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Temporal Specification and Bilaterality of Human Neocortical Topographic Gene Expression

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SUMMARY

Transcriptional events involved in the development of human cerebral neocortex are poorly understood. Here, we analyzed the temporal dynamics and laterality of gene expression in human and macaque monkey neocortex. We found that interareal differences exhibit a temporal hourglass pattern, dividing the human neocortical development into three major phases. The first phase, corresponding to prenatal development, is characterized by the highest number of differential expressed genes among areas and gradient-like expression patterns, including those that are different between human and macaque. The second, preadolescent phase, is characterized by lesser interareal expression differences and by an increased synchronization of areal transcriptomes. During the third phase, from adolescence onward, differential expression among areas increases again driven predominantly by a subset of areas, without obvious gradient-like patterns. Analyses of left-right gene expression revealed population-level global symmetry throughout the fetal and postnatal time span. Thus, human neocortical topographic gene expression is temporally specified and globally symmetric.

INTRODUCTION

The cerebral neocortex (NCX) is organized into functionally distinct sensory, motor, and association areas that provide the biological substrates underlying perception, behavior, and cognition (Brodmann, 1909; O'Leary and Sahara, 2008; Rakic, 1988; Rash and Grove, 2006; Sur and Rubenstein, 2005). While the basic architecture of this areal map is shared among mammals, important species-specific organizational differences have allowed for the elaboration of human-specific cognition and behavior (Hill et al., 2010; Judaš et al., 2013; Kaas, 2012; Kennedy and Dehay, 2012; Lui et al., 2011; Molnár and Clowry, 2012; Preuss, 2011).

Another key feature of the human NCX is that it covers the surface of the left and right hemispheres, each comprising a topographically matched, though slightly structurally and functionally asymmetric areal map (Amunts et al., 2003; Gazzaniga et al., 1962; Geschwind and Levitsky, 1968). This asymmetric organization plays a crucial role in functional lateralization of many cognitive and motor functions, such as language and handedness, between the hemispheres. Several lines of evidence indicate that these asymmetries are reflected at the molecular (Sun et al., 2005) and cellular (Amunts et al., 2003; Hayes and Lewis, 1993) levels. Structural asymmetry first appears during the late midfetal period (Chi et al., 1977; Kasprian et al., 2011) and becomes more prominent during early postnatal development when functional asymmetries become noticeable (Amunts et al., 2003; Hill et al., 2010).

Multiple lines of evidence indicate that distinct human neocortical areas, and the hemispheres as a whole, mature at different rates (Flechsig, 1901; Giedd et al., 1999; Giedd and Rapoport, 2010; Huttenlocher and Dabholkar, 1997; Sowell et al., 2003). For example, axons in primary sensory-motor areas start to myelinate before those in the association areas (Flechsig, 1901). Other processes such as synaptogenesis also exhibit prominent interareal differences in their maturational trajectories (Huttenlocher and Dabholkar, 1997). Furthermore, the right hemisphere appears to mature faster than the left during late fetal and early postnatal development (Taylor, 1969; Thatcher et al., 1987).

There is increasing evidence to suggest that processes regulating areal patterning and asymmetry, as well as the maturational trajectories of these processes, are affected in major psychiatric and neurological disorders (Cullen et al., 2006; Faludi and Mirnics, 2011; Piao et al., 2004; Rapoport and Gogtay, 2008). Moreover, the progression of certain neuropathologies follows a stereotypic areal pattern (Braak et al., 1993), indicating that the mechanisms involved in patterning and asymmetry may play a role in the manifestation of disease. However, little is known about these developmental processes in normal or diseased human brains, or how they differ among mammals, especially closely related nonhuman primates (NHPs).

Gene expression has previously been profiled in the developing human NCX (Abrahams et al., 2007; Colantuoni et al., 2011; Ip et al., 2010; Johnson et al., 2009; Kang et al., 2011;

Lambert et al., 2011; Sun et al., 2005). However, most of these studies were restricted to a small number of areas and time points. Furthermore, a number of genes was found to be expressed asymmetrically in the early fetal (Sun et al., 2005) NCX, but not in midfetal or adult NCX (Hawrylycz et al., 2012; Johnson et al., 2009; Lambert et al., 2011), suggesting that transcriptional asymmetry may be temporally regulated. In the present study, we analyzed the temporal dynamics and left-right asymmetry of NCX topographic gene expression across the full course of fetal and postnatal development and adulthood.

RESULTS

Interareal Transcriptional Divergence Exhibits a Temporal Hourglass Pattern

Our previous analyses of gene expression in the human brain revealed robust transcriptional differences among topographically defined areas of the fetal and, to a lesser extent, adult NCX (Johnson et al., 2009; Kang et al., 2011). To analyze temporal progression and left-right asymmetry of areal gene expression, we performed a secondary analysis of this data set (Kang et al., 2011) that included 11 topographically defined NCX areas corresponding to the orbital (OFC), dorsolateral (DFC), ventrolateral (VFC), medial (MFC), and primary motor (M1C) cortices of the frontal lobe; the primary somatosensory (S1C) and posterior inferior (IPC) cortices of the parietal lobe; the primary auditory (A1C), posterior superior (STC), and anterior inferior (ITC) cortices of the temporal lobe; and the primary visual (V1C) cortex of the occipital lobe (Figure 1 and Supplemental Experimental Procedures, Note 2 for topographic sampling, available online). This data set was generated using 886 tissue samples isolated from left and right hemispheres of 53 clinically unremarkable postmortem human brain specimens spanning from early fetal development through old age (from 10 weeks of postconception [PCW] to 82 years of age), which corresponded to periods 3–15, as previously designated, an interval during which all of the analyzed putative functional areas were represented (Kang et al., 2011) (Tables S1A and S2).

The areal localization of dissected NCX samples was previously verified by histology in the postnatal brains and matched across fetal periods using the same anatomical landmarks (Supplemental Experimental Procedures, Note 2; see also Kang et al., 2011). A hierarchical clustering of both fetal and postnatal NCX samples confirmed their grouping by topographical proximity and functional overlap (Figure 2A). Principal component analysis also revealed that transcriptional differences across periods account for the majority of the variance among NCX samples (Figure S1A), indicating that NCX areal gene expression is strongly developmentally regulated.

To investigate how putative areal transcriptional differences change over time, we used ANOVA to identify genes that exhibit differential expression (DEX) among areas in each period (henceforth referred to as the interareal divergence; Supplemental Experimental Procedures, Note 3.3). This analysis revealed that interareal transcriptional divergence, but not the total number of expressed genes, exhibits a previously unrecognized temporal hourglass pattern, with robust and dynamic differences found prenatally and, to a lesser extent from adolescence onward, with few in infancy and childhood (Figures 2B and S1B). In contrast, differential expression among other (non-neocortical) brain regions did not exhibit the same temporal hourglass pattern (Figure S1B).

We also analyzed the genotype of individuals using the data previously generated using Illumina HumanOmni 2.5 SNP arrays (Kang et al., 2011) to test whether the hourglass pattern could be explained by a reduction in the genetic diversity among individuals in periods 8 to 11. We observed that the genetic diversity of samples varies throughout development in a random way, without any observable pattern for periods 8 to 11 compared with others. We also found no relation with the number of samples (Figure S1C).

To estimate the contribution of differences in specific putative areas to the overall interareal divergence, Tukey's pairwise comparison was performed after ANOVA to determine the total number of significant ($p < 0.01$) DEX gene comparisons of each area with all the other areas for a given period. The relative contribution of areas to the overall hourglass shape varied across periods. During fetal periods, MFC, ITC, and the primary areas (V1C, A1C, S1C, and M1C) exhibited the most prominent dissimilarity, whereas only MFC and V1C showed robust dissimilarities during adolescence and adulthood (Figure 2C). Together, these findings show that the pattern of interareal transcriptional divergence is specified over time and exhibits an hourglass pattern, with infancy and childhood representing a long phase of minimal divergence. Our results also show that the spatial pattern of interareal divergence is mainly driven by a subset of putative functional areas.

Temporal Transcriptional Hourglass Pattern Reflects Putative Areal and Functional Differences

We hypothesized that increased interareal transcriptional divergence during fetal development and from adolescence onward reflects the differences in the underlying molecular and cellular processes between these two phases. Consistent with this hypothesis, only 848 of 3,125 (27%) interareal DEX genes were DEX both in fetal development and from adolescence onward. To gain insights into the differential organization of the NCX transcriptomes during the two phases of increased interareal differences, we performed weighted gene coexpression network analysis (Supplemental Experimental Procedures, Note 3.7) to identify modules of coexpressed genes with often-shared functional relevance. Within fetal development, we identified 122 modules (M1–M122: Table S4A), and from adolescence on we found 207 modules (M123–M329; Table S4B). Functional annotation of the modules revealed significant differences between the organization of fetal and adolescent/adult differential putative areal transcriptomes. Furthermore, the gene ontology (GO) enrichment analysis (Supplemental Experimental Procedures, Note 3.8) revealed significant differences between fetal and adolescent/adult DEX and also between coexpression modules in the enrichment for GO categories (Tables S3 and S4). Among exclusively fetal GO categories were mostly categories related with developmental processes, such as phosphoprotein, neuron differentiation, cell cycle, neuron development, cell morphogenesis, mitosis, cell morphogenesis