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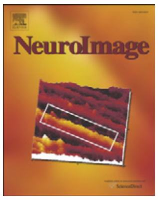

Cytoarchitectural differences are a key determinant of laminar projection origins in the visual cortex

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ABSTRACT

Regularity of laminar origin and termination of projections appears to be a common feature of corticocortical connections. We tested three models of this regularity, originally formulated for primate cerebral cortex, using quantitative data on the relative supragranular layer origins (*SGN%*) of 151 projections from 19 areas (~145,000 neurons) to four areas of cat extrastriate cortex. Predictive variables in the models were: hierarchical level differences (Barone et al., 2000), structural type differences (Barbas, 1986), and distances (Salin and Bullier, 1995) between areas. Global and local hierarchies of cat visual cortex were used to evaluate the hierarchical model. Ranking of areas by their cytoarchitectural differentiation (e.g., relative prominence of layer IV) allowed testing of the structural model, while the distance model was tested for the number of borders separating areas. Laminar projection origins correlated moderately with hierarchical differences, and poorly with border distances, but were strongly and consistently correlated with area differences in cytoarchitectural rank. Moreover, projection densities were moderately and negatively correlated with area distances and structural differences. Our findings suggest that the relative cytoarchitectural differentiation of cortical areas is the main determinant of laminar projection origins in cat visual cortex, and may underlie a general laminar regularity of mammalian cortical connections.

Introduction

Laminar origins and terminations of connections between areas of the mammalian cerebral cortex show a remarkable regularity. This regularity is most clearly demonstrated by the fact that the areas of sensory (e.g., visual, somatosensory) and motor cortex can been arranged into largely consistent global schemes, in which laminar connectivity patterns believed to signify projections in one of three possible directions ("forward," "backward" or "lateral") are repeated across the whole arrangement (Felleman and Van Essen, 1991; Hilgetag et al., 2000b; Scannell et al., 1995). The classification of "forward," "backward," and "lateral" projections is based on the predominant layers of projection origin and termination. Projections have been labeled "forward" if they originate mainly from neurons in upper cortical layers (II + III) and terminate in the granular layer (IV) of their target, while complementary "backward" projections arise mainly from deep cortical layers (V + VI) and terminate outside layer IV of the target area (Rockland and Pandya, 1979). Finally, "lateral" projections originate in a more bilaminar pattern involving roughly equal contributions from upper and deep layers and terminate across all layers, including layer IV (Felleman and Van Essen, 1991). While this classification was originally applied to connections of primate visual areas 17, 18 and 19 (Rockland and Pandya, 1979), later approaches extended the scheme to additional extrastriate cortices and other types of primate sensory cortex (Felleman and Van Essen, 1991), as well as visual cortex in the cat (Felleman and Van Essen, 1991; Scannell et al., 1995) and rat (Coogan and Burkhalter, 1993).

Quantitative studies have demonstrated that there are also gradual shifts in the laminar projection origins within each of these categories. For example, the relative contribution of upper (supragranular) layers to a "forward" projection may vary from 100% to little more than 50%

Abbreviations: AEV, anterior ectosylvian visual area; ALLS, anterolateral lateral suprasylvian area; AMLS, anteromedial lateral suprasylvian area; Δ_{dist}, border distance; Δ_{hier}, structural type difference; Δ_{type}, hierarchical level difference; DLS, dorsolateral suprasylvian area; ER, entorhinal; *IGN*, absolute number of infragranular neurons; *IGN%*, relative number of infragranular neurons; Ins, insular; Its, suprasylvian integration belt; *LB*, laminar bias measure; LS, lateral suprasylvian; MSG, middle suprasylvian gyrus; MSS, middle suprasylvian sulcus; MT, middle temporal area; *N*, absolute number of neurons; *N%*, relative number of neurons; PCG, posterior cingulate gyrus; PeV, peristriate belt; pL, paralimbic; PLLS, posterolateral lateral suprasylvian area; PMLS, posteromedial lateral suprasylvian area; PS, posterior suprasylvian area; *SGN*, absolute number of supragranular neurons; *SGN%*, relative number of supragranular neurons; Ss, suprasylvian sulcus; Ssi, suprasylvian sulcus belt, intermediate division; Ssp, suprasylvian sulcus belt, posterior division; Ssv, suprasylvian sulcus belt, ventral division; SVA, splenial visual area; VLS, ventrolateral suprasylvian area; WGA-HRP, wheat germ-agglutinated horseradish peroxidase.

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(Barone et al., 2000; Grant and Hilgetag, 2005; Vezoli et al., 2004) with the remainder coming from the deep layers. Moreover, there exists considerable variability of projection patterns at the level of individual neurons (Rockland, 1997, 2003).

What explains the systematic but varied patterns of laminar projection origins? Three principal models have been proposed. First, the graded distribution may depend on the hierarchical level difference between the linked areas in global cortical hierarchies (Barone et al., 2000; Vezoli et al., 2004). Such hierarchical schemes (Felleman and Van Essen, 1991) are popular summaries of the organization of cortical association fibers and their putative function, despite caveats for their construction (Hilgetag et al., 1996) and interpretation (Hegde and Felleman, 2007). In the hierarchical model (Barone et al., 2000) it is argued that projections with a strong laminar bias of origin (i.e., close to 100% upper or deep layer) occur between areas occupying the top and bottom levels of a global hierarchy, while projections of more equal laminar origin (i.e., approximately 50% bilaminar) occur between areas on the same or adjacent hierarchical levels.

In the second, structural model it is proposed that graded laminar patterns derive from differences in the cytoarchitectonic differentiation of the cortical areas forming the source and target of a given projection (Barbas, 1986; Barbas and Rempel-Clower, 1997; Medalla and Barbas, 2006; Rempel-Clower and Barbas, 2000). Specifically, projections from structurally more differentiated to less differentiated areas arise predominantly from the upper cortical layers, while projections in the reverse direction originate mainly from deep layers, and projections between architecturally similar areas possess a balanced bilaminar character (Barbas, 1986).

Finally, it has been an influential idea that the existence or absence of inter-connections between cortical areas (Klyachko and Stevens, 2003; Young, 1992) as well as laminar projection features (Salin and Bullier, 1995) vary systematically with physical distance and the number of intervening area borders.

So far, these different models have been primarily tested for connections of the visual cortex (hierarchical model) or prefrontal cortex (structural model) or both (distance model) in the primate brain. Here an opportunity arose to test all three models on the same quantitative material concerning both the relative densities (i.e., strength) and laminar origins of widespread projections to cat extrastriate visual areas (Grant and Hilgetag, 2005; Hilgetag and Grant, 2000). This allowed us to directly evaluate the selective applicability of these concepts for graded laminar connections in another class of mammal with a well developed cerebral cortex.

Materials and methods

Projection data

Quantitative measures of relative projection densities and laminar origins were based on published connection tracing data derived from WGA-HRP injections in the vicinity of the middle suprasylvian sulcus (MSS) in 11 adult cats; 10 with single injections in different areas (PMLS, AMLS, PLLS) of the lateral suprasylvian (LS) cortex, and one with a similar injection in area 21a which adjoins area PMLS caudally. Each injection resulted in the labeling of projection neurons in 13–19 identified cortical areas. A total of 151 projections were evaluated from counts of ~145,000 of labeled cells (from an average of ~13,000 per injection site). Detailed accounts of the experimental procedures and methods of quantification can be found in Grant and Shipp (1991), Hilgetag and Grant (2000), Grant and Hilgetag (2005).

The laminar origin of each projection was quantified as *SGN%* ($SGN\% = SGN/(SGN + IGN)^{*}100$), where *SGN* and *IGN* are the absolute numbers of neurons labeled in the supragranular (II + III) and infragranular (V + VI) layers, respectively, of a given area. Typically, no labeled projection neurons were found in the granular layer (IV), which provided a useful separator between supra- and infragranular layers. The *SGN%* measure thus quantified the laminar projection patterns, as used previously (e.g., Barbas, 1986; Barone et al., 2000; Grant and Hilgetag, 2005). The relative density, *N%*, of each projection was calculated by normalizing the total number of counted neurons in each projection (i.e., *SGN* + *IGN*) by the absolute number of neurons analyzed for each injection ($N_i\% = (N_i/\sum N_i)^{*}100$, where N_i was the absolute number of projection neurons labeled in a particular area a_i).

Five of the injections were in area PMLS (in different cats), and four in area PLLS (also in different cats). Data from these same-area injections were averaged in order to avoid repeated measure biases in subsequent correlation analyses. Averages were conservatively calculated as the median of *SGN%* or *N%* (for a detailed discussion of the variability and distribution of corticocortical projection densities see Hilgetag and Grant, 2000; Scannell et al., 2000). Table 1 provides the *SGN%* and *N%* values for all projections, together with the three investigated predictive measures which are introduced in the following sections.

Hierarchical ranking of cat cortical areas

Hierarchical ranks were assigned to each of the 19 projection origin areas according to the cat visual hierarchy shown in Hilgetag et al. (2000b, Fig. 12), which was compiled from a database of the anatomical literature (Scannell et al., 1995). The hierarchy was produced by arranging the connected areas in such a way that as many as possible “forward” connections (typically possessing a supragranular origin and a granular layer termination) pointed upwards, while as many as possible “backward” projections (possessing infragranular origins and terminating outside the granular layer) pointed down. Note that this scheme is only one optimal arrangement out of more than 20,000 arrangements that can be computed for the underlying qualitative laminar projection pattern. For a detailed account of the hierarchical analysis see Hilgetag et al. (2000b). We also computed and analyzed a unique, local hierarchy of MSS areas PMLS, PLLS, AMLS and 21a for the present quantitative projection data, as described in the next section.

Hierarchical model analyses

The concept proposed by Barone et al. (2000) suggests that the *SGN%* origins of cortical projections reflect a gradual metric of the difference in hierarchical rank between interconnected areas. The authors tested this idea with quantitative data for various projections to area V1 or V4 in the macaque, by rank-correlating the *SGN%* of the projection origins with the level difference between the respective connection source and V1 or V4, as appropriate. The hierarchical level difference Δ_{hier} (corresponding to $\Delta_{\text{hier}} = \text{level}_{\text{source}} - \text{level}_{\text{target}}$) was calculated from previous global hierarchical schemes for macaque visual cortex (Felleman and Van Essen, 1991; Hilgetag et al., 2000b). We applied an analogous approach to our quantitative data by rank-correlating the *SGN%* of projections from 19 cat visual areas to areas 21a, PMLS, AMLS and PLLS with the difference in the areas′ rank in the global hierarchical scheme of Hilgetag et al. (2000b, Fig. 12).

A principal problem with this approach is that a single, optimal global hierarchy cannot be determined for either macaque or cat visual cortex (Hilgetag et al., 2000b). For the cat, more than 20,000 alternative arrangements fit the available connectional data equally well. For this reason, we extended the hierarchical analysis to a unique, optimal hierarchy derived for the local connectivity of the four injected areas of the middle suprasylvian sulcus (MSS) cortex (cf. Grant and Hilgetag, 2005). In this scheme, 100% *SGN%* represents a strictly “forward” projection, 0% *SGN%* is considered strictly “backward,” and intermediate values either side of strictly “lateral” (i.e., 50% *SGN%*) are either increasingly “forward” (51 to 99% *SGN%*) or increasingly “backward” (49 to 1% *SGN%*). This definition can also be expressed by an index *LB* representing the “laminar bias,” where $LB = SGN\% - 50$.