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# Rules relating connections to cortical structure in primate prefrontal cortex

# H. Barbas<sup>a,b,c,\*</sup>, C.C. Hilgetag<sup>d</sup>

<sup>a</sup>Department of Health Sciences, Boston University, 635, Commonwealth Ave., RM 431, Boston, MA 02215, USA <sup>b</sup>Department of Anatomy and Neurobiology, Boston University School of Medicine, 700 Albany Street W746, Boston, MA 02130, USA <sup>c</sup>NERPRC, Harvard Medical School, Boston, MA, USA <sup>d</sup>International University Bremen, Campus Ring 1, D-28759 Germany

#### Abstract

According to the structural model of prefrontal cortex, the pattern of corticocortical connections is intricately linked to cortical architecture (Cereb. Cortex 7 (1997) 635). We further explored this model by using quantitative methods to describe the structure and connections of prefrontal cortices. Multi-parameter analyses distinguished different cortical types, positioning at one extreme medial and orbitofrontal (limbic) cortices, and at the other extreme, lateral (eulaminate) cortices. The structural model accurately predicted the laminar pattern of connections, and the relative distribution of connections within cortical layers, based on cortical type. This model may provide the foundation to predict the nature of corticocortical processing and its disruption in psychiatric and neurologic diseases, where neuropathology affects specific types of neurons and layers. (c) 2002 Elsevier Science B.V. All rights reserved.

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# 1. Introduction

Neural connections within the cerebral cortex in primates form a massive and largely reciprocal communication system, underlying processes ranging from elementary sensory perception to complex processes of learning, memory and emotion (for review

<sup>\*</sup> Corresponding author. Department of Health Sciences, Boston University, 635, Commonwealth Ave., RM 431, Boston, MA 02215, USA.

E-mail address: barbas@bu.edu (H. Barbas).

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see [3]). It is, therefore, important to identify the neuronal populations involved in this extensive communication system and to determine if there are rules that govern its organization. In the multilayered cortex of primates, this question involves identification of the specific laminar origin and termination of connections. Laminar connection patterns are likely to have functional significance, since the chemical and physiological properties of neurons differ across layers within a single column of cortex (for review see [15]), so that projections originating and terminating in different layers are likely to interact with a different local environment.

# 1.1. Cortical structure and neural communication

It has become increasingly apparent that the organization of cortical connections is intricately linked to cortical structure (for review see [3]). Structure in this context refers to the classic parceling of the cortex into many architectonic areas, or into a few cortical types. Parceling into architectonic areas relies on analysis of cellular morphology and the relative distribution of cells in cortical layers, which give each area a unique signature. On the other hand, parceling the cortex by type is based on broad structural features shared by several architectonic areas, including the number and distinction of identifiable layers. However, classic architectonic approaches rely on subtle qualitative features, which may account for disagreements in different studies. Quantitative approaches are needed to describe reliably structural features and their relationship to cortical connections.

# 2. Methods and results

#### 2.1. Quantitative architecture

To circumvent methodological difficulties of qualitative approaches to describe cortical architecture, we used quantitative stereologic methods to investigate whether architectonic areas of the prefrontal cortex in the rhesus monkey can be characterized by a set of systematic criteria. We focused on features examined in classic architectonic studies, including the density of neurons and glia, as well as neurochemical markers for the calcium binding proteins parvalbumin and calbindin, which label distinct classes of cortical inhibitory interneurons and are useful in architectonic studies (e.g., [13]). We asked whether prefrontal areas have unique structural profiles, on the one hand, and whether groups of architectonic areas share similar features that may suggest common functions, on the other. We addressed the latter question by using multi-parameter analyses to determine if, and how, prefrontal areas form clusters when multiple features are considered simultaneously. The study of even a few architectonic features within the context of the complex areal and laminar features of the prefrontal cortex provided up to 18 parameter dimensions, as explained in greater detail elsewhere [9]. Conventional and multi-parameter statistical analyses distinguished at one extreme the agranular and dysgranular (limbic) type cortices, which were characterized by prominent deep layers (5-6), the lowest overall neuronal density, highest ratio of glia to neurons, the highest

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Fig. 1. Hierarchical cluster tree based on similarity of normalized laminar profiles of prefrontal cortical areas using experimental measures for: neuronal, glial, parvalbumin, and calbindin density in layers 1, 2-3, 5-6, and cortical depth.

density of calbindin, and the lowest for parvalbumin positive interneurons. At the other extreme, lateral eulaminate-type cortices were characterized by the highest density of neurons, a prominent granular layer 4, denser supragranular (2-3) than infragranular (5-6) layers, and a balanced distribution of neurons positive for parvalbumin or calbindin. Global similarities among prefrontal cortices in terms of these structural features are shown in the cluster tree in Fig. 1.

# 2.2. The structural model for corticocortical connections

The significance of cortical type can be linked to previous findings, indicating that the relative distribution of corticocortical connections in different areas is neither purely 'feedforward'(bottom–up) or 'feedback' (top–down) but is graded, and can be predicted on the basis of the broad laminar features of the interconnected areas (e.g., [2,5]). The structural model for the pattern of connections emerged from our findings that the pre-frontal cortical system is composed of areas belonging to different cortical types. Some prefrontal areas have three identifiable layers and lack a granular layer 4 (agranular cortex), others have four layers, including an incipient granular layer 4 (eulaminate cortex) [4].



Fig. 2. Summary of the pattern of connections predicted by the structural model. (A) Connections between cortices with large differences in laminar definition show a readily distinguishable pattern. Top: Projection neurons originate mostly in the deep layers of cortices with low laminar definition (e.g., agranular-type cortices, bottom cartoon) and their axons terminate mostly in the upper layers of cortices with high laminar definition (eulaminate areas). Bottom: The opposite is true for the reciprocal connections. (B) A less extreme version of the above pattern is predicted in the interconnections of cortices with moderate differences in laminar definition.

We tested the structural model for pairs of interconnected prefrontal areas, by classifying areas into five levels (types), based on the number and definition of their layers: (1) agranular; (2) dysgranular; (3–5) eulaminate areas with low (3), intermediate (4) and high (5) laminar definition. We then used the ratings to test if the structural relationship of pairs of connected prefrontal areas could predict the pattern and relative laminar distribution of intrinsic corticocortical connections (origin level-destination level= $\Delta$ ). Two predictions of the structural model were tested (Fig. 2). First, for most pairs of connected cortices, the model predicted accurately whether projection neurons would originate predominantly in the supragranular layers and terminate mostly in the deep layers (when  $\Delta > 0$ ), or originate predominantly in the deep layers and terminate



Fig. 3. The application of the structural model to interconnections of prefrontal areas. Normalized density of anterograde label in the deep layers (4–6) differed as a function of differences in level between pairs of connected areas (dots). Points -4 to -1 show terminations in areas with comparatively higher laminar definition than the origin (when  $\Delta < 0$ ); points 1-4 show terminations in areas with lower laminar definition than the origin (when  $\Delta > 0$ ). (From [5].)

mostly in the upper (supragranular) layers (when  $\Delta < 0$ ). We found that when areas with six layers and high laminar definition projected to areas with fewer than six layers or less laminar definition, projection neurons originated mainly in the upper layers (2–3) and their axons terminated predominantly in the deep layers (4–6). In contrast, in the reciprocal pathways when areas with fewer layers or lower laminar definition projected to areas with more layers or better laminar definition, projection neurons originated mostly in the deep layers (5–6) and their axons terminated most densely in the upper layers (1–3) [5]. Second, the structural model predicted that the relative distribution of projection neurons or axonal terminals within cortical layers would vary as a function of the number of levels between the interconnected cortices, or the value of  $\Delta$ , as shown for pairs of connected prefrontal cortices (Fig. 3).

#### 3. Discussion

Our findings indicated that a given prefrontal area does not have one, but rather many modes of anatomic communication, in a pattern that depends on the structural relationship of the interconnected cortices. The model has the advantage of predicting the connectional relationship of two areas solely on the basis of their respective architecture, and can be applied to the sensory and motor cortical systems as well, because their structure also varies systematically in primates (for review see [16]). Within the conceptual framework of the structural model, feedforward projections in sensory areas always originate in areas with higher laminar definition in comparison with the site of termination, while the opposite is true for projections proceeding in the reverse direction. We recently tested the structural model in the connections between prefrontal areas with medial temporal and inferior temporal visual areas [21], and superior temporal auditory areas [6], and the same patterns appear to hold.

The quantitative approaches to cortical architecture provided key insights into factors that define cortical structure, which may justify the use of similar approaches to cortical mapping. Further, findings based on quantitative approaches that combine structural features and connections are likely to have functional implications. In our own material, the differential prevalence of inhibitory interneurons that express parvalbumin or calbindin in different types of cortex has implications for inhibitory control in the cortex. Parvalbumin is expressed in neurons that are most densely distributed in the middle layers of the cortex, labeling basket and chandelier cells, which synapse with cell bodies, proximal dendrites, or the axon initial segment of pyramidal neurons (e.g., [8,14,23]). Calbindin positive neurons include inhibitory double bouquet cells, which are most prevalent in cortical layers 2 and 3, and innervate distal dendrites and spines of other neurons (e.g., [18]). Physiologic studies suggest that there are differences in the pattern of excitation and inhibition in different cortical layers, so that stimulation of 'bottom up' pathways (when  $\Delta > 0$ ), leads to monosynaptic excitation followed by disynaptic inhibition [10,23]. In contrast, in pathways that terminate in layer 1 (top-down, or when  $\Delta < 0$ ), excitatory influences predominate [22,23]. Corticocortical pathways originating and terminating in different layers are likely to interact in a microenvironment with a bias for interneurons that express parvalbumin or calbindin, which differ in efficacy in inhibitory control.

The relationship of axonal terminations to local inhibitory interneurons is particularly relevant for the prefrontal cortex, in view of its posited role in selecting relevant information and suppressing irrelevant information to guide behavior (for review see [11]). In a previous study we noted that most inhibitory interneurons in the prefrontal cortex labeled with one of the three calcium binding proteins were distributed in layers 1-3 in both eulaminate and limbic prefrontal cortices [9]. However, eulaminate and limbic areas differ markedly in the mode of their connections, according to the rules of the structural model [2,5,21]. In limbic areas the deep layers are the principal sources and targets of corticocortical connections, whereas in eulaminate prefrontal areas it is the upper layers that primarily issue and receive cortical connections [5]. This evidence suggests that there is a match in the preponderance of connections and inhibitory interneurons in eulaminate prefrontal cortices, but a mismatch in limbic areas. This pattern may have functional consequences, in view of the fact that inhibitory interneurons expressing calcium binding proteins also have an important role in sequestering, buffering, and transporting intracellular calcium (for reviews see [1,12]). The mismatch in the focus of connections and prevalence of inhibitory interneurons with calcium buffering capacity may provide an important clue as to why limbic areas have a predilection for epileptiform activity [17].

The presented evidence indicates that by virtue of their structure, limbic cortices issue projections mostly from their deep layers and target mostly the upper layers of eulaminate areas, suggesting a predominant role in feedback communication [2,5]. Because limbic areas are preferentially affected in several neurologic and psychiatric diseases, including Alzheimer's disease, epilepsy, schizophrenia, obsessive compulsive disorder and Tourette's syndrome (for reviews see [20,24]), their pathology is likely to disrupt a massive feedback system to the neuraxis. This would essentially change the ubiquitous bidirectional mode of neural communication into a unidirectional mode, with potentially profound consequences on behavior.

The structural differences that appear to underlie the pattern of corticocortical connections may arise during development. The lower overall density of neurons in limbic areas in comparison with the eulaminate areas can be explained if limbic areas complete their development earlier than the eulaminate, at a time when cell cycle duration is longer and fewer cells migrate to the cortex [7]. Conversely, the higher density of neurons in eulaminate areas is consistent with a prolonged developmental period in lateral prefrontal areas. Consistent with this idea is our finding that the higher density in eulaminate areas could be accounted for by a higher density in layers 4, 3 and 2, which are formed after the deep layers, at a time when more neurons migrate to the cortex (for review see [19]). The different pattern of connections in limbic than in eulaminate prefrontal cortices is also consistent with a differential temporal development of these cortices. If our hypothesis of differential development of limbic and eulaminate cortices is substantiated through further studies, it will have implications for diseases that have their root in development, including dyslexia, schizophrenia, and some forms of epilepsy, and may help explain the varied symptomatology in these diseases.

The combination of structural and neurochemical features and connections in future analyses may provide the basis for predicting the pattern of corticocortical connections in humans, where invasive procedures are precluded. These analyses may also be relevant to understanding the nature of the loss in cortical excitatory and inhibitory control in neurologic and psychiatric diseases where neuropathology affects specific types of neurons and layers (for review see [3]).

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Helen Barbas received a Ph.D. degree from McGill University and is now Professor of Neuroscience at Boston University and School of Medicine, and affiliated scientist at NERPRC, Harvard Medical School. Her research focuses on the prefrontal cortex and the pattern, organization and synaptology of prefrontal pathways associated with cognitive, mnemonic and emotional processes in primates.

**Claus C. Hilgetag** studied Biophysics in Berlin and Neuroscience in Edinburgh, Oxford and Newcastle. He is now an Assistant Professor of Neuroscience at the newly founded International University Bremen. His current research focuses on computational analyses of neural architecture and connectivity, and on understanding the mechanisms of spatial attention and inattention in mammalian brains.