions are confirmed. This could indicate the decisive role played by an external stimulative environment and the effects of socialization, not only on cerebral structure, but also on its physiology.

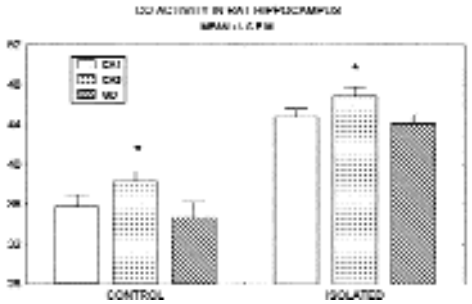

Figure 2. Cytochrome oxidase activity measured in the different hippocampal regions studied in an isolated rats and a control group * P<0.05.

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