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Research report

Areal and laminar variations in the vascularity of the visual, auditory, and entorhinal cortices of the developing rat brain

Helen Michaloudi[a,*], Ioannis Grivas[a], Christos Batzios[b], Maria Chiotelli[a], Georgios C. Papadopoulos[a]

[a]*Laboratory of Anatomy and Histology, Veterinary School, Aristotle University of Thessaloniki, Thessaloniki 54124, Greece*
[b]*Laboratory of Animal Production Economics, Veterinary School, Aristotle University of Thessaloniki, Thessaloniki 54124, Greece*

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Abstract

Understanding of place-specific cortical cerebrovascular changes after insult and injury depends on the detailed knowledge of the areal and laminar variations in cortical vascularity. The present study examines comparatively the developmental changes of the total vascular density and the density of capillaries and medium- and large-sized vessels in the primary visual cortex (Oc1), the primary auditory cortex (Te1), and the lateral entorhinal cortex (EntL) of the developing rat brain. Vascular networks in the three cortical areas were marked after transcardial perfusion of India ink and quantified with an image analysis system. Parameters examined exhibited (i) peculiar developmental time course within individual cortical layers and (ii) area- and age-dependent variations. Angioarchitecture in Te1 layers was stabilized earlier than that in Oc1 layers and the period of postnatal development of the vascularity of neocortical sensory areas Oc1 and Te1 appeared to be more protracted compared to that of the phylogenetically older entorhinal cortex. By the end of the first postnatal month, vascular densities in the three cortical areas established a dorsoventral gradient (Oc1 > Te1 > EntL). Finally, in all areas, layer IV was the first layer to obtain adult values of capillary density.

Theme: Development and regeneration
Topic: Cerebral cortex and limbic system

Keywords: Laminar variations; Areal variations; Vascularity; Quantitative analysis; Development

1. Introduction

It is known that structural and functional differentiation of the developing mammalian cerebral cortex is accompanied by vascular proliferation and increases in metabolic activity [5,11,38]. However, despite existing evidence that the process of vascular sprouting during cortical development correlates in time and place with specific structural and functional changes [9,16,32], the study of the developing cortical angioarchitecture has attracted limited attention and much of what is presently known about cortical vascularity derives from qualitative or semiquantitative relative studies in the visual cortex [2,4,19,31,32]. Detailed comparative information on how development of cortical vascular parameters proceeds in cytoarchitectonically and functionally distinct cortical laminae and areas is practically missing.

The present study aimed to examine comparatively areal and laminar variations in the vascular bed of the developing primary visual, primary auditory, and lateral entorhinal cortices of the rat brain. Functional, phylogenetic, and ontogenetic characteristics of these regions provide the appropriate cortical substrate to study comparatively qualitative and quantitative aspects of the developing cortical angioarchitecture in two differentially maturing neocortical regions and in one paleocortical region, in which, among other differences from neocortical regions, neurons tend to be generated earlier than more medially placed neurons [7].

* Corresponding author. Fax: +30 2310999842.
E-mail address: emichal@vet.auth.gr (H. Michaloudi).

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Detailed quantitative developmental profiles of vascular network in various cortical laminae and areas could eventually contribute to the understanding of place-specific cerebrovascular changes that occur after insult and injury, i.e., the existence of regional differences in cerebral angiogenesis after severe chronic hypoxia [29], the selective vulnerability or resistance to cell death in particular layers after injury, brain ischemia, and reperfusion [40], and the laminar distribution of neuropathological lesions found in aging and dementia of the Alzheimer type [8].

In order to study quantitatively the vascularity of the primary visual cortex (Oc1), the primary auditory cortex (Te1) and the lateral entorhinal cortex (EntL) from postnatal day (P) 1 to P60, vascular networks, marked after transcardial perfusion of India ink, were analyzed with the use of an image analysis system linked to a photomicroscope. Measured parameters included total vascular density as well as density of capillaries and medium- and large-sized vessels.

2. Materials and methods

Laminar vascularity of the Oc1, Te1, and EntL cortices was measured in Wistar rats at postnatal days 1, 8, 14, 21, 31, and 60, following the protocol of Yu et al. [42]. A total of 22 animals, three to four from each age group, were used. Briefly, animals under deep ether anesthesia were perfused transcardially with India Ink, preceded by a phosphate-buffered solution containing 0.4% $NaNO_2$, and followed by 10% phosphate-buffered (0.1 M) formalin. $NaNO_2$ is a vasodilator and was given prior to formalin perfusion to compensate occurring vasoconstriction and cerebral hypoperfusion before death. The brain was removed, sliced into blocks, and postfixed in the same fixative overnight. Brain blocks including the Oc1, Te1, and EntL cortices were cut in transverse sections of 100 μm with a Vibroslice. Every second section was mounted on slide, counterstained lightly with cresyl violet, in order to facilitate subsequent identification of cortical regions and layers, cleared, and coverslipped with Entelan.

Quantification of the vascular parameters was performed with the aid of an image analysis system, linked to a Zeiss Axioplan photomicroscope. Twelve sections from each animal were analyzed for all cortical areas and all measures were performed on the same sections with the use of an objective lens of 10×.

For each section/area/layer(s) examined, the size of the analyzed area (area of interest) was manually adjusted each time to the size of the layer(s) examined.

Following categorization principles suggested previously [6,14,20,24], vessels were sorted as capillaries, having a diameter of ≤8 μm, and medium- or large-sized vessels, with diameters of 9–20 μm or >20 μm, respectively. Measured vascular parameters included vascular density (μm^2 of surface occupied by vessels per 100 μm^2 of cortex) and density of the various types of vessels (μm^2 of surface occupied by each vessels group, namely capillaries, medium- and large-sized vessels per 100 μm^2 of the cortex).

2.1. Statistical analysis

Both parametric and nonparametric methods were applied for the statistical analysis and evaluation of the experimental data. As all forms of parametric tests are based on the assumptions that the within-groups data are samples drawn from normally distributed populations with equal variances, both formal tests (Shapiro–Wilk and Lilliefors tests) and graphical displays (normal probability and detrended normal probability plots) were performed for assessing departures from Gaussian distribution, while variances were tested for homogeneity using Levene's test. For accessing the assumptions of normality and stability of variances, data were also transformed to $\log_e$, $\log_{10}$, or sqrt [43].

More particularly, a fixed-effect hypothesis model of analysis of variance was employed with three factors, namely (1) "age", (2) "area", and (3) "layer", to determine possible significant interaction effects between these factors on the mean vascular density of vessels. Variability in the raw data was divided into "types", namely (1) "additive" effects of factors and (2) "two-way interactions" between factors. Putative sources of variability were (1) main effects of age, (2) main effects of area, (3) main effects of layer, (4) two-way interactions of age with area, (5) two-way interactions of age with layer, (6) two-way interactions of area with layer. Higher-order interaction effects were eliminated and pooled into the Error Term.

Furthermore, one-way analysis of variance was used to evaluate possible significant effects of age, layer, and area on the total vascular density, and the density of capillaries, of medium- and of large-sized vessels, while the Kruskal–Wallis nonparametric test was applied where assumptions about either variability or the form of the populations distribution were seriously violated, with or no transformed data. Differences between mean values of specific groups were evaluated using Duncan's new multiple range test, or the nonparametric Wilcoxon rank sum test (Mann–Whitney U test) in case of non-normality and heterogeneity.

All analyses were conducted using the statistical software program SPSS for Windows (v. 11.0). Significance was declared at $P \leq 0.05$, unless otherwise noted.

All animal care and treatment followed the European Community Council Directive of 24 November 1986 (86/609/EEC) and the guidelines for animal experimentations of Aristotle's University Research Committee.

3. Results

Examination of the vascularization pattern in all developing areas revealed that at all ages relatively regularly spaced arterial trunks (arterioles) enter the cortex from the