

## THE REGIONAL SCATTERING OF PRIMATE SUBPLATE

### THE SHADOWS OF SUBPLATE

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The subplate layer is a highly dynamic zone of the developing cerebral cortex that reaches huge proportions in human and non-human primates and has been associated with various brain developmental abnormalities. It contains some of the earliest born neurons of the cortex. Surprisingly, the timing of subplate neuron birth, migration, distribution and degree of programmed cell death has only been analysed in detail in rodents and carnivores, but not in primates. The Duque et al. (2016) study is the first that specifically examines the distribution of subplate cells labeled with the DNA replication marker tritiated thymidine ([<sup>3</sup>H]dT) across different brain regions in valuable archived macaque brain material. They show that macaque subplate neurons, after having completed their migration, become secondarily displaced inward by the arrival of subcortical and cortical axons. Due to regional differences in the magnitude of these developing connections, the subplate shows remarkable variations in width between different cortical areas. This study adds important and novel insights that may become most relevant in understanding the origin and pathogenesis of human neurodevelopmental disorders.

The basic pattern of cortical development was originally described from histological preparations at the beginning of the previous century (reviewed in 2). Transient embryonic cellular compartments including the subplate zone are generated in the proliferative centers near the ventricular cavity on the center of the brain (reviewed in 3). See Figure 1 for the distribution of the transient embryonic zones in the human fetus at mid gestation and in macaque at E50 and 70 (4).

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The subplate and marginal zone situated below the outer (pial) surface contain mature neurons at the time before the majority of cortical plate cells are born or

have completed their migration. The first synapses are formed below and above the forming cortical plate (5). In a study similar to that of Duque et al., (2016), Rakic (6) used [3H]dT in macaque monkeys at various gestational ages to label the cohorts of neurons that were born at that period to establish the inside-first, outside-last generation of cortical layers in primates and produce fundamental insights into the timing of the generation of the macaque cortical neuronal cohorts that we still use today (7). Since then we gained a much better understanding of subplate, its dynamic interactions with incoming afferents and forming cortical circuits in a variety of species (4, 8-11).. Additionally, the subplate has been the subject of imaging studies and transcriptomic analyses because of increasing evidence for its association with various cognitive developmental disorders (7, 12-14).

The majority of recent work on the subplate was conducted on rodents and carnivores, sometimes with clear interspecies differences (15-18), emphasizing the importance of non-human primate and human studies such as the one in this issue (1). Combined birthdating and marker expression studies in mouse suggest that subplate neurons with distinct gene expression patterns have differential birthdates and differential cell death (19). We do not know whether these observations can be generalized to primate subplate.

The developing macaque cortex has special cytoarchitectonic subcompartments and neurogenetic characteristics not found in rodents (reviewed 20 and 21). It gives huge credit to Kostovic and Rakic and their colleagues that they went back to the unique material that they generated over the last four decades and examined the above issues of primate subplate neurogenesis and post-migratory dispersion. Their study in this issue (1) is based on existing archived specimen from the collection of Nonhuman Primates at Yale University (US) and the university of Zagreb human tissue Collection (Croatia). A range of injection ages and post-injection survival times were analysed in macaque, but the paper focuses entirely on those heavily [3H]dT-labelled neurons born on E40, a time when many subplate and some layer 6 cells are born. Subplate has long been known to be exceptionally thick in primates (see 4 for a recent review) and to vary in thickness depending on the brain region. Based on the above primate material, the authors demonstrate that the increased thickness of subplate in primates is primarily due to a massive invasion of fibers, which cause the dispersion of early generated subplate cells after they have finished active migration. In contrast, co-generated neurons in the deep cortical plate remain as a fairly compact band above the future white matter. Similar axons can be demonstrated in human post-mortem material, as show-cased in Figure 3 of Duque et al. (1), suggesting that similar dispersion mechanisms may be at work. Differences in the quantities or properties of ingrowing axons could therefore account for the regional differences in subplate thickness and the regional differences in subplate cell dispersal reported by Duque and colleagues. But the question remains, why subplate cells behave differently from the layer 6 cells generated at the same time? Are these genetic differences or purely mechanical/structural differences? The study also demonstrates that some

subplate and marginal zone cells are co-generated as it was described in cat (15), although marginal zone neurogenesis was previously described to extend for a much longer period in primates (22) and contains “pioneer” neurons not described in non-primate species (3). These issues were extremely important to settle in our field, but still the extent of preferential cell death in the subplate remains unaddressed. Moreover, we do not know what proportion of the early born subplate neurons survive to adulthood as interstitial white matter cells in nonhuman primates under pathological conditions.

It will be important to continue with systematic comparisons of various birthdates at various post injection times (6) and directly compare subplate, marginal zone with other cortical layers. These more integrated studies would provide us with estimates on the range of birthdates and extent of cell death in subplate in nonhuman primates, which is expected to vary widely between different brain regions. Furthermore, there are other transient cell populations in the brain. The archived material used in the Duque et al. study could be valuable to study thalamic reticular and perireticular thalamic nuclei to determine the basis of the apparent reduction in cells.

The subplate is not homogeneous in its depth and various, often arbitrary, subdivisions have been attempted. The authors describe upper, middle and lower sub-divisions of the SP. The compartmentalization was dependent on the tissue level and maturity. In their previous studies the authors described a compact cell-dense layer; transient cell-dense band at E70 macaque within the putative primary visual cortical subplate in non-human primate (8,9 and Fig. 1 right panel). This transient thin cell dense layer divides subplate into upper and lower parts. The nature and possible relevance of this compartmentalization in primary visual cortex is not known. Recent transcriptomic analysis does not show specific differences from other subplate compartments (7). It would be important to examine this transient cell-dense band for birthdates and follow the dispersion and distribution of the cells that comprise it.

One of the most important outstanding questions relates to our understanding of the relationship between subplate and interstitial white matter cells in primate subplate (8, 9). It would be important to investigate what proportion of subplate cells survive to adulthood in primates and why increased numbers of interstitial white matter cells are found in post-mortem material of patients with schizophrenia (12). Which cell types survive and how is this regulated? Are there particular subplate cells that are more vulnerable? How are these altered interstitial white matter cells related to cognitive disorders?

These questions require studies in primates. The material generated in Pasko Rakic’s laboratory is unique and it is very likely that such work will not be performed in the future. The Duque et al. (2016) study emphasizes the need for preserving and digital archiving of these unique materials for the use of future research generations in a proposed Macaque Brain Resource Center at Yale University that shall serve as International Resource for Research on prenatal development of non-human primates. It will also contain EM blocks from multiple

brain regions of fetal and postnatal monkeys The field needs these data and considering the clinical importance of the subplate, this data will keep attracting the attention of a broad group of developmental and evolutionary neurobiologists, neuropathologists and neurologists.

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## FIGURE LEGEND

Figure 1

Compartments and zones of the developing human and macaque cerebral cortex. Left, schematic coronal section showing the relative location and size of the major compartments within the developing human dorsal cortex at 26 post-conception weeks (PCWs), at the peak of neurogenesis and cell migration. The inset box provides a higher-powered view of the cellular make-up of the transient developmental zones within this developing cortical region. Subplate (SP) develops from the deeper stratum of the first generated cell layer, the primordial preplate after its separation (split) from the marginal zone (MZ) situated above by arrival of the cortical plate (CP) neurons from the proliferative ventricular zone (VZ). The intermediate zone (IZ) contains more fibre tracts, whereas the SP is more abundant in postmitotic SP neurons with well-developed cellular processes.

The right panels show Nissl stained coronal sections from E50 and E70 primary visual cortex in the macaque. These panels illustrate the huge increase of subplate zone in primate during development and the E70 panel also depicts the transient cell dense layer that divides the subplate to upper and lower layers in the primary visual cortex. It is currently not known whether subplate zone increases by the dispersion of existing cells or receives more newly generated subplate cells at later stages or both. This issue has key relevance to understand evolutionary origin of primate subplate (23). Abbreviations: VZ, ventricular zone; SVZ, subventricular zone; IZ, intermediate zone; SP, subplate; CP, cortical plate; MZ, marginal zone. Illustration is reproduced from (4) with permission of Nature Publishing Group (United States).



