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Neurocomputing 44–46 (2002) 823–829

NEUROCOMPUTING

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Classes and gradients of prefrontal cortical organization in the primate

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Abstract

Organization and function of the primate prefrontal cortices have been the subject of much discussion and speculation. We quantitatively analyzed the architecture of primate prefrontal cortices in order to provide more reliable foundations for this debate. Stereological data were obtained for more than 20 prefrontal areas in the rhesus monkey, including quantitative information on the densities of neurons, glia and different classes of neurons expressing calcium binding proteins as well as laminar depth. The results of multivariate data analyses indicated that the anatomical organization of prefrontal cortices is determined by categorical factors, such as the existence of a granular layer IV in some areas, and is also shaped by structural gradients, such as a systematic shift of cellular density from deep to superficial cortical layers progressing from medial and orbital to lateral areas. © 2002 Published by Elsevier Science B.V.

Keywords: Prefrontal cortex; *Macaca mulatta*; Quantitative neuroanatomy; Cellular density; Calcium-binding proteins

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0925-2312/02/$ - see front matter © 2002 Published by Elsevier Science B.V.
PII: S0925-2312(02)00479-4

1. Introduction

The prefrontal cortex in primates, which extends from the frontal pole to the premotor cortex, is composed of several structurally heterogeneous areas. The delineation of cortical areas traditionally relies on the recognition of unique morphological and functional features [10]. However, the differences in, for instance, cellular densities among areas of a local brain region might be subtle. Quantitative methods are needed to reliably distinguish architectonic areas on the basis of multidimensional structural parameters [11]. Such approaches might also help to overcome the many existing differences in prefrontal maps and nomenclature, which present a formidable problem in constructing central databases on the functional attributes and connections of areas obtained from different studies [9].

Furthermore, structural differences of prefrontal cortices may underlie the diverse computations of prefrontal cortices in complex cognitive, mnemonic and emotional processes, which have been the subject of continuing debates, e.g. [6,7]. In order to link these processes to prefrontal architecture, it is worthwhile to identify structural features shared by different prefrontal areas, and to recognize features that vary characteristically among prefrontal cortices. Here, we used quantitative approaches, comprising stereological data collection and multivariate analyses, in order to investigate how laminar, cellular, and neurochemical features separate or group prefrontal cortices and how the architectonic characteristics vary among different prefrontal areas of the rhesus monkey.

2. Methods

We employed stereological procedures to estimate the areal and laminar density of neurons, glia and of neurons positive for the calcium binding proteins parvalbumin (PV), calbindin (CB) and calretinin (CR) among 21 prefrontal areas or subdivisions of areas in the adult rhesus monkey (*Macaca mulatta*). We also determined laminar depth (DEP). For details see [5]. Criteria used to determine areal and laminar boundaries were based on architectonic features [2]. Data were derived from five to seven individual cases for each measure, and the data were obtained separately for four laminar subcategories (layer I, layers II–III, layer IV, if present, layers V–VI) for neuronal and glial density as well as laminar thickness, and for two laminar subcategories (layers I–III, layers IV–VI) for the densities of neurons positive for calcium binding proteins.

Three different multivariate analysis techniques were employed to assess global similarities and dissimilarities in the various morphological and cytological features of prefrontal cortical areas: (i) discriminant analysis (DA), (ii) nonmetric multidimensional scaling (NMDS), and (iii) hierarchical cluster analysis (HCA). DA identifies those experimental measures that show the smallest overlap and clearest separation of the distributions of individual data points belonging to different entities (that is, prefrontal cortical areas). NMDS arranges areas in a chosen low-dimensional (typically 2D or 3D) space, based on the pairwise correlation (dis)similarities between areas. The

relative proximity among items in an NMDS diagram represents their relative similarity. HCA hierarchically groups areas based on (dis)similarities in their parameter profiles, which are interpreted as spatial distances. The relative similarity of areas is expressed as the distance between two branching points in a cluster tree diagram; the longer the inter-branch distance, the more dissimilar are the subgroups. Both NMDS and HCA investigations employed squared area (dis)similarity matrices derived from the normalized laminar profiles by Pearson's correlation.

3. Results

At the outset of the data assessment, we performed a discriminant analysis DA, in order to identify the experimental measures that were most informative for distinguishing structural features of prefrontal areas. The DA determined that in particular the absolute values of neuronal densities were highly characteristic for identifying individual prefrontal areas. The analysis also suggested a grouping of prefrontal cortices into at least two clusters of similar regional density. These followed a division of granular orbitomedial and lateral areas, on the one hand, and agranular or dysgranular posterior medial and orbitofrontal areas, on the other [5]. The DA further suggested that some structural measures, such as the density of CR-positive neurons, contained very little additional information, as CR-positive neurons were uniformly distributed among all prefrontal cortices. As a result, this particular measure was excluded from the subsequent multivariate analyses.

Systematic exploration of the distribution of different structural measures indicated a number of global differences and trends, which are described in greater detail elsewhere [5]. Here we concentrate on the features that became apparent in the analysis of relative laminar areal profiles. These profiles were obtained by normalizing, for each area and each experimental measure, the absolute laminar values by their total sum across all layers. (e.g., for relative neuronal density of layer I:ND [layer I]/{ND [layer I]+ND [layers II–III]+ND [layer IV]+ND [layers V–VI]}). This analysis produced indicators for the relative laminar dominance of the different density measures or thickness across the cortical layers. To adjust degrees of freedom for subsequent analyses, one of the relative laminar coordinates resulting from the normalization was omitted.

Multivariate statistical techniques indicated that, despite the many experimental measures used to characterize prefrontal cortices, all areas were grouped into two main classes. Fig. 1 displays an NMDS arrangement of the prefrontal cortices according to the similarity of their normalized laminar profiles for ND, GD, DEP, PV and CB, omitting in all cases information for layer IV. Nevertheless, the dichotomy of the resulting groups corresponds to the existence or absence of a well-defined layer IV in different prefrontal cortices, putting agranular and dysgranular areas (that is, limbic cortices) to the left and eulaminate areas to the right of the diagram. A very similar result was obtained in an independent hierarchical cluster analysis of the same data [1]. Therefore, limbic and eulaminate areas must differ characteristically in further features apart from possessing a granular layer. In addition, Fig. 1 also shows the existence of a smaller

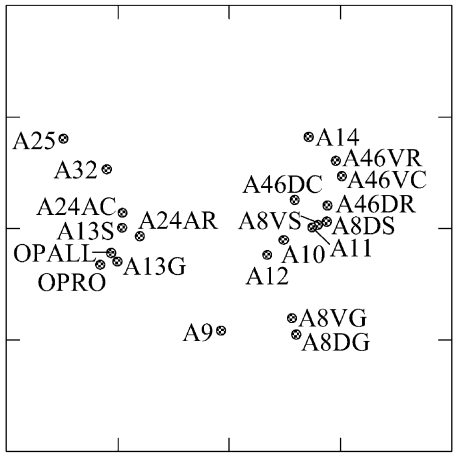

Fig. 1. Similarity (NMDS) plot of prefrontal area profiles, taking into account normalized laminar values for neuronal density, glial density, laminar depth as well as density of the calcium binding proteins parvalbumin and calbindin. The displayed arrangement of areas represents a very good fit of the areas' multivariate similarity in two dimensions: poorness of fit (Guttman/Lingoes coefficient of alienation) is: 0.06 and represented proportion of data variance (RSQ): 0.99.

separate group formed by areas 9 and gyral components of 8, as well as the shaping of the two main clusters by gradual factors (in roughly vertical orientation in Fig. 1).

We investigated these aspects in more detail by looking at laminar profiles for the most defining experimental measure, neuronal density, and considering the similarity of profiles when information for layer IV was either included or excluded. Inclusion of layer IV, as expected, produced a strict separation of cortices into the limbic and eulaminate groups (Fig. 2A). However, more gradual influences on the structure of prefrontal areas became apparent when information on layer IV was eliminated from the laminar profiles (Fig. 2B). The strikingly strict one-dimensional gradient apparent in Fig. 2B was found to correspond to the proportion of relative neuronal densities in superficial to deeper cortical layers (specifically the ratio of density in layers II–III to layers V–VI), with limbic areas such as A14 or A25 possessing a ratio smaller than one, and lateral eulaminate areas such as A8, > 1. Similar linear structural gradients could also be identified in the distribution of other architectonic measures, such as glial density (GD). In the case of GD, the gradient corresponded to the ratio of density in layer I to that in V–VI. Fig. 3 demonstrates how prefrontal cortices are characteristically distinguished by a combination of different structural gradients. Eulaminate areas, which are found entirely above the diagonal in the scatterplot, have a relatively greater neuronal density in their upper layers combined with a roughly balanced relation between GD in layer I and deeper cortical layers. By contrast, limbic areas, below the diagonal, are characterized by a larger content of neurons in the deeper layers compared to the upper ones, and a disproportionately large density of glia in layer I.

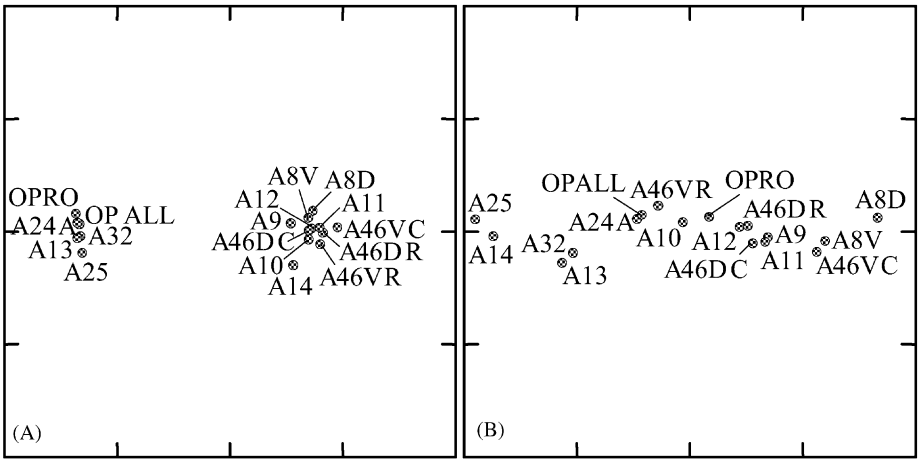

Fig. 2. Global similarity (NMDS) plot of prefrontal areas, based on normalized neuronal density in the different cortical layers. (A) Analysis of profiles including information on relative neuronal density in layer IV. Distortion by dimensional transformation (coefficient of 'alienation'): 0.003; data variability covered in 2D plot (RSQ): 1. (B) Analogous approach as in (A), but excluding neuronal density information for layer IV (coefficient of alienation: 0.012; RSQ: 1).

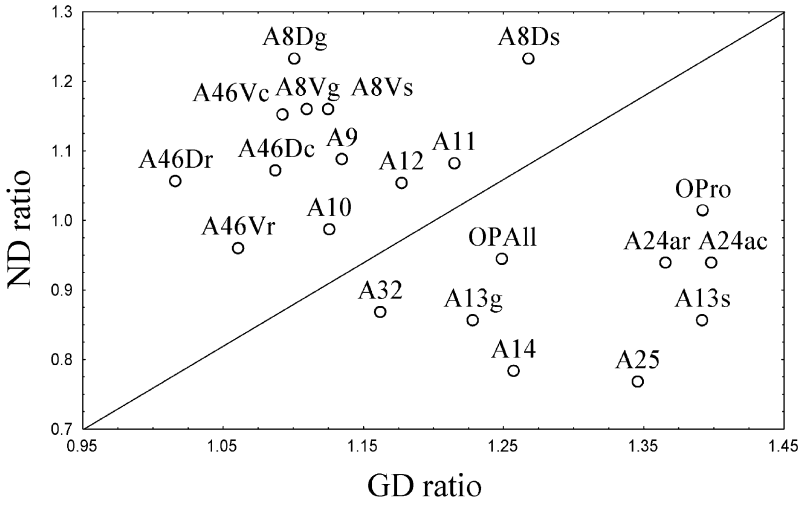

Fig. 3. Combination of structural gradients in prefrontal cortices. Represented in the scatterplot are gradients for ND (ratio relative laminar density layers II–III/V–VI) and GD (ratio relative density layers I/V–VI).

4. Discussion

Previous work has indicated the existence of several distinct classes (or 'types') of prefrontal areas, based on the number and definition of layers in the areas [3].

The current analyses confirm that the existence or absence of a granular layer IV is a characteristic structural indicator that can be used to group prefrontal cortices. Overlaying such categorical features are gradual structural trends, such as the shift of cellular density from deep cortical layers in medial and orbital areas to superficial layers in lateral areas, a characteristic trend to greater glial density in layer I of limbic areas. We found that combinations of these gradients also uniquely characterize limbic and eulaminate cortices (Fig. 3).

Categorical as well as gradual structural characteristics of different prefrontal areas may have their roots in development [5]. Limbic areas may complete their development earlier than eulaminate ones, at a time when cell cycle duration is longer and fewer cells migrate to the cortex [4]. This would explain the lower overall density of neurons in their upper layers, compared to eulaminate areas. In turn, the higher density of neurons in eulaminate areas is consistent with a prolonged development in lateral prefrontal areas, affecting mostly layer IV and the upper layers (II and III), which are formed after the deep layers, and at a time when more neurons migrate to the cortex; for review see [8].

Our results show that 'traditional' architectonic parameters, such as cellular densities or the depth of different cortical layers, can be used to characterize individual areas, and to reveal global structural features in the prefrontal cortex. These results support our idea that quantitative methods can provide an objective approach to construct maps, address differences in nomenclature across studies, establish homologies in different species, and provide a baseline to identify changes in pathologic conditions. Based on the current findings, we are now investigating the relationship between prefrontal structure and the organization of corticocortical connections among different prefrontal cortices, cf. [1,3].

Acknowledgements

Supported by grants from NIMH, NINDS, and the Wellcome Trust.

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