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Extraordinary neoteny of synaptic spines in the human prefrontal cortex

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The major mechanism for generating diversity of neuronal connections beyond their genetic determination is the activity-dependent stabilization and selective elimination of the initially overproduced synapses (Changeux JP, Danchin A (1976) Nature 264:705–712). The longest number of supramunerary synapses has been recorded in the cerebral cortex of human and nonhuman primates. It is generally accepted that synaptic pruning in the cerebral cortex, including prefrontal areas, occurs at puberty and is completed during early adolescence (Huttenlocher PR, et al. (1979) Brain Res 163:195–205). In the present study we analyzed synaptic spine density on the dendrites of layer IIIC cortico–cortical and layer V cortico–subcortical projecting pyramidal neurons in a large sample of human prefrontal cortices in subjects ranging in age from newborn to 91 y. We confirm that dendritic spine density in childhood exceeds adult values by two-to threefold and begins to decrease during puberty. However, we also obtained evidence that overproduction and developmental remodeling, including substantial elimination of synaptic spines, continues beyond adolescence and throughout the third decade of life before stabilizing at the adult level. Such an extraordinarily long phase of developmental reorganization of cortical neuronal circuitry has implications for understanding the effect of environmental impact on the development of human cognitive and emotional capacities as well as the late onset of human-specific neuropsychiatric disorders.

association cortex | critical period | schizophrenia | synaptogenesis

Selective stabilization of developing synapses as a mechanism for specification of neuronal connections was proposed more than 4 decades ago (1). This hypothesis gained considerable support from the discovery that synaptic connections in the cerebral cortex of human and nonhuman primates initially are overproduced to about two times the adult number and are then pruned during puberty to reach the adult level at the onset of adolescence (2–5). The selective elimination hypothesis basically assumes that during a period of overproduction of synapses neuronal activity tunes the molecular structure of individual synapses and determines which will be retained and which removed from the neural network (6, 7). Previous EM analyses in nonhuman primates revealed that synaptic elimination in the monkey prefrontal cortex occurs mainly by removal of asymmetric synapses on spines, whereas the number of symmetric synapses on dendritic shafts remains constant (5, 8).

The tempo of elimination of supernumerary synaptic spines and the identification of the end of this critical period are extremely important, because these factors are related to establishment of cognitive abilities and duration of the window for optimal acquisition of new language and mathematical skills as well as personality transformation from the developmental mode to adult status. In addition, the several leading hypotheses for the explanation of late-onset neuropsychiatric disorders, such as schizophrenia and drug- or stress-induced psychoses, implicate defective pruning of the initially overproduced synapses on dendritic spines (9–13). Furthermore, spine dynamism is the only detectable anatomical phenotype in some human cognitive disorders such as nonsyndromic mental retardation (14). Finally, this biomedically and socially important concept (9) is also the subject of continuing dialogue between proponents of selectionism versus constructionism (15).

The end of the critical period of synaptic spine elimination in the human cortex basically relies on the pioneering study of Huttenlocher and colleagues (2, 4). Thus, it usually is assumed tacitly that the period of synaptic overproduction in the human cerebral cortex is completed by the end of puberty (16), even though Huttenlocher's study contains only a single 19-y-old brain specimen in the age group between 15 and 32 y. In contrast, more recent studies using electroencephalography (15, 16), PET (20), and functional MRI (18, 21–26) have suggested that the dynamic changes in gray matter density and white matter integrity in the human association neocortex extend into the third decade of life (21, 23, 26, 27). These changes, observed by MRI, cannot be explained by a concurrent increase in dendritic length, because the dendritic growth in the human neocortex is limited mainly to early childhood (28). Therefore, it has been assumed that functional plasticity probably reflects reorganization of circuitries, including synaptic elimination (29–32), essential for acquisition of the highest brain functions in humans, including affective modulation of emotional cues, self-conceptualization, mentalization, cognitive flexibility, and working memory (33–36). However, the cellular data supporting this assumption have been missing.

Results

To fill this gap in our knowledge, we analyzed the initial overproduction and subsequent elimination of dendritic spines on layer IIIc and layer V pyramidal neurons in the dorsolateral prefrontal cortex [Brodmann area 9 (28, 37)]. Our focus was on the prefrontal cortex because of the relevance of this region for late-onset, human-specific, neuropsychiatric disorders and the possible implications in understanding the mechanisms of environmental impacts such as education and training on prolonged development of human cognitive capacities (9–12, 38–41). Because dendritic spines are impregnated reliably by the rapid Golgi methods in postmortem brain tissue (*Material and Methods*), we decided to examine their development and measure their density on dendrites in well-preserved human tissue, including ages that were not analyzed in previous studies (Table S1).

We selected to focus on the large pyramidal cells in layer IIIc and layer V (28), that form cortico–cortical and subcortical pro-

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