

# Regional scattering of primate subplate

Zoltán Molnár<sup>a,1</sup> and Anna Hoerder-Suabedissen<sup>a</sup>

The subplate layer is a highly dynamic zone of the developing cerebral cortex that reaches huge proportions in human and nonhuman 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 analyzed in detail in rodents and carnivores, but not in primates. The study by Duque et al. (1) 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 ref. 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 ref. 3). See Fig. 1 for the distribution of the transient embryonic zones in the human fetus at midgestation and in the macaque at embryonic days 50 and 70 (4).

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. (1), Rakic (6) used <sup>3</sup>H)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 the subplate and 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 nonhuman primate and human studies such as the one in PNAS (1). Combined birth dating and marker expression studies in the mouse suggest that subplate neurons with distinct gene expression patterns have differential birth dates and differential cell death (19). We do not know whether these observations can be generalized to the primate subplate.

The developing macaque cortex has special cytoarchitectonic subcompartments and neurogenetic characteristics not found in rodents (reviewed in refs. 20 and 21). It gives huge credit to Duque et al. 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 postmigratory dispersion. Their study in PNAS (1) is based on existing archived specimens from the collection of nonhuman primates at Yale University and the University of Zagreb human tissue collection in Croatia. A range of injection ages and postinjection survival times was analyzed in macaques, but the paper focuses entirely on those heavily <sup>3</sup>H)dT-labeled neurons born on embryonic day 40, a time when many subplate and some layer-6 cells are born. Subplate has long been known to be exceptionally thick in primates (see ref. 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, cogenerated neurons in the deep cortical plate

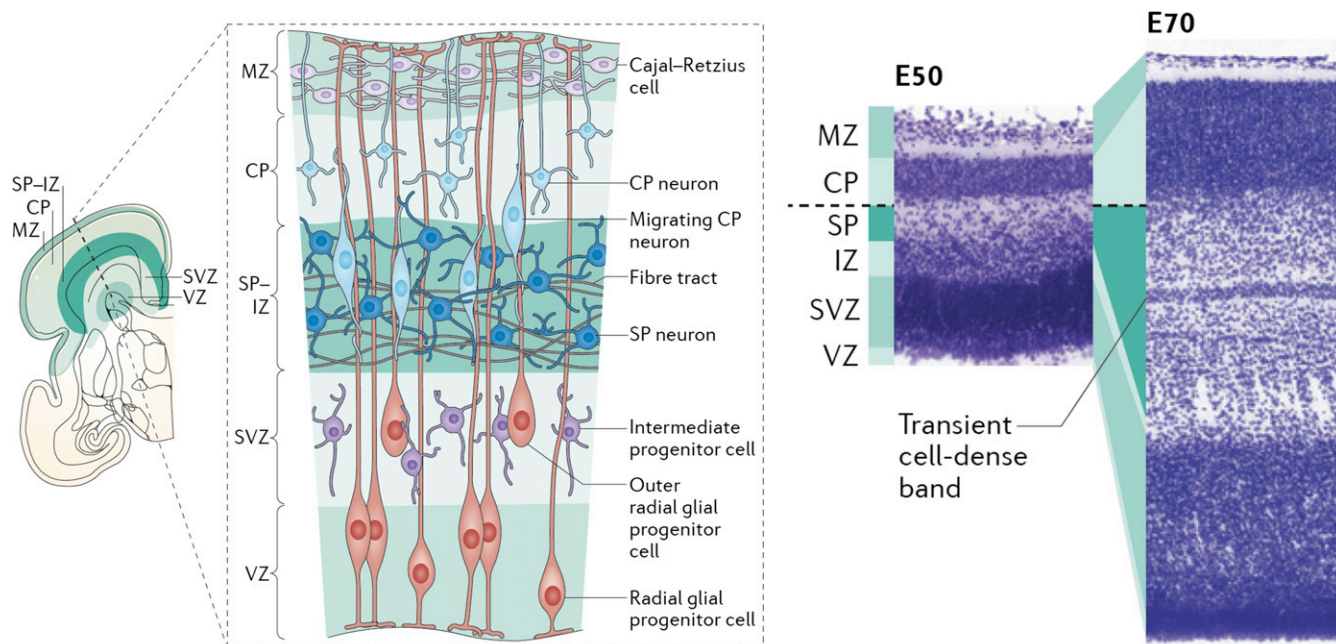
<sup>a</sup>Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford OX1 3QX, United Kingdom

Author contributions: Z.M. and A.H.-S. wrote the paper.

The authors declare no conflict of interest.

See companion article on page 9892.

<sup>1</sup>To whom correspondence should be addressed. Email: zoltan.molnar@dpag.ox.ac.uk.



**Fig. 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 postconception weeks, at the peak of neurogenesis and cell migration. (Inset) A higher-powered view of the cellular makeup 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 fiber tracts, whereas the SP is more abundant in postmitotic SP neurons with well-developed cellular processes. (Right) Nissl-stained coronal sections from embryonic day (E) 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 understanding the evolutionary origin of the primate subplate (23). SVZ, subventricular zone. Modified with permission from ref. 4.

remain as a fairly compact band above the future white matter. Similar axons can be demonstrated in human postmortem material, as showcased 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 et al. (1). However, the question remains of 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 cogenerated as was described in cats (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 nonprimate 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 birth dates at various postinjection times (6) and directly compare the subplate and marginal zone with other cortical layers. These more integrated studies would provide us with estimates on the range of birth dates and extent of cell death in the 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 study by Duque et al. (1) 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 subdivisions of the subplate. The compartmentalization was dependent on the tissue level and maturity. In their previous studies the authors described a transient cell-dense band at embryonic day 70 in the macaque within the putative primary visual cortical subplate in nonhuman primates (refs. 8 and 9 and Fig. 1, Right). 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 birth dates and follow the dispersion and distribution of the cells that compose it.

One of the most important outstanding questions relates to our understanding of the relationship between the 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 postmortem 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 (6) and it is very likely that such work will not be performed in the future. The Duque et al. (1) study emphasizes the need for preservation 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 would serve as an international resource

for research on prenatal development of nonhuman 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 these data will keep attracting the attention of a broad group of developmental and evolutionary neurobiologists, neuropathologists, and neurologists.

- 1 Duque A, Krsnik Z, Kostović I, Rakic P (2016) Secondary expansion of the transient subplate zone in the developing cerebrum of human and nonhuman primates. *Proc Natl Acad Sci USA* 113:9892–9897.
- 2 Judaš M, Sedmak G, Pletikos M (2010) Early history of subplate and interstitial neurons: From Theodor Meynert (1867) to the discovery of the subplate zone (1974). *J Anat* 217(4):344–367.
- 3 Bystron I, Blakemore C, Rakic P (2008) Development of the human cerebral cortex: Boulder Committee revisited. *Nat Rev Neurosci* 9(2):110–122.
- 4 Hoerder-Suabedissen A, Molnár Z (2015) Development, evolution and pathology of neocortical subplate neurons. *Nat Rev Neurosci* 16(3):133–146.
- 5 Molliver ME, Kostović I, van der Loos H (1973) The development of synapses in cerebral cortex of the human fetus. *Brain Res* 50(2):403–407.
- 6 Rakic P (1974) Neurons in Rhesus monkey visual cortex: Systematic relation between time of origin and eventual disposition. *Science* 183(4123):425–427.
- 7 Bakken TE, et al. (2016) A comprehensive transcriptional map of primate brain development. *Nature* 535(7612):367–375.
- 8 Kostovic I, Rakic P (1980) Cytology and time of origin of interstitial neurons in the white matter in infant and adult human and monkey telencephalon. *J Neurocytol* 9(2):219–242.
- 9 Kostovic I, Rakic P (1990) Developmental history of the transient subplate zone in the visual and somatosensory cortex of the macaque monkey and human brain. *J Comp Neurol* 297(3):441–470.
- 10 Allendoerfer KL, Shatz CJ (1994) The subplate, a transient neocortical structure: its role in the development of connections between thalamus and cortex. *Annu Rev Neurosci* 17:185–218.
- 11 Kanold PO, Luhmann HJ (2010) The subplate and early cortical circuits. *Annu Rev Neurosci* 33:23–48.
- 12 Akbarian S, et al. (1996) Maldistribution of interstitial neurons in prefrontal white matter of the brains of schizophrenic patients. *Arch Gen Psychiatry* 53(5):425–436.
- 13 Hoerder-Suabedissen A, et al. (2013) Expression profiling of mouse subplate reveals a dynamic gene network and disease association with autism and schizophrenia. *Proc Natl Acad Sci USA* 110(9):3555–3560.
- 14 Kostović I, et al. (2014) Perinatal and early postnatal reorganization of the subplate and related cellular compartments in the human cerebral wall as revealed by histological and MRI approaches. *Brain Struct Funct* 219(1):231–253.
- 15 Luskin MB, Shatz CJ (1985) Studies of the earliest generated cells of the cat's visual cortex: Cogeneration of subplate and marginal zones. *J Neurosci* 5(4):1062–1075.
- 16 Wood JG, Martin S, Price DJ (1992) Evidence that the earliest generated cells of the murine cerebral cortex form a transient population in the subplate and marginal zone. *Brain Res Dev Brain Res* 66(1):137–140.
- 17 Valverde F, López-Mascaraque L, Santacana M, De Carlos JA (1995) Persistence of early-generated neurons in the rodent subplate: Assessment of cell death in neocortex during the early postnatal period. *J Neurosci* 15(7 Pt 1):5014–5024.
- 18 Price DJ, Aslam S, Tasker L, Gillies K (1997) Fates of the earliest generated cells in the developing murine neocortex. *J Comp Neurol* 377(3):414–422.
- 19 Hoerder-Suabedissen A, Molnár Z (2013) Molecular diversity of early-born subplate neurons. *Cereb Cortex* 23(6):1473–1483.
- 20 Smart IH, Dehay C, Giroud P, Berland M, Kennedy H (2002) Unique morphological features of the proliferative zones and postmitotic compartments of the neural epithelium giving rise to striate and extrastriate cortex in the monkey. *Cereb Cortex* 12(1):37–53.
- 21 Geschwind DH, Rakic P (2013) Cortical evolution: Judge the brain by its cover. *Neuron* 80(3):633–647.
- 22 Zecevic N, Rakic P (2001) Development of layer I neurons in the primate cerebral cortex. *J Neurosci* 21(15):5607–5619.
- 23 Montiel JF, et al. (2011) Hypothesis on the dual origin of the mammalian subplate. *Front Neuroanat* 5:25.