Functional Magnetic Resonance Imaging: a study of malnourished rats

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Abstract

Malnutrition is a main public health problem in developing countries. Incidence is increasing and the mortality rate is still high. Malnutrition can leads mayor problems that can be irreversible if it is present before brain development is completed. We used BOLD Functional Magnetic Resonance Imaging to investigate the regions of brain activity in malnourished rats. The food competition method was applied to a rat model to provoke malnutrition during lactation. The weight increase is delayed even if there is plenty milk available. To localize those regions of activity resulting from the trigeminal nerve stimulation, the vibrissae-barrel axis was employed due to the functional and morphological correlation between the vibrissae and the barrels. BOLD response changes caused by the trigeminal nerve stimulation on brain activity of malnourished and control rats were obtained at 7T. Results showed a major neuronal activity in malnourished rats on regions like cerebellum, somatosensorial cortex, hippocampus, and hypothalamus. This is the first study in malnourished rats and illustrates BOLD activation in various brain structures.

1 Introduction

The discovery of the cerebral processes of cerebral maturation in mammals, have held the opportunity to investigate that exists a certain vulnerability in the cerebral development when there is a malnourished problem, causing cerebral damage [1]. Among the important is that we can see damage at both morphological and neurotransmitter levels [1].

Different studies in malnourished animal models, such as post-mortem, histological and imaging have helped to discover some morphological changes including a decrease in the prenatal brain size [2], the postnatal brain size [3], the hippocampal neural cells [4], the granular volume at the cerebellar cortex [5], abnormalities in the dendritic spines at the cerebellar cortex [6], reduction in the myelin content [7], and the protein synthesis of myelin [8], among others.

Other specific consequences in brain function are principally alterations in neurotransmitters. Reduction of serotonin levels [9] [10], increase of GABA concentrations at the hippocampus [11], reduction in the cholinergic cells [12], increase of the AChE at the hippocampus [13], decrease in the segregation of dopamine [14] [15], have been found such as hypothalamus, hippocampus, and cerebral cortex, areas related to memory and learning.

Almost all of the functional in vivo records have been made with electroencephalography methods, using both, conventional and invasive [1]. Electroencephalographic methods can give us many noisy

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signals that can lead into a malinterpretation of results if there is not an expertise. The quality of the electroencephalographic signals depends on the type and number of electrodes employed, that can carry out problems at the moment of placing them. We have to consider that at the moment of inserting the electrodes we can damage some brain tissues. Magnetic Resonance Imaging (MRI) is a powerful imaging tool which has many advantages in comparison to other imaging techniques. MRI is a non-ionizing technique, able to produce anatomical and functional information. Image contrast depends on the tissue intrinsic characteristics making it ideal to study brain activity. In this work, we used functional Magnetic Resonance Imaging (fMRI), which provides a method for mapping brain functional activity based on the blood oxygen level-dependent effect (BOLD). The BOLD MRI technique, allows indirectly to study the whole brain activation, in an animal model under a malnourished program.

As far as we know, this is the first fMRI study on malnutrition. To provoke malnutrition, the food competition method was applied [16]. This method consists in inducing malnutrition during lactation through food competition. A large number of pups cannot be sufficiently fed by one nursing mother. Then, a delay in the weight increase is observed even if there is plenty milk available. We chose the stimulation of the vibrissae-barrel axis because it is suitable for studying structure, function, development and plasticity within the somatosensory cortex, due to the functional and morphological correlation between the vibrissae and the barrels [17].

2 Materials and methods

2.1 Animal preparation

All animal procedures were performed according to the federal guidelines of the Animal Care and approved by the local authority. Twelve male Wistar rats (provided by the closed colony of breeding of the Division of Biological and Health Sciences of the Autonomous Metropolitan University, Mexico) were used. The animals were kept in an environment at a temperature of 22 - 25°C, with 45% relative humidity. Animals were kept under 12 hours controlled light-darkness cycles. Animals were divided into two groups according to nutritional manipulation: control and experimental groups. Both groups consisted of 6 rats each (aged 18 to 21 days). To provoke malnutrition, the food competition method was applied [17]. Control rats weighted 41.94±4.9g and malnourished rats weighted 29.09±3.3g. The weights of the experimental group correspond to a second degree malnourished level, according to [16]. This setup can be seen in Figure 1.

Figure 1. Animal Setup employed for all BOLD experiments. Stimulating electrodes were placed on the whiskers (anode) and in the masticatory muscles (cathode). The anesthesia was delivered through the tube attached to the stereotactic frame-like. To minimise motion artefacts, the rat’s head and ears were fixed to an Agilent stereotactic frame. This setup was also used to administer anesthesia at 1% of isoflurane with 2 l/min of O2 during all the fMRI experiment. EKG, breathing monitoring, and rat’s temperature control (37°C ± 1°C) were done with a small animal monitoring and gating system (Model 1025, SA Instruments, NY, USA).
2.2 Trigeminal nerve stimulation

The trigeminal nerve (the fifth cranial nerve, also called the fifth nerve, or simply CNV or CN5) contains both sensory and motor fibers. It is responsible for sensation in the face and certain motor functions such as biting, chewing, and swallowing. Sensory information from the face and body is processed by parallel pathways in the central nervous system. In animal models, more specific in rodents, it has been used as an attractive model the whisker sensory system for studying this nerve. This structure helps us to study and understand structure, function, development, and plasticity within the somatosensory cortex [17].

2.3 fMRI Acquisition and data processing

All experiments were performed on a 7T/21cm Agilent system (Agilent Technologies, Inc, Palo Alto, CA) equipped with DirectDrive technology and a transceiver 16-rung birdcage coil (12 cm long and a 6 cm diameter). Rat’s brain images were acquired using a standard gradient echo sequence and the following parameters: TR/TE=107.82/3.8 ms, Flip angle=200, FOV=30x30mm, matrix size= 128x128, thickness=0.3mm, and NEX=1, thus a spatial resolution of 0.23 x 0.23 x 0.3 mm3 was achieved. Gradient-echo BOLD fMRI has reasonably high spatiotemporal resolution can be routinely performed on this type of animal models at high field.

Images for BOLD fMRI were taken for both control and experimental groups. The left trigeminal nerve was stimulated using percutaneously inserted stainless steel electrodes, whose cathode was positioned in the whiskers and the anode was inserted in the masticatory muscles. The trigeminal nerve was stimulated using a stimulator with 10 ms constant current pulses (500 mV and 2 mA) applied every second (Grass S-48 Stimulator, Grass Technologies, RI, USA) and at 1Hz. 60s OFF was alternated with 60s ON periods [17].

To determine the regions of interest in the somatosensory cortex where neuronal activity is expected to happen, all brain images were digitally processed using the toolbox SPMMouse [18]. Data were overlaid onto anatomical images acquired in the same image plane. For comparison between control and experimental somatosensory cortex we used a p < 0.005, and for the analysis in the experimental group a p < 0.05 was used, for estimating the SPM results.

3 Results

3.1 fMRI maps

To study the possible areas of activation, representative activation maps of stimulus-correlated intensity changes in the rat brain of both groups were obtained. Fig. 2 shows five different brain regions from the olfactory bulb to the cerebellum on both groups, so we can appreciate different activation between groups. Schematics diagrams taken from Paxino’s rat brain atlas were added to facilitate localization of the coronal cuts in the rat’s brain [19]. Under normal conditions, the expected responses are similar to the response of the control group, as shown in Figure 2.
This is an important activation only in somatosensory cortex per se. But in the case of a malnourished brain, it can be appreciated responses from other brain structures, such as hippocampus, hypothalamus, and cerebellum besides somatosensory cortex. In general, all BOLD fMRI maps of malnourished rats show more activation than those in the control group in the entire brain. However, the brain may be regulated by a fine mechanism of excitatory and inhibitory entries at the interneuron level [20]. Our results indicate an abnormal increment in the electrical activity among different regions of the cerebral tissue. This is probably the result of a decrease on the neural inhibitory discharges of the inhibitory postsynaptic potentials (IPSP) and/or an increase in the excitatory postsynaptic potentials (EPSP) [20]. Results reported by Segura and collaborators [21] demonstrated the existence of a demyelinating process in the peripheral nervous system in malnourished rats and, that a similar effect is happening at the encephalic level. Malnutrition probably is affecting the inhibitory interneurons, so that their demyelination processes cause a decrease in the IPSP, and then increasing the neuronal activity in a random way as depicted in Figure 2. From our results, it is not yet possible to determine if abnormal activation from different brain regions is a consequence of brain’s tissue damage or these activations are the result from adaptive process of neural plasticity. The changes are so dramatic that suggest the existence of preexistent synaptic pathways that are not normally expressed, while in a brain of a malnourished subject these pathways are unmasked.

3.2 BOLD response

Control and experimental data at the somatosensory cortex were then fitted to a non-linear regression over time and shown in Figure 3. Nonlinearity of the BOLD response is observed for both groups. There is great similarity in the pattern of BOLD response for both groups. These results showed a great concordance with results already reported by Silva and Korestky [22]. The BOLD responses experiment a similar time delay for both groups and reach their maximum value at 5 seconds during the stimulation. Experimental group results suggest larger oxygen consumption when compared with the control group. Fig. 3c) shows the averaged BOLD signal intensity time course for control and experimental groups were computed with 6 rats: 30 seconds per rat. A sign change can be appreciated between the regions I and II of Fig. 3.c), roughly after 12 s for both cases.
This has been already reported as a negative BOLD response and it is caused by inhibition mechanisms [23]. A t student test was run to investigate statistical independence of the measurements of the response amplitude. At the 0.05 level, the difference of the population means (mean\textsubscript{control}=1879.39 & mean\textsubscript{exp}=1254.34) is statistically different. In order to quantify the BOLD response of the malnourished rats, the method reported in [24] was used. Then, the response amplitudes were calculated from the peak of BOLD response intensity. Additionally, the response integral was defined as the product of the amplitude and the full width at half maximum (FWHM) for both groups of rats the response integral values are 5638.18 and 3763.01 for the control and malnourished rats, respectively. Also a simple trapezoid-integration under the positive curve was made; the results were 20460.15 and 13708.85 for control and malnourished rats, respectively. This parameter indicates a clear increment in the neuronal activity for the malnourished rats.

We also analyzed responses in the experimental group, in Figure 4, we can see BOLD responses for different structures that were activated, and we can see responses from cerebellum, hypothalamus, hippocampus, and somatosensory cortex. We made the same measurements of area under the positive curve of the mean responses and the results obtained by simple trapezoid-rule integration were: cerebellum≈21625.49, hypothalamus≈15606.45, hippocampus≈15272.86, and somatosensory cortex≈13708.85.
Figure 4. Adjusted BOLD signals from different brain areas from experimental (malnourished) group. Also there is represented the mean BOLD signal from each brain areas. All figures show same maximum intensity scale so we can notice the differences between structures. The lower figure show areas from which this signals were taken.

A similar behavior was obtained with the FWHM method, described for the comparison between control and experimental groups; in the malnourished analysis the results were cerebellum\(\approx 5936.09\), hypothalamus\(\approx 4283.9\), hippocampus\(\approx 4192.33\), and somatosensory cortex\(\approx 3763.01\). A greater area at the cerebellum region can be appreciated, hypothalamus and hippocampus have a very similar behavior, and somatosensory cortex area has a lower area in comparison to the cerebellum one, and larger area means a greater activity in the region. Our results agree with the results obtained with invasive techniques and demonstrated that cerebellum is one of the structures more affected by malnutrition as demonstrated by Hillman [5]. Other structures related to learning and memory processes are also affected because of the malnutrition process, such as hippocampus and hypothalamus [2-14]. Finally a three-dimensional rendering process was computed to observe anatomical differences between both groups. In Fig. 5 we can clearly observe significant differences in brain structure, mainly at the cerebellum development. Also we can notice a big difference at the development of both brain hemispheres.

Figure 5. 3D reconstruction from the anatomical images obtained from the a) top row, control and b) bottom row, experimental group. We can see an important difference between both brains, at the cerebellum (solid line arrow), in which a notorious low development is present. Also an irregular development from both cerebral hemispheres (dashed line arrow) can be noticed in the experimental brain.
4 Discussion

This fMRI study is the first performed in rats and illustrates a similar pattern of BOLD responses between malnourished and control rats. Negative BOLD responses can be also appreciated for the two groups of rats caused by inhibition mechanisms. Vascular nonlinearities are the major contribution to the observed nonlinearities and they are neuronal in origin. Our results make a pathway to continue study this health problem and its consequences in brain function, because we cannot still say if the resulting changes in the brain correspond totally to a brain damage or if there is a process of adaptive plasticity.

Results obtained were not the expected ones, although it has been described brain damages by malnutrition in structures like cerebellum, hypothalamus, and hippocampus; according to the experimental protocol used. We only expected limited alterations at the somatosensory cortex at first, and no significant changes in the function of other structures. The brain changes observed with this experiment may imply that the mechanisms of neurotransmitters like GABA (inhibitory) or Glutamate (excitatory) are affected, but others neurotransmitters like ACh, Dopamine, or Noradrenaline may be not working as they should.

Also, our results may imply that exist problems with the hypothalamic pathway with other structures. These pathways are shown in the brain scheme of Fig. 5. These problems may be involved with the neurotransmitters cycle.

5 Conclusion

We used BOLD fMRI and trigeminal nerve stimulation to study the BOLD changes during sensory stimulation in malnourished rats. This study is the first performed in malnourished rats and illustrates BOLD activation in various brain areas, specially cerebellum is the structure with more activation. Further investigation should be carried out to determine whether plasticity or demyelination, or even combinations of both are responsible for the activation of the brain areas reported in this work. These results may pave the way into the treatment of this health problem and other possible rehabilitation procedure.

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