Chapter 1

Reflections on the origins of the human brain

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Introduction

Human beings belong to the biological species *Homo sapiens*. The definition of the species includes the description of the characteristic anatomy and physiology of the body, as well as of the functional organization of the brain together with the multiple facets of behaviors unique to human beings. The human brain is obviously a fascinating object of scientific investigation.

The aim of this chapter is to debate the origins of the human brain. This raises an overwhelming challenge. First of all, one should attempt to delineate what makes the human brain "human," even in the newborn, and to identify the features that distinguish it from the current living primates and from its fossil antecedents. It is intriguing, on the one hand, to find ways of specifying the universal traits of "human nature" in objective terms. On the other hand, the broad diversity between individuals, in particular as a consequence of their past and recent personal and/or cultural history, raises a second challenge. Does such diversity break the unity of the human brain within the human species?

A tension thus exists in neuroscience, as well as in the humanities, between two main lines of research: one that aims at defining the *universal* characteristics of the human species, for instance at the level of the infant brain, and the other that stresses the *variability* of cognitive abilities in adults, such as the language they speak and the social conventions they adopt. It is a formidable task to deal with these contrasting approaches with the aim of achieving a meaningful scientific understanding of the human brain, its learning capacities, and its higher functions, and it seems plausible that a realistic picture of the human brain will require the synthesis of these divergent approaches. I shall therefore consider successively these two aspects of research on the human brain.

Universality, diversity, complexity

A primary difficulty, raised by the philosopher John Searle (1995), concerns the notion of function, specially psychological function in the case of humans. One should never underestimate the conceptual and experimental predicaments posed by any attempt to singularize a behavioral or mental trait. Searle even suggests that functions are not intrinsic, but might be assigned to, or imposed on, living objects or organisms, and in particular the human brain, by the observer. This problem must be taken seriously. Even though, since Darwin, attempts have been made to eliminate teleology from the life sciences, one has to be aware of a possible observer bias in the definition of a function. The difficulty is real for the pediatrician who has to objectively evaluate newborn perceptive abilities and behavior. In my opinion, Searle's criticism could go beyond the definition of function and equally applies to the description of the anatomy or to the dynamics of the electrical and chemical activities that take place within the brain. To overcome these difficulties, a recommended strategy is to elaborate theoretical models, even within the clinical framework of pediatrics. Furthermore, the theoretical models that will primarily aim at resolving brain complexity, to my view, should not to be confined to a given discipline or technique. On the contrary, they have to systematically *bring together* well-defined molecular, anatomical, physiological, behavioral, and psychological data (see Changeux *et al.*, 1973; Changeux, 2004). In any case, the aim of the modeling enterprise will not be to give an exhaustive description of brain reality: one has to remain humble! It will, furthermore, in any case be limited by its scope and by its formulation. Moreover, one should never forget that the modeling process involves the selection of theoretical representations… by the brain of the model builder!

The Newborn Brain: Neuroscience and Clinical Applications, 2nd edn., eds. Hugo Lagercrantz, M. A. Hanson, Laura R. Ment, and Donald M. Peebles. Published by Cambridge University Press.

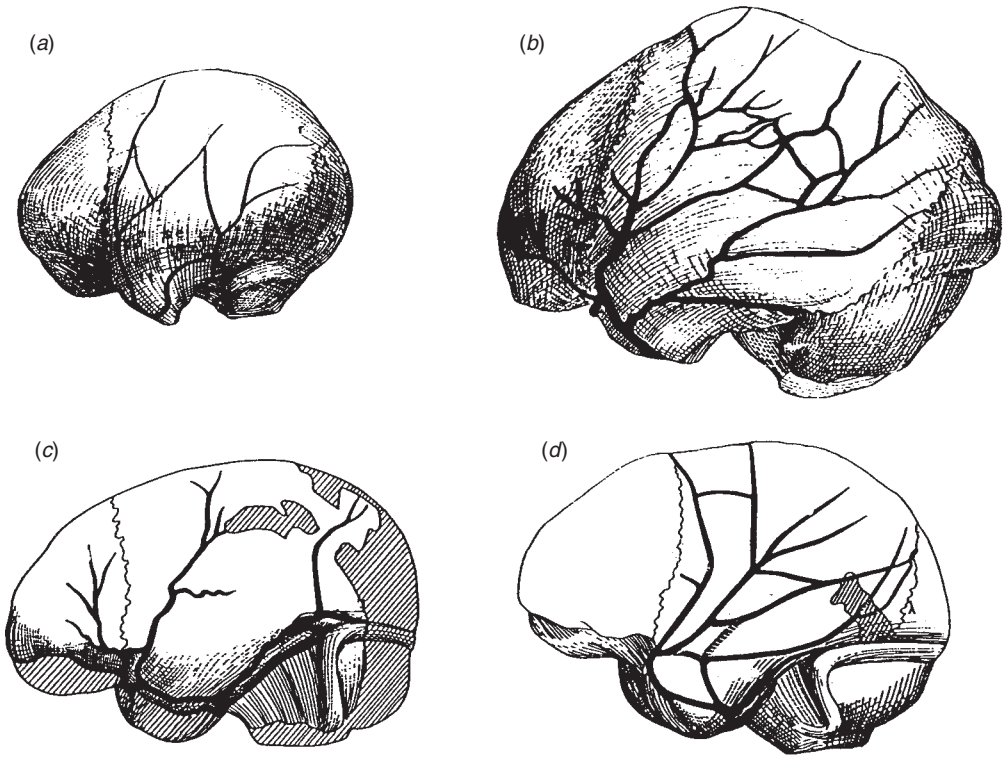

Fig. 1.1 Comparison of the impression of the meningeal vessels on endocranial casts of 40-day-old infant (*a*) and 1-year-old infant (*b*) with the endocranial casts of *Australopithecus gracilis* (*c*) and *Homo habilis* (*d*). (From Saban, 1995.)

Another difficulty resides in the very attempt to establish an appropriate causal link, or "bridge," between the structural elements of the system and the function considered. The reductionist approach, as mentioned, has to be "fair." One should deliberately avoid frequently encountered statements such as "the gene(s) of intelligence" or the "neurotransmitters of schizophrenia!" Such simplistic and incorrect proposals bypass one essential feature of brain organization; that is, there is no direct and unequivocal link between the molecular and the cognitive levels. In between these, there exist parallel and hierarchical levels of organization nested within each other and with abundant cross-connections. These levels develop step by step from the molecule to the cell, from elementary circuits to populations (or assemblies) of neurons, up to complex global neuronal patterns engaged in higher cognitive functions. The definition of these relevant parallel and hierarchical levels is in itself a difficult theoretical problem that should be made explicit (Changeux & Connes, 1989). A critical conceptual and practical issue in any investigation of the newborn brain will thus be to specify the selected hierarchical level (or, more probably, levels) of organization at which a relevant *causal link* will be established between anatomy, physiology, and behavior, together with the massive parallelism and strong lateral interactions that potentially contribute to coherent unitary brain processes.

I will make one last remark about brain complexity, which may seem far-fetched to the pediatrician: my conviction is that investigations on the human brain, in particular that of the newborn, should deal with our current understanding of biological evolution (Fig. 1.1). This requirement is obviously of practical interest, since it may lead to a fair evaluation of the commonly adopted (sometimes erroneous) use of lower animal species as models for human diseases, in particular for neuropsychiatric deficits. But the evolutionary perspective also points to interesting empirical questions. Take, for instance, the case of brain anatomy. No simple apparent logic accounts for the actual morphology, distribution, and interrelation of the multiple areas (or nuclei) that compose the brain (see Changeux, 1985). For instance, the actual nesting of the archeo-, paleo-, and neocortices within human

brain anatomy cannot be understood without considering that they have been derived by some kind of evolutionary "tinkering" (Jacob, 1981) from prior ancestral brains. The older structures have not been eliminated, but rather incorporated and nested within the newer ones. Millions of years of evolutionary history under extremely variable environmental conditions thus introduce, indirectly, *contingencies in the anatomy* such that the intrinsic logic of the functional organization of the brain may no longer be apparent by simple inspection. This situation frequently invalidates attempts to infer function from anatomy or to relate a given behavior to a single brain structure, such as, for instance, the current debates about the role of the hippocampus, amygdala, or prefrontal cortex in behavior. It also illustrates why the best models may not be the simplest or the most minimalistic ones. Model building thus has to rely on concrete observational approaches, to be "neurorealist," and it becomes a particularly difficult, though necessary, process to progress in the understanding of the human brain.

Finally, one should not limit the evolutionary perspective to the context of the biological – or genetic – origins of the human brain. Rather, as discussed in the following sections, the brain of a human subject may be more appropriately viewed as the synthesis of multiple nested evolutions by variation selection (Changeux, 1983a,b, 2004). These developments include not only the past genetic evolution of the species, but also the epigenetic development of the brain of each individual, within the framework of his or her personal history, as well as the more recent social and cultural evolution of the social environment with which the newborn interacts. The data are scarce, but the potential outcomes of future research could be richly rewarding.

Genes and the newborn brain

The brain of the newborn is often taken as holding the innate features that characterize "human nature." In reality, many more characteristics proper to the human brain develop after birth, in particular through learning during postnatal development, which is one of the longest known among living species. Even though, as we shall see, epigenetic regulations may take place which involve specific interactions with the environment, strictly innate, DNA-encoded mechanisms contribute, in a definite manner, to the prenatal and postnatal development of the adult brain (Watson *et al.*, 2007). But these genes are not expressed all at once in the egg or the embryo or the newborn, as postulated by the extreme views of the eighteenth-century preformationists, views that assumed that the adult organism was already present in a miniaturized form in the sperm and in the egg. On the contrary, they are activated (or suppressed) throughout embryogenesis and postnatal development in a sequential and combinatorial manner.

The straightforward inspection of the genetic endowment of the species compared with the organization of the brain raises, however, two apparent paradoxes (Changeux, 1983a,b, 2004; Edelman, 1987; Miklos & Rubin, 1996). The total amount of DNA present in the haploid genome comprises approximately 3.1 billion base pairs, but no more than 20 000–25 000 genes sequences (Lander *et al.*, 2001; Venter *et al.*, 2001). The coding exons represent only 1.2% of our genome, yet alternative splicing may increase the number of mRNA protein-coding sequences up to 100 000. On the other hand, the total number of cells in the brain is in the order of 170 billion, including 86 billion neurons (Herculano-Houzel *et al.*, 2007, Azevedo *et al.*, 2009), each neuron possessing its particular connectivity – or "singularity" (Changeux, 1983a). There is thus a striking parsimony of genetic information to code for brain complexity.

Another paradox is raised by the relation between the total number of genes and the evolution of brain organization. The 97 million bases that constitute the total sequence of the genome of a small invertebrate, the nematode *Caenorhabditis elegans* (Miklos & Rubin, 1996; Chervitz *et al.*, 1998; Hodgkin *et al.*, 1998; Thompson *et al.*, 2001) with its humble 302-neuron nervous system, contains a predicted 18 266 protein-coding genes. *Drosophila* possesses a much larger nervous system, with about 250 000 neurons, but with a similar number of genes (13 338; Rubin *et al.*, 2000). Even more striking, the gene number from bony fish, through the laboratory mouse, to the human is roughly constant. Yet, notwithstanding the increase of cell numbers (from about 70 million in the mouse to 86 billion in humans [Azevedo *et al.*, 2009]), mammalian brain anatomy has evolved dramatically from a poorly corticalized lissencephalic brain with about 10–20 identified cortical areas to a brain with a very high relative cortical surface, multiple gyri, and sulci and possibly as many as 100 identified cortical areas (Mountcastle, 1998). Thus, there exists a remarkable nonlinearity between the

evolution of brain anatomy and that of the total number of genes (Changeux, 1983a,b, 2004; Edelman, 1987; Miklos & Rubin, 1996).

The molecular genetics of the early stages of embryonic development in *Drosophila*, *Xenopus*, chick, and mouse offers at least one major perspective on resolving these paradoxes. For example, in *Drosophila* a variety of genes have been identified that control the cartesian coordinates of the embryo, the segmentation of the body, and the identity of its segments (Nüsslein Volhard, 1990; Lawrence, 1992). A significant fraction of these "homeotic genes," which are also found in *C. elegans* (Ruvkun & Hobert, 1998), are absent in bacteria and yeast but conserved throughout the evolution of higher animal species and possibly act in equivalent regulatory cascades in mammals. In the course of embryonic and postnatal development, these developmental genes become expressed according to well-defined spatiotemporal patterns, in a hierarchical and parallel manner with cross-regulatory interactions and reutilizations. Such a view of morphogenesis, as a developing network of gene interactions (Koentges, 2008), may account, at least in part, for the parsimony paradox. An enormous diversity, indeed, may arise from such combinatorial expression of a limited number of genes.

Development of the body plan

As a consequence of the combinatorial gene expression described in the previous section, the plan of the body's embryo develops. At defined critical stages, anteroposterior and dorsoventral polarities, and sharp boundaries between territories and/or of patterns of stripes become established. The symmetry of the embryo evolves in the course of development. "Symmetry breakings" (Turing, 1952; Meinhardt & Gierer, 1974) take place. On theoretical grounds, such defined and reproducible patterns can be generated from a set of chemical substances, or morphogens, which cross-react and diffuse throughout the organism (Turing, 1952). For instance, gradients of diffusible morphogens are thought to contribute to the unfolding of developmental gene expression resulting in anteroposterior polarity (Meinhardt & Gierer, 1974). The main factors (but not the only ones) are the products of the developmental genes: regulatory proteins referred to as transcription factors that control gene transcription at the level of the core RNA polymerase II transcription complex (see Mannervik *et al.*, 1999). These protein molecules may have played a critical role in the phylogenetic evolution of the body form (Koentges, 2008). They bind to DNA elements (enhancers or silencers) that lock or unlock the transcription of adjacent structural genes and are themselves often conserved across species. Interplay between morphogens and transcription factors (coactivators and/or corepressors) builds up an intracellular network of protein–protein interaction (Rual *et al.*, 2005; Stelzl *et al.*, 2005) and thus of gene regulation, together with membrane receptors and the relevant second messengers. Models have been proposed according to which particular sets of such molecules may contribute to the "reading" of a gradient of morphogen by some kind of all-or-none switch in both a noncellularized (Kerszberg & Changeux, 1994) and a cellularized embryo (Kerszberg, 1996) (Fig. 1.2). It has been further suggested that such reading may require particular kinds of molecular interconnections at the level of the transcription factors: the assembly of molecular partners into hetero-oligomers between, for instance, one morphogen molecule from the gradient and a transcriptional coregulator now coded by a gene expressed in the embryonic nuclei. Nonlinear relationships between transcription factor concentration and morphogenesis may thus emerge from these combinations. Such a concept of nonlinear networks of transcription factors (Kerszberg & Changeux, 1994) has been recently documented with particular reference to *Drosophila* (Mannervik *et al.*, 1999) and may plausibly contribute to morphogenesis, together with receptors, kinases, phosphatases, G-proteins, and second messengers (Lisman & Fallon, 1999) within and between the developing embryonic cells (Koentges, 2008).

Many developmental genes are expressed in the nervous system. They are part of a still largely uncharacterized population of genes concerned with brain morphogenesis: its segregation into definite patterns of areas and nuclei and even the differentiation of asymmetrical hemispheres. The concepts mentioned for embryonic development may also apply to brain morphogenesis, in particular to the very early stage referred to as neurulation (see Kerszberg & Changeux, 1998). The process of neurulation differs strikingly in invertebrates and vertebrates. In the former case, the neuroblasts delaminate from the neural ectoderm to form progressively the ventral solid ganglion chain of the adult (which may reach up to 520 million

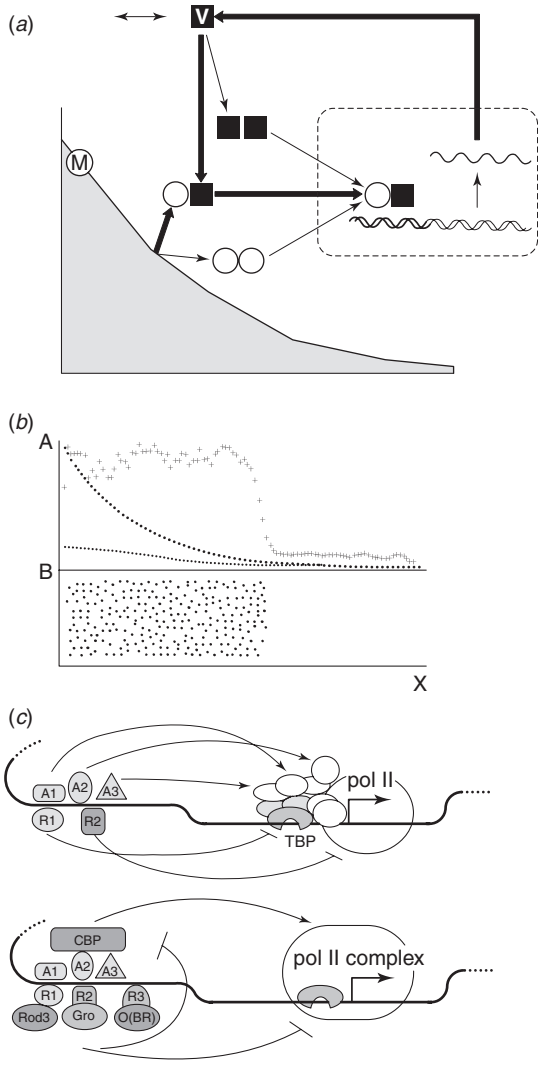

Fig. 1.2 A hypothetical model for reading morphogenetic gradients underlining the importance of protein–protein interaction and combinatorial information between transcription factors at the gene level. (*a*) A morphogen M (circles) is initially distributed along a smooth anteroposterior gradient; a 'vernier' molecule V (square) is coded by a gene present in the embryonic nucleus (broken line). Morphogen and vernier may form heterodimers but may also exist as homodimers. These various dimers form transcription factors which diffuse in the cytoplasm and bind to a promoter element regulating, in *cis*, the transcription of the vernier gene. Each dimer contributes to activation/inactivation of the vernier gene transcription thus yielding autocatalysis and competition and, as a consequence, sharp boundaries (*b*) and/or stripes (from Kerszberg & Changeux, 1994; see also Smolen et al., 2000). (*c*) Plausible schemes for the integration of combinatorial information from various transcription activators and repressors (various letters) at the level of the RNA polymerase (pol II) transcription complex (from Mannervick et al., 1999).

neurons in *Octopus*). In the latter, the neural plate invaginates en bloc dorsally to form a hollow neural tube, which may facilitate considerable growth of the central nervous system through surface expansion. This is observed from cyclostomes to mammals, primates, and humans (see also Chapter 2).

Such a decisive evolutionary step is not fully understood. Yet, on strictly theoretical grounds, one may propose the hypothesis that it does not require a large number of molecular changes at the gene transcription level. Arendt and Nübler-Jung (1997) have presented evidence that the ventral–dorsal transition of the nervous system from invertebrates to vertebrates can be reduced to simple changes in the gastrulation movements of the embryo, themselves under the control of a few homeotic genes. These discrete molecular transitions may, for instance, affect transcription factor switches, which themselves regulate cell motion (Kerzsberg & Changeux, 1998) as well as cell adhesion (see Edelman, 1987). As a consequence, either the whole neural plate infolds into a tube (in vertebrates) or individual neuroblasts delaminate, yielding a solid nervous system (in invertebrates). This illustrates hypothetically how only a few gene changes may contribute to the critical transition between the invertebrate and vertebrate nervous systems.

Another example, even less well understood, is that of the increase in size and fast expansion of the cerebral cortex and cerebellum that took place in the course of the evolution of the vertebrate brain, from fish to reptiles, birds, and mammals (see Changeux, 1983a,b; Mountcastle, 1998; Reiner *et al.*, 2004). First of all, the biochemical differences between these adult brains look negligible: from mouse to human brain, the region-specific genes are strikingly conserved at both the sequence and gene expression levels (Strand *et al.*, 2007). Moreover, in the cerebral cortex, the number of neurons per cortical column appears uniform throughout vertebrates (Rockwell *et al.*, 1980). Thus, the surface area of the cortex, i.e., the number of columns, appears as a primary target of the evolutionary changes (Rakic, 1988). One may then further speculate that the fast expansion of the frontal lobe and parietotemporal areas, which contributed to the evolutionary origins of the brain in *H. sapiens*, has resulted from the exceptionally prolonged action of some developmental genes (or of slight variation of concentration of morphogens) (Changeux, 1983a,b, 2004), the genomic

evolution underlying this process engaging a rather small set of genes.

In the course of the subsequent development of the cellular organization of the nervous system, large populations of cells project into other large ensembles of neurons, and neural maps develop in regular species-specific patterns. The molecular mechanisms governing the formation of ordered connections of neural maps are progressively being understood and, again, it is anticipated that a few molecular events will greatly modify the developmental patterns (for a review, see Tessier-Lavigne & Goodman, 1996; Drescher *et al.*, 1997). For example, in the case of the cerebral cortex, four transcription factors – COUP-TFI, Emx2, Pax6, and Sp8 – display graded expression across the embryonic cortical axes and determine the sizes and positions of cortical areas by specifying (or repressing) area identities within cortical progenitors (Bishop *et al.*, 2000; O'Leary *et al.*, 2007).

Phylogenetic ancestors of the human brain

As mentioned above, many important anatomical features of our brain have been inherited from our direct ancestors (see Changeux, 1983a, b; Mountcastle, 1998). The soft parts of their brains may be lost forever, but comparison of the endocranial casts of modern humans and their fossil ancestors provides interesting information. It reveals striking analogies between the various stages of the phylogenetic evolution of the ancestors of *H. sapiens* and the ontogenetic development of the brain in the modern human (Saban, 1995). The observations are limited to the impression of the meningeal veins and thus yield only limited information. Yet, the simplified topography of the *human newborn* meningeal system strikingly resembles the arrangement in *Australopithecus robustus* (who lived about three to two million years ago). The meningeal topography of *Homo habilis*, who lived two million years ago (cranial capacity 700 ml), is rather similar to that of a modern 40-day-old infant. *Homo erectus*, who lived one million years ago (cranial capacity of about 1000 ml), has a meningeal system topography similar to that of a modern 1-year-old child. Neanderthals (brain volume about 1500 ml, larger than modern *H. sapiens*) retained many archaic features of *H. erectus*. Recent DNA studies indeed suggest that they were only our cousins (Krings *et al.*, 1997; Carroll, 2003).

Making inferences from these rather scarce paleontological data about how spoken language and higher cognitive functions including self-consciousness emerged is a highly controversial issue. Chimpanzees use tools, have intricate social lives, and show rudiments of self-awareness (Hauser, 1999, 2005; Jensen *et al.*, 2007; Premack, 2007). They utilize a number of vocalizations, but lack rapid manipulation of symbolic representations, as well as the capacity to form and organize abstract concepts. Monkeys have been claimed to possess the equivalent of Broca's and Wernicke's areas, although without the rich connectivity that characterizes language processing in humans (Aboitiz & Garcia, 1997; Deacon, 1997; Gil-da-Costa *et al.*, 2006). An analysis of fossil skulls supports the view that the early evolution of the hominoid brain included three major reorganizations (Holloway, 1995): a relative enlargement of the inferior parietal lobe, an expansion of the frontal lobe, and a greater hemispheric specialization occurring before major increases in brain volume. Similar conclusions have been reached from a neuroanatomical perspective (Aboitiz & Garcia, 1997). This study more specifically points to the development of strong corticocortical cross-modal interactions in the postrolandic cortex, providing the basis of a semantic neural device that converges into a prospective Wernicke's area in which concepts acquire their specific link with sound. These phonological representations project into inferoparietal areas which connect to Broca's area and the premotor representations of orofacial movements. Finally, a fundamental element in the evolution of cognitive and linguistic capacity would be the coordinated operation of these networks, which is required to generate higher levels of syntax and discourse associated with the expansion of the prefrontal cortex, together with the development of its interconnections with the above-mentioned cortical (and subcortical) areas (see Chapter 23).

The morphology of the face and skull, as well as many body characteristics of human adults, resembles that of the face and skull of newborn chimpanzees (Stack & Kummer, 1962). It has thus been suggested that neoteny, i.e., access to sexual maturity at early stages of development and/or the persistence of embryonic or fetal characters in the adult, together with the prolonged development and increase in brain size after birth, contributed to the evolution of the human brain (see Gould, 1977). In any case, the

intrinsic changes in the cellular organization of the brain that make us human are already present before birth and cannot be derived exclusively from neoteny; the proliferation of nerve cells largely (but not definitively) stops at around eight months of prenatal development (except in the hippocampus and cerebellum). Without doubt, in the newborn the brain already possesses a highly organized neuronal architecture determined by an envelope of genetically coded processes.

The identification of the genetic events that have been at the origin of the brain in *H. sapiens* is still in its infancy but data that are now available on the genome and transcriptome of the rhesus monkey (Rhesus Macaque Genome Sequencing and Analysis Consortium, 2007) and the chimpanzee (Herlyn & Zischler, 2006), as well as humans (Levy *et al.*, 2007), offer interesting possibilities. From a comparative analysis it appears that about one-third of our genes started to evolve as human-specific lineages *before* the differentiation of humans, chimpanzees, and gorillas took place. This may account for the findings of very old human-specific morphological traits in the fossil record, which anticipate the recent emergence of the human species by about five to six million years (Ebersberger *et al.*, 2007). Comparative analysis further reveals that, if humans and chimpanzees show high similarity in sequence between orthologous genes, the rate of gene turnover in humans is more than two and a half times faster than in other mammals. Several gene families have expanded or contracted by shaping copy-number more rapidly than expected even after accounting for an overall rate acceleration in primates (Hahn *et al.*, 2007). Quite surprisingly, some gene families have decreased in size in the human lineage. This is the case of the olfactory genes family, of which the proportion of inactive (pseudogenes) genes is larger in humans than in other apes and larger in apes than in the mouse (Gilad *et al.*, 2000; Rouquier *et al.*, 2000). Another interesting difference is that alternative splicing is larger in humans than in chimpanzees in the corresponding tissues from the two species (Calarco *et al.*, 2007). Last, genomic surveys in humans identify a large amount of recent positive selection, to the extent that, using the 3.9-million HapMap single nucleotide polymorphism (SNP) dataset, positive selection has, unexpectedly, accelerated greatly during the past 40 000 years in human populations (Hawks *et al.*, 2007).

"Phylogenetically old" genes and neuropediatric disorders

Few of the fast-changing genes of human lineage have been investigated in detail. Particularly interesting for the pediatrician are the primary microcephaly genes that cause a reduction in brain volume to a size comparable with that of early hominids (Ponting & Jackson, 2005; Bond & Woods, 2006). Microcephalin and abnormal spindlelike-associated microcephaly are among the several genes for which mutation causes pathology by affecting neurogenic mitosis and fetal brain growth. These genes appear to alter neural progenitor cell number at a rather simple molecular target: the microtubular organization at the centrosome and the mitotic pathway. This modification may alter the action of neural progenitors when switching from symmetrical to asymmetrical cell division. The rapid evolution of these genes in the human lineage has been related to the increase in relative brain size during primate evolution (Evans *et al.*, 2005).

Childhood apraxia of speech or verbal dyspraxia is an impairment of speech production with a broad profile of linguistic deficits caused by alteration of the gene coding for the transcription factor FOXP2. In this context, an ancient DNA analysis of the *FOXP2* gene, undertaken on two Neanderthal specimens from El Sidrón, northwestern Spain, and dated to around 43 000 years ago, showed two evolutionary changes in the derived human form of FOXP2 (Krause *et al.*, 2007). Since FOXP2 has undergone recent positive selection in human history, it has been speculated that the evolution of human *FOXP2* gene might be related to the emergence of modern speech capacities (Enard *et al.*, 2002, 2009).

Other genes of relevance for the newborn brain are the genes involved in axon guidance and target selection. Most (though not all) of them have been identified in the fly and their homolog recognized in high vertebrates and humans. They comprise a finely tuned code of attractive and repulsive cues and their receptors that include, for instance, semaphorins/plexins, netrins/DCC or Unc5a-d, and slit/Robo, among many others (Tessier-Lavigne & Goodman, 1996). Interestingly, these cues also help blood vessels to navigate to their target often alongside nerve fibers (Carmeliet & Tessier-Lavigne, 2005). Other types of molecule involved in axon guidance are the cell adhesion molecules of the neural cell adhesion molecule (NCAM) and of the L1 family type that interact with

attractant and repellent guidance receptors to control growth cone and cell motility in a coordinated fashion (Maness & Schachner, 2007).

Several genes associated with autism have been recently identified. Among the many genes implicated in this disorder, particularly relevant ones are the neuroligins NLG4 and 3 and their binding partner, the scaffolding protein SHANK3, which are critically involved in synapse formation and stabilization (Persico & Bourgeron, 2006). The neurological basis for dyslexia, or reading disability, is due in large part to genetic factors. Among the several candidate genes for dyslexia (Paracchini *et al.*, 2007), *ROBO1* is orthologous to a *Drosophila* gene widely expressed in the developing nervous system. It encodes an axon guidance receptor protein that is essential for directing the outgrowth of both the axon and dendrite during development and in particular the neuronal axon crossing the midline between hemispheres. The exact functions of the other three candidates genes (*DCDC2*, *KIAA0319*, and *DYX1C1*) have yet to be elucidated, but they have all been shown to be involved in glia-guided neuronal migration during the formation of the cortex.

In short, these studies on the molecular genetics of human brain evolution have unexpected important medical consequences: more than 3000 genetic diseases that correspond to gene mutations or defects are known in humans. Many of them affect brain functions in one way or another and are already expressed in the infant brain (see Mandel, 1995). At variance with a commonly accepted "empiricist" point of view, *the brain of the newborn is not a* tabula rasa *but a richly organized structure*.

Individual variability of the human brain and the activity-dependent epigenesis of neuronal networks

The concept of synaptic epigenesis

Recent studies on human genomes carried out at the level of individuals have revealed an important inherent variability that may not systematically result in disease phenotypes. Indeed, about one base pair in 400–500 is polymorphic in the nuclear genome. Thus, two copies of the genome from different individuals will show about 1×10^6 to 2×10^6 sequence differences. The vast majority of these differences are selectively neutral but others may, as discussed, alter in a subtle way the function, or regulation, of a gene. Most of the polymorphism of the human leukocyte antigen (HLA) region was already present in the ancestors of chimpanzees and gorillas before the separation of the human lineage (Gyllensten & Erlich, 1989). Genome-scanning technologies have recently revealed an unexpectedly large extent of "structural blocks" variations in chromosomes that include deletions, duplications, and large-scale copy number variants as well as insertions, inversions, and translocations. These variants can comprise millions of nucleotides of heterogeneity within every genome and plausibly contribute to human diversity and disease susceptibility (Paabo, 2003; Feuk *et al.*, 2006). Heredity is thus often stated to be a major source of individual variability in the human brain. It may possibly account for important variations in the precise topology of defined Brodmann's areas noticed among the few brains of different individuals investigated with the required anatomical accuracy (see Mountcastle, 1998). An important variability also exists at the functional level. Joint positron emission tomography (PET) and magnetic resonance imaging (MRI) have revealed significant intersubject variability of functional areas in the visual cortex in the range of 5 mm (Hasnain *et al.*, 1998).

To analyze further the relative contribution of genetic versus "epigenetic" or environmental factors in this variability, three-dimensional maps of gray matter and models of cortical surface anatomy were derived from high-resolution three-dimensional magnetic resonance images from groups of unrelated subjects, dizygotic and monozygotic twins (Thompson *et al.*, 2001). Their comparison revealed a genetic continuum in which brain structure was found to be increasingly similar in subjects with increasing genetic affinity. Brain structures under considerable genetic control include Broca's and Wernicke's language areas, as well as frontal brain regions. In a series of anatomical (see Steinmetz *et al.*, 1995; Traino *et al.*, 1998; Eckert *et al.*, 2002) and behavioral (see Kee *et al.*, 1998) investigations carried out with genetically identical monozygotic twins who were discordant for handedness, both the in vivo measurements of the planum temporale by MRI and the results of behavioral tasks (e.g., finger tapping with anagram load) collected with left- and right-handed monozygotic co-twins yielded convergent results. The right-handers showed leftward hemispheric asymmetry, whereas the left-handers lacked symmetry. Early

epigenetic events taking place during early embryogenesis may thus contribute extensively to variability in the development of the anatomo-functional laterality of the cerebral hemispheres in genetically identical twins. In other words, "cloned" humans are not anticipated to be neurally identical.

Another rather important cause of variability originates from the way the entire network of connections becomes established between neurons during embryonic and postnatal development. The million billion (10^{15}) synapses that form the human brain network do not assemble like the parts of a computer according to a plan that defines precisely the disposition of all individual components. If this were the case, the slightest error in the instructions for carrying out this program could have catastrophic consequences. The mechanism appears, on the contrary, to rely on the progressive setting of robust interneuronal connections through trial-and-error mechanisms that, even though they are typically nongenetic, i.e., epigenetic, formally resemble an evolutionary process by variation selection (Changeux *et al.*, 1973; Changeux & Danchin, 1976; Edelman, 1978, 1987; Changeux, 2004).

Information about synaptogenesis in the developing brain may be provided by examining in adults the variance in synaptic phenotype of genetically identical individuals. The analysis has to be carried out at the level of the single nerve cell and of the exact topology of all the synaptic contacts it establishes with its partners. Such an analysis was achieved by serial sectioning of the brain of parthenogenetic animals such as the water flea *Daphnia magna* and the fish *Poecilia formosa* (see Levinthal *et al.*, 1976). At the electron microscope level, there exists a fringe of variability – a "graininess" – in the details of the axonal or dendritic branching of an identifiable neuron, the variability between left and right arborization being smaller than that found between one individual and another.

In a mammal such as the mouse – the situation might be even more extreme in humans – the number of cells is much greater and there are no longer any identifiable single cells recognizable from one individual to another. Despite common principles in gross architectural features delimited by a species-specific genetic envelope, the individual variability of the fine anatomy observed between individuals from genetically homogeneous strains increases greatly, to the extent that one wonders how such an apparently scrambled connectivity may result in reproducible behavior, even in identical twins!

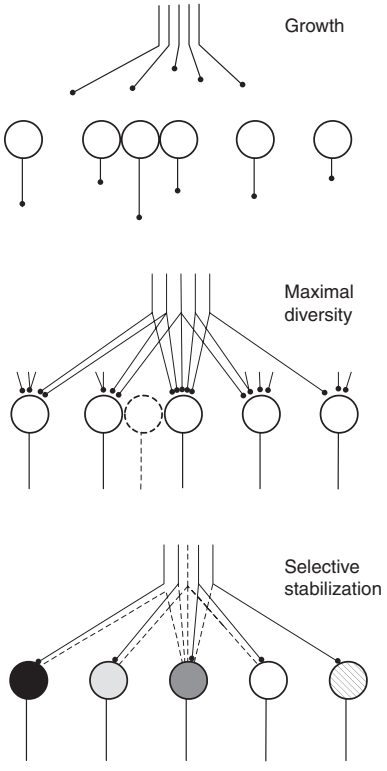

Fig. 1.3 A simple representation of the model of epigenesis by selective stabilization of synapses (from Changeux, 1983b). An interesting outcome of the formal model is that after selection different patterns of connections may yield the same behavioral input–output relationship. The different shadings of the cell bodies indicate different neuron individualities or singularities.

One plausible solution (among many others) is that the state of activity of the developing nervous system contributes to the organization of the adult network by trimming up synapse formation at sensitive periods of development. The formal model suggested (Changeux *et al.*, 1973; Changeux & Danchin, 1976; Edelman, 1978, 1987; Purves & Lichtman, 1980; Changeux, 1983a,b, 2004; Luo & O'Leary, 2005; Low & Cheng, 2006) relies on the observation that synapses do not form en masse, at once, but progressively, through exuberance followed by pruning steps, under the control of the state of activity of the developing network (Fig. 1.3). Throughout the overall development of the cortical mantle in primates and humans, several distinct phases may be recognized

(Bourgeois *et al.*, 2000; Huttenlocher & Dabholkar, 1997; see also Chapter 5).

Programmed neuronal cell death and synaptic pruning

Concomitant phases of proliferation and regression already take place in the generation of the nerve cell layers of the spinal cord, and of the cerebral cortex. The classic observation of Hamburger (1975) that about 40% of the motor neurons in the chick embryo die between the sixth and the ninth days of embryonic life is now further documented in the mouse for the cerebral cortex. Enzymes such as caspase 3 and caspase 9 must be expressed for a cell to die (see Chapter 17). Interestingly, inactivating their genes in the mouse reduces apoptosis, and increases the number of founder and precursor cells, and of radial cortical units and thus of cortical neurons. As a result, the cortical surface expands and even forms the beginnings of sulci and microgyri in this lissencephalic brain (Kuida *et al.*, 1996, 1998). The extent to which the state of activity of the developing nervous system controls such proliferative versus apoptotic steps is still being debated. Yet, correlations have been established between cell death and neuronal activation, a well-documented case being that caused by calcium entry mediated by glutamate *N*-methyl-D-aspartate (NMDA) receptors (Nicotera *et al.*, 1999). The example of the neuromuscular junction is particularly simple since only a single synaptic contact persists in the adult. On the other hand, in the newborn rat, each muscle fiber receives four or five active motor axon terminals. As the rat begins to walk, the number of these functional terminals progressively decreases until for the adult only one is left and the state of activity of the innervated muscle controls this elimination (e.g., Benoit & Changeux, 1975, 1978; Sanes & Lichtman, 1999). Axons disappear through an unusual cellular mechanism, in which they shed numerous membrane-bound remnants by engulfment of axon tips by Schwann cells (Bishop *et al.*, 2004). A similarity exists between axonal pruning and wallerian degeneration, which involves microtubule breakdown and mobilizes the ubiquitin–proteasome system (Luo & O'Leary 2005). Axonal pruning phenomena have also been documented at the synaptic level in other systems such as the sympathetic ganglia (Purves & Lichtman, 1980) or the climbing-fiber Purkinje cell synapse in the cerebellum (for reviews, see Changeux & Mikoshiba, 1978; Crépel, 1982; Kano *et al.*, 1997). For the latter, a mutation that inactivates a specific neurotransmitter receptor (the type 1 metabotropic glutamate receptor [mGluR1]) delays the regression of supernumerary climbing fiber innervation (Kano *et al.*, 1997).

The contribution of synaptic activity (evoked and/or spontaneous) to the formation of cortical circuits has been well documented since the classic experiments of Wiesel and Hubel (1963a, b), which demonstrated the important role of visual experience in fixing the organization of ocular dominance columns (for reviews, see Katz & Shatz, 1996 and Chapter 10), yet within a "functional validation" framework. At variance with this scheme, an exuberant sprouting and proliferation of axon branches, accompanied by limited although critical elimination of collaterals, has been visualized at different locations along the visual pathway (retinogeniculate, thalamocortical, and pyramidal cell arbors) at sensitive periods of development (see Stretavan & Shatz, 1986; Katz & Shatz, 1996). The state of activity of the developing cortical circuits controls synaptic evolution by more than simply validating preformed circuits. Such epigenetic regulation may concern the overall development from the early interaction with the physical world up to the elementary social experience (see Hadders-Algra *et al.*, 1996 and Chapter 21).

The model suggested (Changeux *et al.*, 1973; Changeux, 1983a, b; see also Harris *et al.*, 1997; Elliott & Shadbolt, 1998; Miller, 1998) posits that during synapse formation the genetic envelope controls, in addition to the division, migration, and differentiation of cell categories, the behavior of the growth cone, the outgrowth and formation of widespread connections, the recognition of the target cells, and the onset of spontaneous activity; it also determines the structure of the molecules that enter into the architecture of the synapse, the rules governing their assembly, and the evolution of this connecting link by the activity of the network. Yet, at sensitive periods of circuit development, the phenotypic variability of nerve cell distribution and position, as well as the exuberant spreading and the multiple figures of the transiently formed connections originating from the erratic wandering of growth cone behavior, introduce a maximal diversity that is then reduced by the selective stabilization of some of the labile contacts and the elimination (or retraction) of the others. The crucial hypothesis of the model is that the evolution of the connective state of each synaptic contact is