Published in "Developmental Neuroscience 29(1-2): 179-192, 2007"
which should be cited to refer to this work.

Postnatal Development of the Primate Hippocampal Formation

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Key Words
Hippocampus · Neural development · Monkey · Declarative memory · Neurodevelopmental disorders

Abstract
The hippocampal formation is a multicomponent region of the medial temporal lobe preferentially involved in declarative and relational memory processing. Behavioral studies have suggested a protracted functional maturation of these structures in primates, and postnatal developmental abnormalities in the hippocampal formation are thought to contribute to neurodevelopmental disorders, such as autism, schizophrenia, epilepsy and Down syndrome. Despite all that we know about the functional organization of the adult hippocampal formation, notably absent is a systematic study of its postnatal maturation in primates. In this article, we review current knowledge of the structural development of the primate hippocampal formation and present new data on its postnatal neuroanatomical development. We summarize what is known about the neurobiological processes, such as the addition of new neurons, the establishment and elaboration of connectivity, and the neurochemical changes, that underlie the structural development and functional maturation of the primate hippocampal formation. We conclude that there is yet insufficient information to identify distinct developmental windows during which different hippocampal regions undergo specific maturational processes. For this reason, it is currently impossible to determine the ages at which specific hippocampal circuits become structurally mature and potentially capable of supporting defined, age-specific functional processes. Together with work in rodents, systematic studies of the structural development and functional maturation of the monkey hippocampal formation will be necessary to gain insight not only into the types of information processing that it subserves, but also into the specific maturational processes that might be affected in human neurodevelopmental disorders.

The hippocampal formation is comprised of a group of cortical regions located in the medial temporal lobe that includes the dentate gyrus, hippocampus, subiculum, presubiculum, parasubiculum and entorhinal cortex (fig. 1A). Damage to these structures in adult humans and animals causes a profound loss of declarative memory function without other sensory, motor or cognitive impairments [Milner et al., 1998]. Indeed, episodic memory has been shown to be sensitive to hippocampal damage [Squire and Zola, 1996] and the retrieval of autobiographical memories activates the hippocampus in adults [Maguire, 2001]. Nonetheless, despite all that we know about the functional organization of the adult hippocampal formation (see Amaral and Lavenex [in press] for a review), notably absent is a systematic study of its postnatal maturation in primates. To date, most studies of the emergence of declarative memory in infants have focused on infantile amnesia (the inability to recall early life events), specifying the age at which episodic memory de-

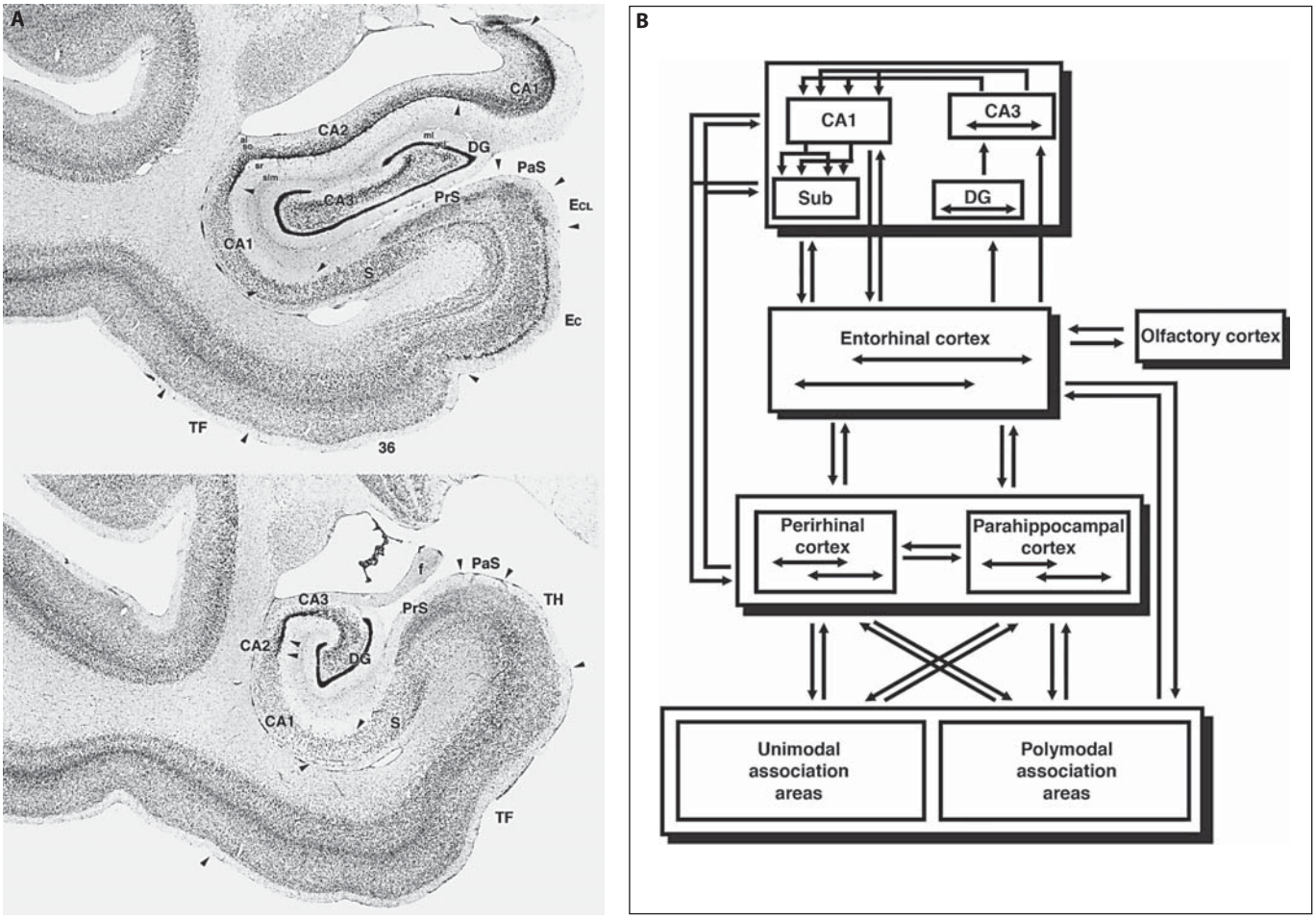

Fig. 1. A Coronal Nissl-stained sections through the medial temporal lobe of the macaque monkey. **B** Schematic representation of the primate medial temporal lobe illustrating the hierarchical organization of the associational networks constituting the neocortical-hippocampal loop. 36 = Area 36 of the perirhinal cortex (area 35 not shown); CA1, CA2, CA3 = fields of the hippocampus; al = alveus; pcl = pyramidal cell layer; so = stratum oriens; sr = stratum radiatum; slm = stratum lacunosum moleculare; DG = dentate gyrus; gcl = granule cell layer; ml = molecular layer; pl = polymorphic cell layer; EC = entorhinal cortex, caudal division; ECL = entorhinal cortex, caudal limiting division; f = fimbria; PaS = parasubiculum; PrS = presubiculum; S = subiculum; TF = area TF of the parahippocampal cortex; TH = area TH of the parahippocampal cortex.

velops (generally around 3–5 years of age [Rubin, 2000]), or on determining its accuracy and reliability [Peterson, 2002]. There has been no study, however, attempting to establish a direct link between the emergence of autobiographical memories in infants and the maturation of specific brain systems [Nelson, 1998]. Other behavioral studies of declarative memory in monkeys and humans have suggested a protracted functional maturation of the primate hippocampal formation [Harlow, 1959; Hayne et al., 2000; Malkova et al., 2000; Overman and Bachevalier, 2001; Overman et al., 1996; Rudy et al., 1993], but no specific anatomical evidence exists to substantiate this hypothesis [Bauer, 2006]. Clearly, elucidating the postnatal structural development of the primate hippocampal formation would allow a greater understanding of the emergence of declarative memory processes and provide critical insight into the concept of infantile amnesia, the organization of memory and the function of the medial temporal lobe structures across the life span.

The importance of specifying the postnatal development of the primate hippocampal formation also stems from studies of neurodevelopmental disorders, such as autism, schizophrenia and Down syndrome, which suggest postnatal developmental abnormalities in these structures

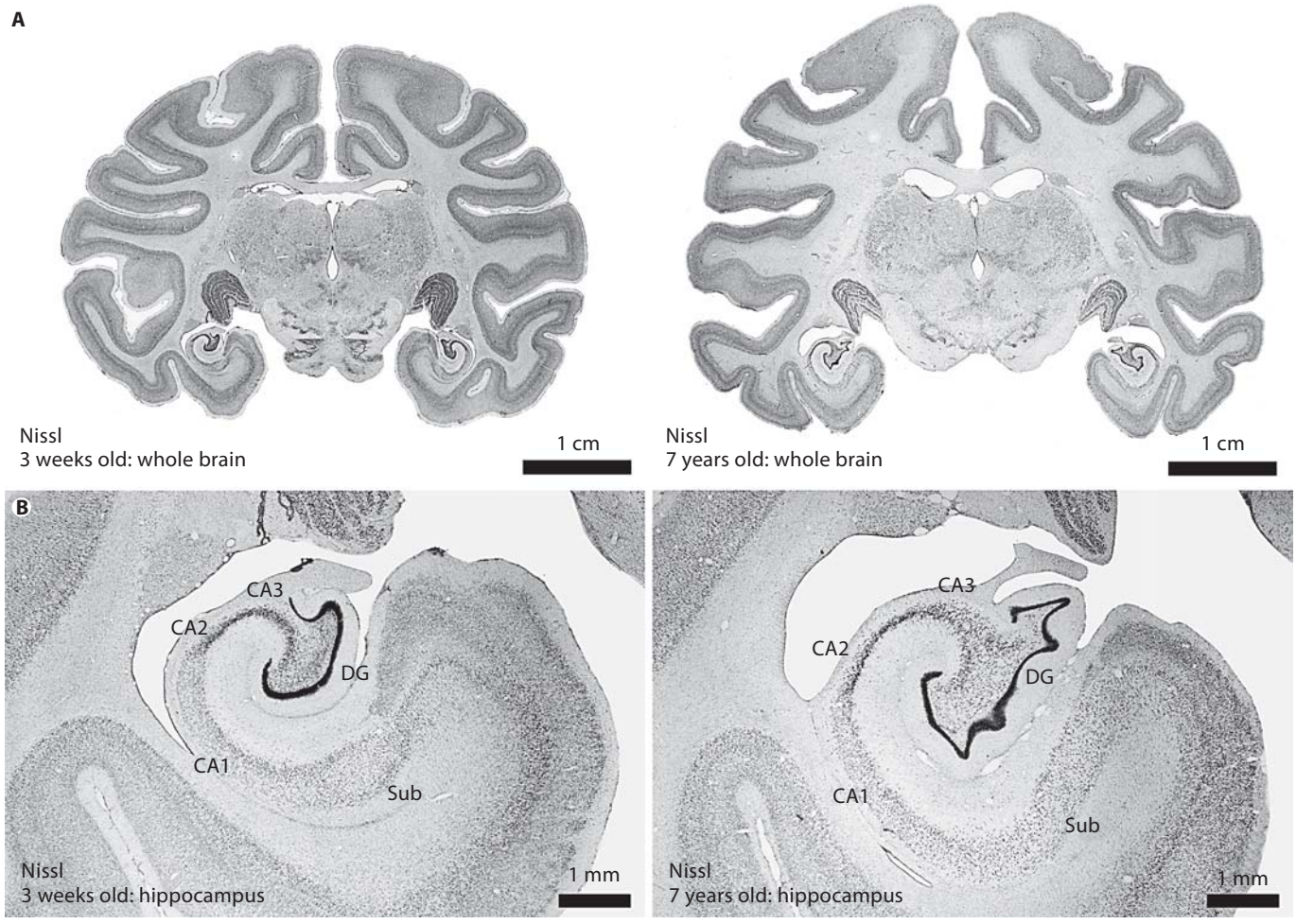

Fig. 2. A Coronal Nissl-stained sections through the brain of a 3-week-old and a 7-year-old monkey at comparable midrostrocaudal levels of the hippocampus. The magnification is the same for both images. **B** Higher-magnification photomicrographs at the same level illustrating four major subdivisions of the monkey hippocampal formation. Note in particular the large increase in size and complexity of the dentate gyrus between 3 weeks and 7 years of age. DG = Dentate gyrus; CA3, CA2, CA1 = fields of the hippocampus; Sub = subiculum.

[Bauman and Kemper, 1985; Dierssen et al., 1996; Harrison, 1999; Leverenz and Raskind, 1998; Raymond et al., 1996; Saitoh et al., 2001; Uecker et al., 1993]. Although the structures of the primate hippocampal formation are easily recognizable at birth (fig. 2) [Rakic and Nowakowski, 1981; Arnold and Trojanowski, 1996], they undergo substantial postnatal maturation throughout infant and juvenile life [this article; Giedd et al., 1996; Grateron et al., 2002, 2003; Saitoh et al., 2001]. It is reasonable to predict, therefore, that during this critical postnatal maturational period these structures might be particularly sensitive to factors capable of altering the expression of specific genes, thus affecting normal development. Consequently, defining the normal maturation of the primate hippocampal formation is of particular import in order to identify processes, substrates and critical periods of maturation that might be sensitive to perturbation.

In this article, we first review the literature on the development of the primate hippocampal formation and present new preliminary data on its postnatal neuroanatomical development. We then discuss specific issues that remain to be addressed to establish direct links between the structural maturation of the brain and the emergence of declarative memory systems and to further our understanding of the etiology of neurodevelopmental disorders.

The Adult Primate Hippocampal Formation

The hippocampal formation is an important component of a distributed brain network that carries out memory function. Neuroanatomical studies in the adult monkey have defined the major components of this system and have indicated that the medial temporal lobe structures are organized as a hierarchy of associational networks [Lavenex and Amaral, 2000]. The hippocampal formation, along with the perirhinal and parahippocampal cortices, are thought to participate in the consolidation of information in higher-order associational cortices via feedback projections [Lavenex et al., 2002]. Associational connections within the perirhinal and parahippocampal cortices enable significant integration of unimodal and polymodal inputs, resulting in only highly integrated, polysensory information reaching the hippocampal formation [Lavenex et al., 2004b]. The entorhinal cortex, in turn, occupies a pivotal neuroanatomical position and constitutes the interface for bidirectional interaction between the neocortex and the hippocampal formation (fig. 1B).

Postnatal Development of the Primate Hippocampal Formation

Even a cursory examination of the neonatal macaque monkey hippocampal formation is sufficient to demonstrate that these structures are far from mature at birth (fig. 2). Despite gross morphological changes, however, little is known about the neurobiological processes, such as the addition of new neurons, the establishment and elaboration of connectivity, and the neurochemical changes, that underlie the structural development and functional maturation of the hippocampal formation in primates. We will address these issues in turn.

Neurogenesis

The majority of the neurons in the primate hippocampal formation are generated prenatally [Arnold and Trojanowski, 1996; Eckenhoff and Rakic, 1988; Nowakowski and Rakic, 1981]. Seminal studies by Rakic and Nowakowski [1981], using ^{3}H-thymidine autoradiographic techniques, established that although the first neurons destined for the different subdivisions of the monkey hippocampal formation are generated nearly simultaneously around 36 days of gestational age (E36; total gestation is 165 days), proliferation ceases at different times for each subdivision: between E56 and E65 for the subiculum and area CA2; between E65 and E70 for area CA3; between E70 and E75 for the entorhinal cortex and the presubiculum; between E70 and E80 for area CA1; between E75 and E80 for the parasubiculum and the polymorphic layer of the dentate gyrus.

In the granule cell layer of the dentate gyrus, in contrast, studies have shown that even though neuron production decreases significantly within early postnatal life, a substantial number of neurons continue to be generated postnatally [Eckenhoff and Rakic, 1988; Nowakowski and Rakic, 1981; Rakic and Nowakowski, 1981], even into adulthood [Gould et al., 1999; Kornack and Rakic, 1999]. However, neither the precise number of new neurons added nor the exact postnatal temporal profile of neuronal addition have been empirically investigated. We thus designed an experiment to quantify the increase in neuron number in the monkey granule cell layer between infancy and adulthood. We used the optical fractionator technique [Lavenex et al., 2000a, b; West et al., 1991] to count the number of neurons in the granule cell layer of 3-week-old (n = 9), 3-month-old (n = 2) and adult (n = 6) monkeys. A total of 24–36 sections per animal (1 in 16 sections, 480 μm apart) were analyzed, with the first section selected randomly within the first four sections through the dentate gyrus. We used a ×100 Neofluar oil objective (N.A. 1.30) on a Nikon Optiphot microscope linked to the PC-based StereoInvestigator 4.5 (Microbrightfield, Williston, Vt., USA) analysis program. The sampling scheme was as follows: average section thickness: 17 μm (range 14–21 μm); stepping frame: 300 × 300 μm; counting frame: 15 × 15 μm; dissector height: 6 μm, and guard zones: 3 μm.

Our stereological study provides the first unbiased quantitative estimates of neuron number in the dentate gyrus of infant and adult monkeys (fig. 3A). We found that the number of granule cell neurons differs significantly between 3-week-old and 3-month-old monkeys: 3-week-old monkeys have on average 9.13 ± 0.80 million neurons in the dentate gyrus whereas 3-month-old animals have on average 13.02 ± 0.54 million neurons. These results provide direct evidence that a substantial number of neurons, more than 30%, are added to the granule cell layer within the first 3 months of life. By 3 months of age, however, neuron number appears to have reached adult levels (12.97 ± 0.70 millions). Interestingly, the stabilization of neuron number by 3 months of age suggests that adult neurogenesis functions as a replace-

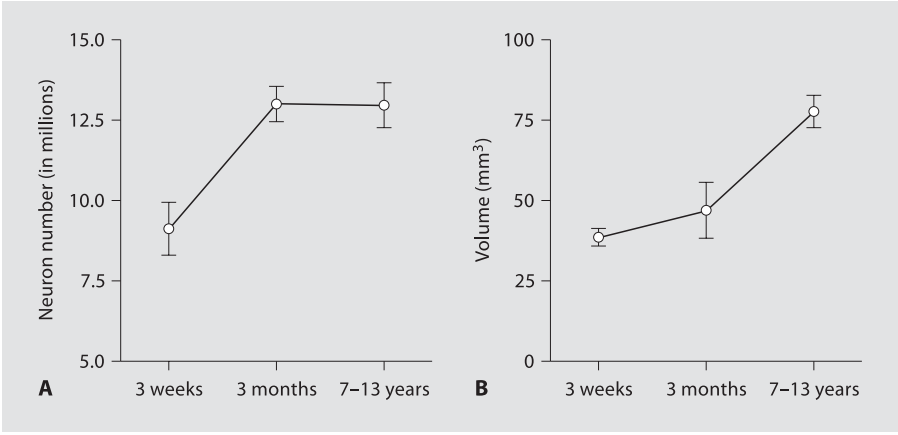

Fig. 3. A The number of dentate granule cells differs significantly between 3-week-old and 3-month-old monkeys. By 3 months of age, however, the neuron number reaches adult levels: $F(2, 14) = 7.16$, $p = 0.0072$; 3 weeks < 3 months = 7–13 years. **B** The volume of the dentate gyrus does not differ between 3-week-old and 3-month-old monkeys, but it almost doubles between 3 months of age and adulthood: $F(2, 14) = 28.31$, $p = 0.0001$, 3 weeks = 3 months < 7–13 years.

ment mechanism for neurons, or for a subpopulation of neurons, in the dentate gyrus throughout life [Banta Lavenex et al., 2001].

Our preliminary evaluation of cell proliferation using the endogenous cell division marker Ki67 reveals a substantial number of labeled cells in the hilus and the subgranular zone of the dentate gyrus in 3-week-old monkeys (not shown; polyclonal antibody anti-Ki67 from Zymed No. 18-019, San Francisco, Calif., USA). We also see numerous labeled cells in the white matter of the fornix and corpus callosum that are likely glial cells associated with myelination processes taking place during early postnatal life. This pattern is similar to that observed in newborn humans [Seress, 2001]. The adult monkey shows far fewer Ki67-labeled cells in the dentate gyrus as compared to the infant, but we have not yet performed a quantitative evaluation of these differences. Information concerning the time of origin of neurons in the human hippocampal formation is rather scarce and primarily based on qualitative observations of the morphological characteristics of neuronal populations [Arnold and Trojanowski, 1996; Humphrey, 1967; Seress, 1992, 1998] and on endogenous markers of cell proliferation such as Ki67 [Seress et al., 2001]. Similar to the monkey, a low rate of neurogenesis continues throughout life in the human dentate gyrus [Eriksson et al., 1998]. Several studies [Arnold and Trojanowski, 1996; Humphrey, 1967; Seress, 1992, 1998] reported that the fundamental cytoarchitectonic appearance of the hippocampal subfields is stable after birth, although there is progressive neuronal enlargement and a decrease in neuronal density throughout childhood and adulthood.

Volumetric Changes and Dendritic Growth

The volumetric changes we observe in the monkey dentate gyrus reveal a very different developmental profile than that of neuron number (fig. 3B). Neither the volume of the dentate gyrus, nor that of any of its layers, differs significantly between 3-week-old (n = 9) and 3-month-old (n = 2) monkeys, but it almost doubles between 3 months of age and adulthood (n = 6; as measured with the Cavalieri method on Nissl-stained sections cut at 30 μm on a freezing sliding microtome). These volumetric changes suggest that although an 'adult' number of granule cell neurons have been generated by 3 months of age, these neurons undergo significant morphological maturation (including dendritic elaboration and synaptogenesis) beyond 3 months of age in order to achieve adult characteristics.

Indeed, all available data indicate that there is significant postnatal maturation throughout the hippocampal formation [Giedd et al., 1996; Grateron et al., 2002, 2003; Saitoh et al., 2001]. In the monkey, studies by Duffy and Rakic [1983] and Seress [1992] indicate that the dendritic arborization of the granule cells, in the molecular layer of the dentate gyrus, matures during the first 6 months of life. In newborns, the total dendritic length is substantially less than in adults (1,275 ± 92 μm in newborns [Duffy and Rakic, 1983] versus 1,977 ± 708 μm in adults [Seress, 1992]), although the total number of dendritic segments may be similar [Duffy and Rakic, 1983; Seress, 1992]. At 6 months, the appearance and numerical parameters of the entire dendritic tree are thought to be comparable to those of 1-year-old and 3-year-old monkeys [Seress, 1992]. Although methodological issues such as inconsistent impregnation of neurons by the rapid Gol-

Table 1. Morphological characteristics of monkey CA1 pyramidal cells

	Age				
	E85	E105–E109	E134	E154	adults
Soma volume, μm^3 (derived from data on surface area)	856 ± 24	2,692 ± 124	3,695 ± 117	3,009 ± 176	3,562 ± 192
Total dendritic length, μm	954 ± 135	5,776 ± 381	15,404 ± 1,206	15,316 ± 1,976	19,393 ± 981
Number of terminal branches (basal dendrites)	2 ± 1	16 ± 6	30 ± 5	28 ± 1	55 ± 3
Number of terminal branches (apical dendrites)	9 ± 1	20 ± 4	45 ± 3	59 ± 2	46 ± 3

Gestational ages (E85, E105–109, E134, E154): data from Khazipov et al. [2001]. Adults: data from Altemus et al. [2005], excludes monkeys less than 3 years old.

gi method in monkeys of different ages [Duffy and Rakic, 1983; Seress, 1992], and possible laboratory differences [Scorcioni et al., 2004] might advise caution in the interpretation of these data, these studies suggest the lack of a regressive period in the elaboration of the dendritic arborization of the granule cells [Seress, 1992].

Following the hippocampal circuitry, the targets of the granule cell projections in the hilus and the CA3 field of the hippocampus also show significant postnatal maturation. The morphological characteristics of the mossy cells in the hilus [Amaral, 1978; Buckmaster and Amaral, 2001] evolve gradually during the first 9 months of life [Seress and Ribak, 1995a]. At birth, only a few thorny excrescences (synaptic specializations for mossy fiber contact on the dendrites of the mossy cells) are found on the proximal dendrites of the mossy cells and their distal dendrites display a low density of pedunculate spines. Thorny excrescences increase in number and complexity until the third postnatal month. Seress and Ribak [1995a] reported that by 3 months of age, mossy cells appeared to be mature. They display large, complex thorny excrescences, similar to 7-month-old, 9-month-old and adult monkeys. In contrast, the pedunculate spines on the distal dendrites continue to increase in number at least during the first 9 postnatal months [Seress and Ribak, 1995a]. There is an average of 33 ± 7 spines per 100 μm dendritic length of mossy cells in the deep polymorphic layer in 1-day-old monkeys, increasing gradually to 54 ± 7 at 1 month, 73 ± 10 at 3 months, 76 ± 10 at 7 months, 93 ± 9 at 9 months, 105 ± 6 at 1 year, 118 ± 14 at 4 years, and 117 ± 12 at 20 years of age.

The mossy fibers (the axons of the granule cell neurons) also exhibit significant postnatal maturation [Seress and Ribak, 1995b]. At birth, Timm-stained mossy fiber terminals are present, but staining intensity is weaker than observed in 3-month-old and adult monkeys. Furthermore, the stratum lucidum (the layer of mossy fiber trajectory and termination located just above the pyramidal cell layer) increases in width after 6 months of age. Interestingly, the mossy fibers that are already present at birth have mature-looking synapses, and the thorny excrescences present on the CA3 pyramidal cells are also adult-like [Seress and Ribak, 1995b]. The number of spines per 100 μm of dendritic length of CA3 pyramidal neurons follows a developmental pattern similar to that observed for the mossy cells. Spine density on CA3 apical dendrites in the stratum lacunosum moleculare is: at 1 day, 47 ± 10 spines; at 1 month, 45 ± 7 spines; at 3 months, 81 ± 10 spines; at 7 months, 70 spines (no SD); at 9 months, 81 ± 12 spines; at 1 year, 95 ± 7 spines; at 4 years, 97 ± 10 spines; at 20 years, 100 ± 10 spines. Spine density on CA3 basal dendrites in the stratum oriens is: at 1 day, 48 ± 11 spines; at 1 month, 58 ± 11 spines; at 3 months, 76 ± 12 spines; at 7 months, 80 spines (no SD); at 9 months, 83 ± 6 spines; at 1 year, 93 ± 8 spines; at 4 years, 97 ± 11 spines; at 20 years, 110 ± 10 spines. Altogether, these findings suggest a protracted period of synaptogenesis in the polymorphic cell layer of the dentate gyrus and the CA3 field of the hippocampus.

A morphological and electrophysiological study of the early development of the monkey hippocampus revealed that the CA1 pyramidal neurons are highly differentiated 1 month before birth [Khazipov et al., 2001], but not necessarily adult-like. Interestingly, the electrophysiological maturation (sequential expression of GABAergic and glutamatergic synaptic currents) correlates with the morphological maturation of the pyramidal cells [Khazipov et al., 2001]. Comparison with the morphological characteristics of adult monkey CA1 pyramidal cells studied in our laboratory [Altemus et al., 2005] suggests that further growth and remodeling of the axonal and dendritic arbors continues after birth (table 1). This conclusion is in

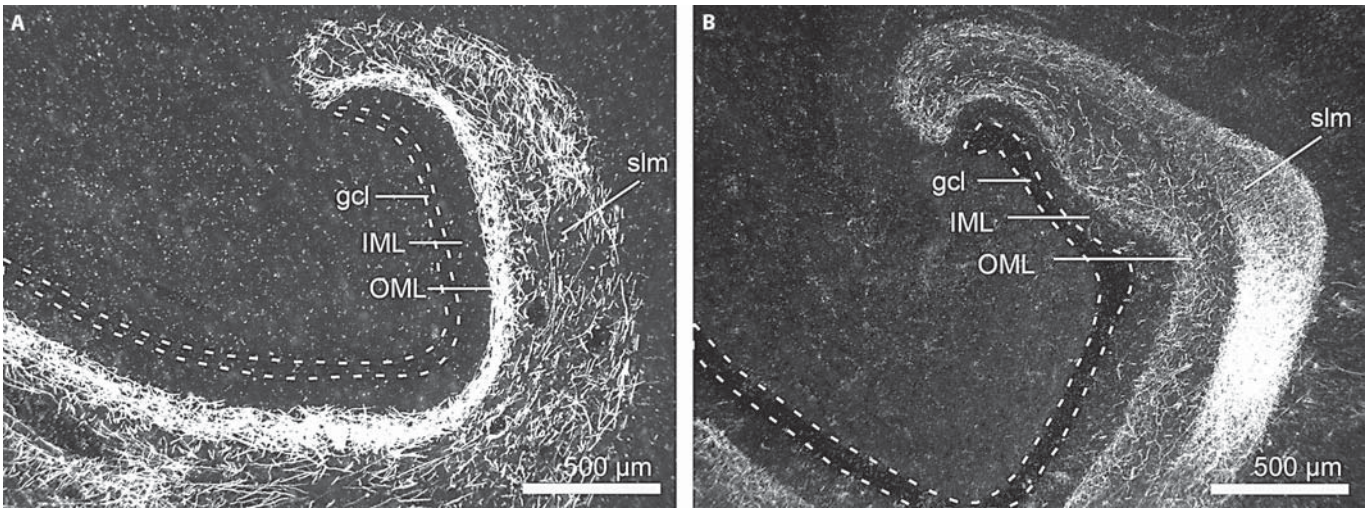

Fig. 4. A Anterogradely labeled fibers in the dentate gyrus and hippocampus of a 3-week-old monkey following a PHA-L injection in the caudal portion of the entorhinal cortex. **B** Anterogradely labeled fibers in the dentate gyrus and hippocampus of an adult monkey following a PHA-L injection in the caudal portion of the entorhinal cortex. Note the similar laminar pattern of projections in infant and adult monkeys. slm = Stratum lacunosum moleculare; gcl = granule cell layer (outlined); OML = outer molecular layer; IML = inner molecular layer.

agreement with Seress [2001] who reported changes in spine density and myelin formation up to the seventh postnatal month in area CA1. Altogether, these findings indicate that the neurons of the monkey hippocampal complex undergo substantial morphological maturation during the early postnatal period and do not achieve adult-like characteristics until between 6 months and 1 year of age.

Connectivity

The development of connectivity in the nonhuman primate hippocampal formation has not yet been evaluated. Despite extensive literature searches, we have not found a single publication dealing with the development of hippocampal connectivity in nonhuman primates. There is one publication on the development of entorhinal-hippocampal connections in the midgestational human fetus [Hevner and Kinney, 1996]. This postmortem study used the lipophilic bidirectional tracer DiI, and revealed that direct connections from the entorhinal cortex to the CA1 field of the hippocampus and the subiculum might be present between 19 and 22 weeks of gestation. In contrast, the entorhinal projections to the dentate gyrus or the connections between the entorhinal cortex and the neocortex were sparse, suggesting later maturation. Interestingly, although all six layers of the entorhinal cortex are identifiable at birth in humans, they are not as clearly laminated as in the adult [Grateron et al., 2002]. In particular, the superficial layers (layers I–III) are not clearly differentiated, suggesting that the entorhinal neurons that originate the main projections to the dentate gyrus and the hippocampus (layer II and III neurons) are not yet fully mature. Neurochemical findings also suggest protracted maturation of entorhinal neurons (see below) [Grateron et al., 2003].

We have begun to investigate the relative maturity of entorhinal connections in the neonatal rhesus monkey. In one series of experiments, we injected the anterograde tracers PHA-L (*Phaseolus vulgaris*-leucoagglutinin) and BDA (biotinylated dextran amine) into the entorhinal cortex of infant (n = 7) and adult (n = 15) monkeys (see Chrobak and Amaral [2006] and Lavenex et al. [2004b] for a detailed description of the methods). These experiments revealed that the entorhinal projection directed towards the dentate gyrus (fig. 4) is established by 3 weeks of age and is directed primarily to the appropriate zone in the outer molecular layer; the projection to CA3 and CA1 also terminates appropriately in the stratum lacunosum moleculare (see Witter and Amaral [1991] for a detailed description of the adult pattern). A study aimed at characterizing the entorhinal connectivity in the newborn monkey, as compared to the adult [Chrobak

and Amaral, 2006; Insausti et al., 1987a, b; Lavenex and Amaral, 2000; Suzuki and Amaral, 1994; Witter and Amaral, 1991; Witter et al., 1989], is currently under way in our laboratory. This study will provide definitive data regarding the degree of maturation of the major input and output pathways of the hippocampal formation at birth.

Neurotransmitters

Previous studies of the neurochemical maturation of the monkey hippocampal formation have focused on establishing the temporal profiles of the appearance of particular neurotransmitter pathways during gestation [Berger and Alvarez, 1994, 1996; Berger et al., 1993, 1999, 2001]. In the entorhinal cortex, several neuroactive substances, such as somatostatin, calbindin D-28K and serotonin, are detected relatively early, i.e., during the first half of gestation [Berger et al., 1993]. Parvalbumin-like immunoreactive interneurons are progressively detected throughout the different structures of the hippocampal formation during the second half of gestation, which indicates that a major GABAergic component of the hippocampal inhibitory circuitry is present several weeks before birth [Berger et al., 1999]. In most cases, however, the lack of direct comparison with adult brains makes it difficult to determine when these systems reach an adult level of maturity. In one study, however, comparison with the expression patterns in adult monkeys was available [Berger et al., 2001]. A thin band of calretinin-immunoreactive puncta occupied the innermost part of the dentate gyrus molecular layer at E109. However, the pattern of calretinin immunoreactivity along the granule cell layer of the dentate gyrus increased in density sometime between E142 and 4 years of age when it constitutes a very dense band occupying the inner third of the molecular layer.

We found a similar trend for the pattern of cholinergic innervation in the infant and adult monkey dentate gyrus. Acetylcholinesterase enzymatic activity, a cholinergic marker, intensely labels the inner molecular layer of the dentate gyrus in both infant and adult monkeys, whereas the outer molecular layer stains relatively lightly (not shown; see Bakst and Amaral [1984] for a description of the methods). However, even though the overall pattern of cholinergic innervation is similar at both ages, densitometric measurements [normalized between sections and between individuals based on the background level determined in the corpus callosum: normalized density = (signal – background)/background] reveal that the intensity of labeling is about 1.6 times greater in the molecular layer of adult monkeys (n = 2) as compared to infant monkeys (n = 2; data not shown). This suggests that the density of cholinergic fibers and terminals increases substantially after birth.

For the inhibitory GABAergic system, the finding that the general pattern of innervation is identifiable in the infant monkey has been taken as evidence that the GABAergic circuitry of the hippocampal formation is already established [Berger et al., 1999] and functional [Khazipov et al., 2001] at birth. Our initial evaluation of GAD67 immunohistochemistry (glutamic acid decarboxylase is the enzyme necessary for the synthesis of the inhibitory neurotransmitter GABA), however, reveals distinct differences in hippocampal labeling patterns between infant (n = 2) and adult (n = 2) monkeys (not shown; polyclonal antibody anti-GAD67 from Chemicon AB5992, Temecula, Calif., USA). These preliminary assessments suggest that although many neurotransmitter systems may be detectable at birth, they undergo substantial postnatal developmental changes that are likely to affect the functional properties of the hippocampal circuitry.

Synapses

As might be predicted, significant modification at the synaptic level can also be expected in the maturing hippocampal formation. For instance, we have found that the labeling pattern of synaptophysin (a presynaptic vesicle glycoprotein) in the molecular layer of the dentate gyrus exhibits striking differences between infant (n = 2) and adult (n = 2) monkeys (fig. 5; monoclonal antibody anti-synaptophysin from Chemicon MAB329). In infants, the inner molecular layer is more darkly stained than the outer molecular layer, whereas in adults the outer molecular layer stains more darkly than the inner molecular layer. Although this method does not provide a direct evaluation of the total number of synapses, it suggests a differential developmental profile of synaptogenesis in the inner and outer molecular layers of the dentate gyrus, which might reflect differential maturation of distinct functional circuits. Specifically, this finding supports the idea that associational connections are established prior to entorhinal afferent connections, as is the case in the infrapyramidal blade of the rat dentate gyrus [Tamamaki, 1999].

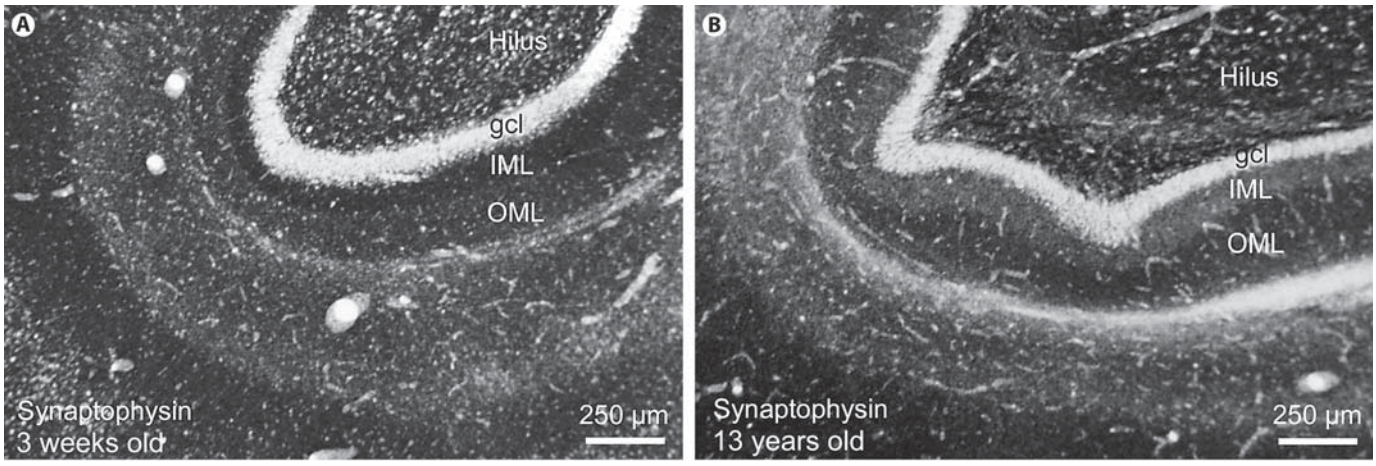

Fig. 5. A Pattern of synaptophysin immunoreactivity in the dentate gyrus of a 3-week-old monkey: heavier staining in the inner molecular layer. **B** Pattern of synaptophysin immunoreactivity in the dentate gyrus of a 13-year-old monkey: heavier labeling in the outer molecular layer. gcl = Granule cell layer; IML = inner molecular layer; OML = outer molecular layer.

Previously, Eckenhoff and Rakic [1991] evaluated synaptogenesis in the molecular layer of the monkey dentate gyrus using electron microscopy. They reported an increase in synaptic density within the first 5 postnatal months, followed by a decline over the next 5 months to reach levels comparable to those observed in adults. The increase in synaptic density is believed to be due to the addition of asymmetrical synapses to dendritic spines. However, the authors reported synaptic densities and not the total number of synapses. Moreover, they did not find any volumetric changes in the molecular layer of the dentate gyrus postnatally. Our own data, acquired using design-based stereological techniques, contradict this finding. We have observed a large increase in the volume of the molecular layer between 3 weeks of age and adulthood [3 weeks old (n = 9): 22.10 $\pm$ 1.34 mm^3; 3 months old (n = 2): 27.28 $\pm$ 2.74 mm^3; 7–13 years old (n = 6): 44.03 $\pm$ 2.24 mm^3; measured with the Cavalieri method on Nissl-stained sections cut at 30 µm on a freezing sliding microtome]. These calculations suggest a selective overproduction of asymmetrical, axospinous synapses during infancy. However, the observed decrease in synaptic density after 5 months of age [Eckenhoff and Rakic, 1991] might be due to an increase in neuropil volume (the number of synapses remaining the same), rather than a decrease in the number of synapses. Clearly, additional information at both the light and electron microscopic levels is essential to resolve this issue.

Neuroskeleton

Finally, dramatic neurostructural differences exist between infant and adult monkeys [Lavenex et al., 2004a]. Neurofilaments, for example, are neuron-specific cytoskeletal components essential for the establishment and maintenance of axonal and dendritic structure [Carden et al., 1987; Lee and Cleveland, 1996]. Neurofilaments assemble as heteropolymers requiring the obligatory subunit low-molecular-weight neurofilament (NF-L) and substoichiometric amounts of medium-molecular-weight neurofilament (NF-M) and/or high-molecular-weight neurofilament (NF-H) [Lee and Cleveland, 1996]. An immature form of neurofilaments, composed of NF-L and NF-M, appears to function in establishing the neuronal phenotype and in initiating and maintaining neurite outgrowth, whereas the slow and gradual expression of NF-H is indicative of the maturation and stabilization of neuronal circuitries [Carden et al., 1987; Lee and Cleveland, 1996; Lopez-Picon et al., 2003].

We found that in the adult monkey hippocampal formation (n = 4), nonphosphorylated neurofilaments (NF-H visualized by SMI-32 immunoreactivity) are prominent in the subiculum and the entorhinal cortex (fig. 6). In infants ($\leq$3 months, n = 6), the subiculum stains heavily for nonphosphorylated NF-H, but there is little or no labeling in the entorhinal cortex [Lavenex et al., 2004a]. These findings suggest that different subregions of the primate hippocampal formation mature at different times

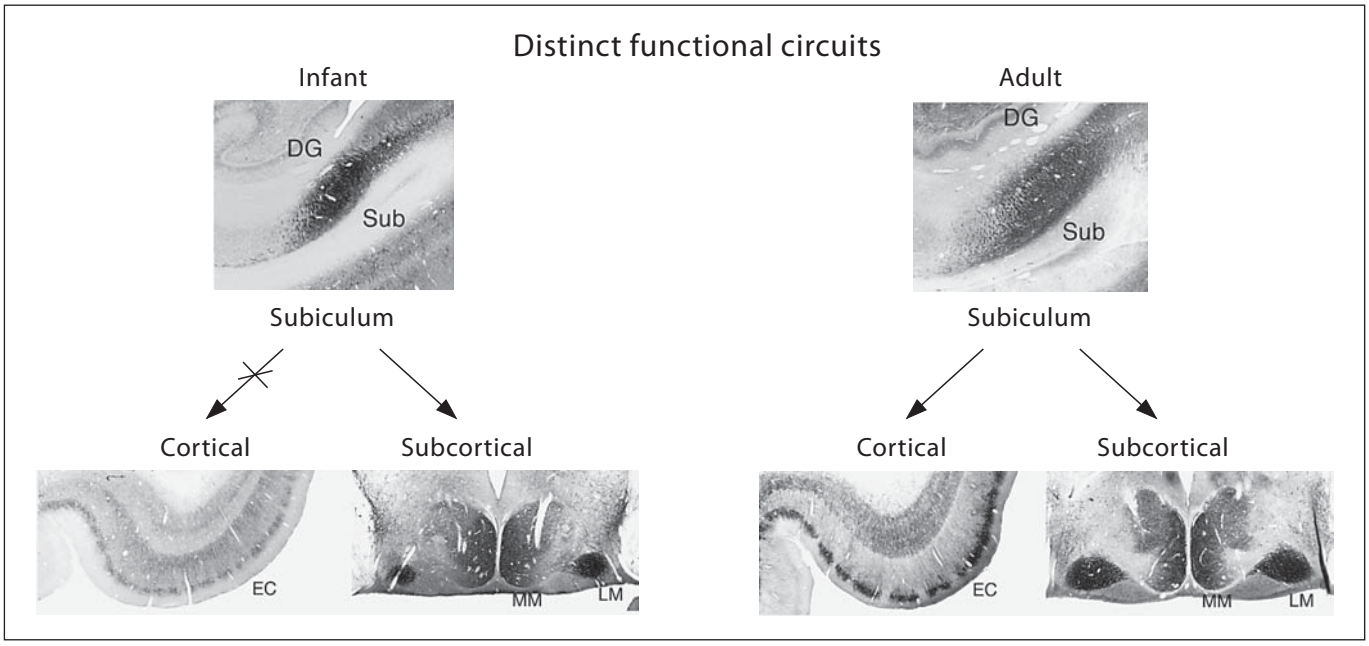

Fig. 6. Patterns of nonphosphorylated NF-H expression in the infant and adult monkey hippocampal formation suggest differential maturation of distinct functional circuits: early maturation of the subicular projection towards subcortical structures and later maturation of the subicular projection towards the entorhinal cortex. DG = Dentate gyrus; Sub = subiculum; EC = entorhinal cortex; MM = medial mammillary nuclei; LM = lateral mammillary nuclei.

during development. The subiculum, the major source of efferent projections from the hippocampal formation towards subcortical structures, matures early during development. In contrast, the entorhinal cortex, the main interface of the hippocampal formation with the neocortex, matures relatively later. These findings have direct implications for the type of information processing that might be subserved by the primate hippocampal formation shortly after birth, as well as for the emergence of particular behavioral and memory processes during postnatal development.

Another intriguing finding, however, is that although the subiculum exhibits a high level of expression of nonphosphorylated NF-H shortly after birth, comparison with the adult reveals distinct differences in the pattern and density of neurofilament expression [Lavenex et al., 2004a]. These differences might reflect varying levels of maturation of distinct functional circuits within the subiculum (fig. 6). It is reasonable to hypothesize, for example, that the subicular circuits that are integral parts of the hippocampal-subcortical network (such as the efferent projection towards the medial mammillary nuclei, which shows a mature pattern of neurofilament expression in infant monkeys) might be relatively mature shortly after birth; whereas the subicular circuits that are integral parts of the neocortical-hippocampal loop (i.e., the projection towards the entorhinal cortex) might not be mature in infant monkeys. This hypothesis will need to be confirmed by the use of neuroanatomical tracers and double-labeling techniques.

Functional Implications

One point that this review makes clear is that data describing the structural development of the primate hippocampal formation, while intriguing, are scant. Based on the available data, for instance, it is not possible to determine the ages at which specific hippocampal circuits become structurally mature and capable of supporting defined, age-specific processes and functions. Neither is it possible to determine the ages at which different subregions of the primate hippocampal formation undergo specific postnatal maturational processes, during which the hippocampal formation may be particularly vulnerable to intrinsic and/or extrinsic factors capable of alter-

ing normal hippocampal development. We believe that a systematic investigation of the structural development of the primate hippocampal formation will enable researchers to begin to address these critical issues.

For example, our stereological studies have demonstrated that more than 30% of the neurons destined for the granule cell layer of the dentate gyrus are generated postnatally. This is especially intriguing considering that the general pattern of projections from the entorhinal cortex to the dentate gyrus in the infant monkey resembles that observed in the adult. In what manner, structurally, is this large number of neurons incorporated into the dentate gyrus and how are the projections from the entorhinal cortex to the dentate gyrus organized, or reorganized, following this addition? Similarly, whereas the mossy fibers (the axons of the granule cell neurons) that are already established at birth have mature-looking synapses, and the thorny excrescences present on the CA3 pyramidal cells are also adult-like, the dendritic arborization and the synaptic organization in the molecular layer of the dentate gyrus demonstrate significant postnatal maturation. Which metric, then, should be used to define the maturity of the dentate gyrus? As alluded to above, it is important to recognize that all hippocampal components need not reach an adult level of maturity in order for the hippocampal formation to be 'functional'. It is crucial, however, to determine when particular developmental milestones are reached in order to suggest ages at which specific hippocampal circuits become capable of subserving age-appropriate functional processes. Together with the development of behavioral assessments documenting the emergence of distinct memory processes in primates [Lavenex and Banta Lavenex, 2006], this information can provide critical insight into the relationship between structure and function of the medial temporal lobe memory system across the life span.

Defining developmental windows or neurodevelopmental critical periods during which particular developmental processes occur (such as when large numbers of new neurons are added to a structure, or when synapses are produced and/or eliminated) is also essential in order to make predictions regarding pathologies that might derive from the influence of inappropriate intrinsic or extrinsic factors at these various stages during development. For example, we have recently shown that scrub jays *(Aphelocoma californica)* subjected to nutritional deficit during early postnatal development had smaller hippocampi with fewer neurons in adulthood as compared to nondeprived controls. Moreover, these nutritionally deprived birds exhibited cognitive deficits later in life, despite nutritional rehabilitation from 2 months to 1 year of age [Pravosudov et al., 2005], suggesting that a neurodevelopmental critical period had been surpassed, and that neuroanatomical and functional rehabilitation was not possible. Similarly, deprived rearing conditions modulate neurogenesis in neonatal mice, leading to neuroanatomical deficits (smaller hippocampi with fewer neurons) as well as functional deficits (spatial learning impairments) in these mice when tested in adulthood [Kempermann et al., 1997]. When these same deprived living conditions are imposed on adult mice, neither the structural nor functional deficits are observed [Kempermann et al., 1998]. From our preliminary data on the developing monkey hippocampus, one could already predict that adverse environmental factors present during the first 3 months of life could negatively affect postnatal neuron production, thus significantly impacting structural development. These structural abnormalities could persist into adulthood and would likely translate into functional abnormalities in the form of cognitive deficits in the adult animal. In contrast, adverse environmental factors after the third postnatal month would be unable to affect the first wave of neuron production, but could impact the normal maturation of the synaptic organization of these structures, impacting adult structure and function by a completely different mechanism. As suggested by our studies in jays, even potentially rehabilitating conditions later in life may not allow the animal to overcome structural and cognitive deficits once the specific developmental critical period has passed. We believe that a comprehensive and systematic study of the postnatal development of the monkey hippocampal formation, in combination with the development of early diagnostic tools for various neurodevelopmental disorders, will have a tremendous impact on the identification and implementation of therapeutic approaches to treat patients suffering from these disorders.

Finally, it is appropriate at this point to acknowledge the critical importance of the work on the development of the rodent hippocampal formation for establishing the validity and generalizability of the work on the development of the monkey hippocampal formation. Data defining the development of the rodent hippocampal formation are absolutely critical to pinpointing the particular systems and molecular mechanisms that should be the focus of the primate research. Moreover, while neither neurobiological observations in rodents nor in monkeys can substitute for direct knowledge of the human brain, neuroanatomical, functional and developmental consistencies between rodents and monkeys likely point to evo-

lutionarily conserved traits that are likely conserved in humans. Thus, comparative work on rodents and monkeys is invaluable to our understanding of the structure and function of the human hippocampal formation.

Conclusion

Review of the literature and original new data indicate that morphological and neurochemical markers exhibit specific changes in the developing primate hippocampal formation. Clearly, a comprehensive neuroanatomical analysis throughout postnatal development will provide fundamental information about the structural maturation of specific functional pathways within the hippocampal formation, and help generate testable hypotheses about the neurobiological mechanisms underlying the emergence of declarative memory. By studying the normal postnatal structural development and functional maturation of the primate hippocampal formation, we can gain insight not only into the types of information processing that it subserves, but also into the specific maturational processes that might be affected in neurodevelopmental disorders. Rhesus monkeys, with their phylogenetic proximity to humans, represent an unparalleled model in which empirical and systematic investigations of the normal and pathological development of brain-cognition interactions can be undertaken.

Acknowledgements

The preparation of this article was supported by grants from the Swiss National Science Foundation (PP00A-106701), the National Alliance for Research on Schizophrenia and Depression (NARSAD Young Investigator Award), and the NIH (RO1-NS16980). The original work was carried out at the California National Primate Research Center (RR00169).

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