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Review

Effects of prenatal protein malnutrition on the hippocampal formation

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Abstract

In this review we have assessed the effects of prenatal protein malnutrition on the hippocampal formation of the developing brain. In investigating this insult in the hippocampal neuronal model we have concentrated on aspects of enhanced inhibition we have shown in our earlier studies. Since this involves particular attention to the GABAergic interneurons we have examined the complex interneuronal networks of the hippocampal formation and their neurotransmitter afferent inputs, particularly the serotonergic system from the midbrain raphé nuclei. A variety of combinations of specialized interneurons are discussed in terms of how malnutrition insults perturb function in these inhibitory and disinhibitory networks. Pathological enhancement of inhibition manifests itself by diminished plasticity, alterations in theta activity and deficits in long-term learning behaviors. Long-term inhibition in select GABA interneuron systems may form a major derangement seen following prenatal protein malnutrition. The focus of this study is to relate enhanced inhibition to the several forms of inhibitory systems present in the hippocampal formation and develop hypotheses as to the primary derangements that may account for pathological inhibition in prenatal malnutrition.

Keywords: Protein malnutrition; GABA interneurons; Hippocampal formation; Serotonin; Brain development; Vigilance state plasticity; Hippocampal inhibitory networks; Neuronal peptides; Calcium binding proteins; Feedback inhibition; Feed-forward inhibition; Plasticity; Long-term potentiation; Long-term inhibition; Raphé nuclei; Effects of insults on the brain

Contents

1. Introduction

Malnutrition is a worldwide problem affecting millions of unborn and young children during the most vulnerable stages of their brain development. As such it alters various maturational events in the brain resulting in behavioral abnormalities, altered cognitive functioning and disturbances in learning and memory. The goal of this review is to identify and briefly define key issues relating to the effects of prenatal malnutrition on the early developing brain. Our earlier reviews [30,40,41,51] cover a voluminous literature in critical detail. Here, several additional important issues will be discussed pertaining to the effects of dietary insults on the developing central nervous system, including how nutritional deficiencies act on the brain and questions relating to the potential amelioration of the effects of early,

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mainly prenatal, malnutrition. The focus of this study is to relate enhanced hippocampal inhibition to the several forms of inhibitory systems present in the hippocampal formation and develop hypotheses as to the primary derangements that may account for pathological inhibition.

Nutritional inadequacy is one of the principal non-genetic factors affecting development of the brain. The effects of malnutrition and undernutrition are of continued interest due to the widespread incidence of fetal and infantile nutritional deficiencies and the growing body of evidence that the effects of nutritional insult on the developing brain are long-lasting and lead to permanent deficits in learning and behavior [28,50,53]. Additionally, there are numerous reports that cognitive impairment results from prenatal and early postnatal malnutrition, especially in underprivileged socio-economic groups [40,41]. It has generally been held that maturation of the central nervous system, and development of optimal intelligence, depend on three critical factors: (1) inborn or genetic directives; (2) environmental stimulation and enrichment; and (3) proper nutrition. Alterations in prenatal brain development in humans from any one or combination of these factors may result in various degrees of behavioral impairment and brain dysfunction. However, more numerous and therefore more important for society, are the borderline cases of sub-optimal brain development, i.e. the non-fulfillment of the genetic potential of the individual.

The term, 'undernutrition' indicates that, while all the nutrients required by the species are available in the diet, the amounts are insufficient. The term 'malnutrition' implies that one or more of these essential nutrients is missing or that the nutrients are present but in the wrong proportions. The biological effects of undernutrition and malnutrition usually overlap, but not in all cases. The term 'brain development' is frequently confused in the literature with pure overall growth or gain in total brain weight. In reality, brain development includes the synthesis of cellular components, such as nucleic acids and proteins, in parallel with neurogenesis and gliogenesis, migration of neurons and glial cells and cellular differentiation, this latter being accompanied by an increase in cell size. The terms 'brain growth' and 'brain growth spurt' are vague and represent imprecise resultants since they comprise a wide variety of underlying processes. These include cellular proliferation, increase in cell size, migration of neurons and glial cells away from germinal zones, the proliferation and ramification of dendrites and axons, myelinization, and synaptogenesis. Given that all of these processes are basically the result of various chemical reactions, they include critical or rate-limiting steps. Both maternal malnutrition and undernutrition may interfere with any of these developmental phases of the brain. In contrast to actual starvation, chronic caloric insufficiency (undernutrition) and imbalanced diets (malnutrition) are seen throughout the entire world, including the United States and other industrialized nations. Within practically every community there exists a subpopulation of malnourished, under-educated, economically deprived, and disease-prone individuals or entire families, which tend to perpetuate themselves and their nutritional inadequacies from one generation to the next [1]. Thus, there is an accumulated disadvantage in most malnutrition and undernutrition situations.

Impairments of intellectual development need to be considered in terms of the community, i.e. how environmental or nutritional insufficiencies within a social group affect both the neurological and social maturation of the individual [29]. Even in industrialized nations chronic, often generational, intellectual underdevelopment is a pervasive threat in specific subpopulations. It is clear that socio-economically disadvantaged populations are most affected and at risk not only for nutritional but for other social factors such as drug and alcohol abuse, emotional stress, inadequate education and impaired parent–child relationships, all of which may combine to influence the outcome of development. Considering the high incidence of moderate to severe malnutrition among underprivileged, pregnant women, it is probable that most of the infants and children in low economic groups have sustained nutritional deprivation in utero with many individuals continuing to be undernourished or malnourished throughout infancy and childhood. Both animal and human studies indicate that a combination of prenatal and postnatal mal- or undernutrition is more deleterious than nutritional insult occurring during either period alone. However, a majority of studies also show that prenatal malnutrition results in greater permanent mental deficiencies than postnatal malnutrition. Further, pregestational combined with gestational malnutrition results in more severe effects on behavior than gestational malnutrition alone [48].

2. Basic pathology of developmental nutritional inadequacies and neuronal insults

A clear distinction should be made between two major types of central nervous system developmental disorders. The first involves inadequacies of substances, such as nutrients, that are essential for cellular formation and tissue organization. Such inadequacy interferes with basic developmental programs by depriving the brain of critical ingredients needed for cellular structure and growth, in particular for the fundamental programs of cytogenesis and histogenesis, which occur early in gestation. The second developmental disorder involves lesions or toxic agent pathologies resulting in destructive processes contemporaneous with rapid brain growth and differentiation, which occur later in gestation (Fig. 1).

Maturational development of the brain evolves through a series of temporally overlapping stages. Accordingly, the impact of malnutrition on brain development must be analyzed in the context of the stage or stages during which the pathologic process is judged to be initially and maximally active. The development of the central nervous

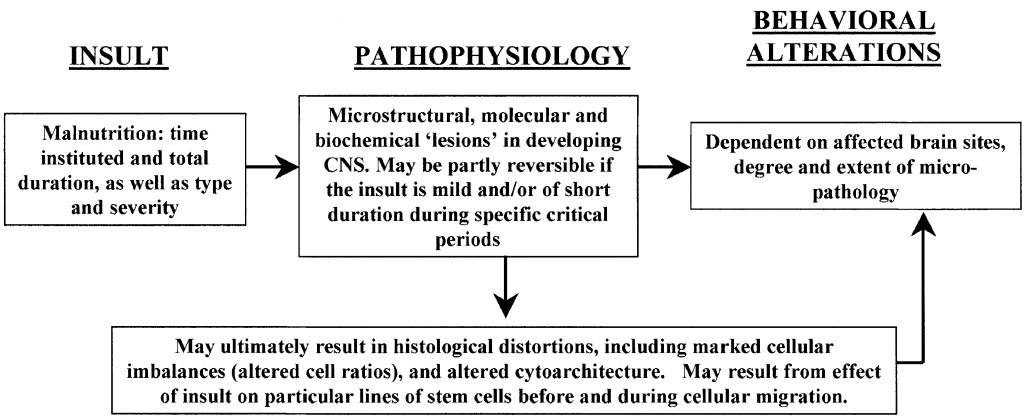

Fig. 1. Schema illustrating pathophysiology of central nervous system inadequacies resulting from malnutrition or direct neural insults. Distributed 'lesions' resulting from malnutrition insult are subtle and not usually directly observed at the light or electron microscopic levels. They principally involve distortions of neuron ratios, cellular atopias resulting from altered migration of neurons, disorders of cellular alignment or orientation, alterations of the lamination phase of cortical organization, neurotransmitter imbalances, aberrancy of regressive events such as axonal and dendritic pruning, synaptic elimination or cell death. These types of pathologies can best be defined by morphometric techniques and thus represent distortions and imbalances in aspects of development rather than direct focal lesions.

system occurs in phases, which follow a precise sequence, which is different in various brain regions and even within a particular region, and varies in time from one animal species to another. The essential sequence of brain developmental processes, i.e. the genotypically ordained schedule of development, does not vary fundamentally among mammals [45,46]. Across species the only substantial differences with respect to brain development are represented by the timing of birth in relation to the stage of brain maturation (Fig. 2). This consideration is of special consequence due to the use of animal models to examine brain processes affected by malnutrition.

The definitive structure of the brain arises during the stage of histogenesis, which occurs as postmitotic brain cells migrate from germinal zones, differentiate and interact with each other, and surrounding non-neural tissues, in a series of highly ordered, sequential steps. Normal brain development not only depends on this exact sequence of developmental phases but also on the multiple metabolic reactions that regulate these cellular events [45,46]. These sequences are primarily determined by the genome so that genetic regulation of brain development must also be considered to be under the influence of nutritional factors. Thus, genes code for the formation of enzyme proteins and determine their catalytic functions as well as control the intracellular sites and timing of activation and deactivation of enzymatic actions during development.

Exogenous factors, such as malnutrition, can alter the activity of enzymes and interfere with protein synthesis and protein structure and, thereby, also interfere with the proper incorporation of lipids into various brain structures. Distortions of the coordinated maturation of different brain components, such as alterations in the sequential production of particular classes of neurons, will disrupt the orderly growth and elaboration of neuronal circuitry. This results in malfunction of inhibitory circuits that modulate the strong excitatory actions of pyramidal cells [32]. Delay in even a few isolated neurological events resulting from malnutrition may also cause a chain reaction amplifying functional errors. Misdirected, mistimed or absent developmental cues can cascade to increasingly perturb the normally ordered progression of brain development, thereby impacting the highly complex expressions of brain function, including compromising logic and memory circuits, and thereby affecting cognitive processes.

The idea that there are times during development when the organism is particularly vulnerable is one that underlies the entire modern concept regarding the effects of early insults on the brain. We have particularly examined the effects of moderate prenatal protein malnutrition on the developing brain. This type of nutritional insult does not result in abnormal anatomical shape of the brain, focal brain pathologies equivalent to definable microscopic lesions, nor in gross mental retardation or psychopathology, but rather results in permanent sub-optimal intellectual and behavioral development (Fig. 1). Under such nutritional restriction, derangements tend to be subtle [50] rather than gross and largely involve alterations in cellular parameters and connectivity that can best be assessed by quantitative anatomical measures, functional analyses, and behavioral studies. As noted below, these derangements are usually studied in well-known brain model systems such as the hippocampal formation where much of the internal and external connectivity and cellular architecture and chemistry are becoming known and, to a considerable extent, well examined quantitatively in normal animals, and in our studies in malnourished animals [3,4,10,11–14,38–41].

3. How protein malnutrition affects the developing brain

Except for certain immunoglobulins, proteins do not

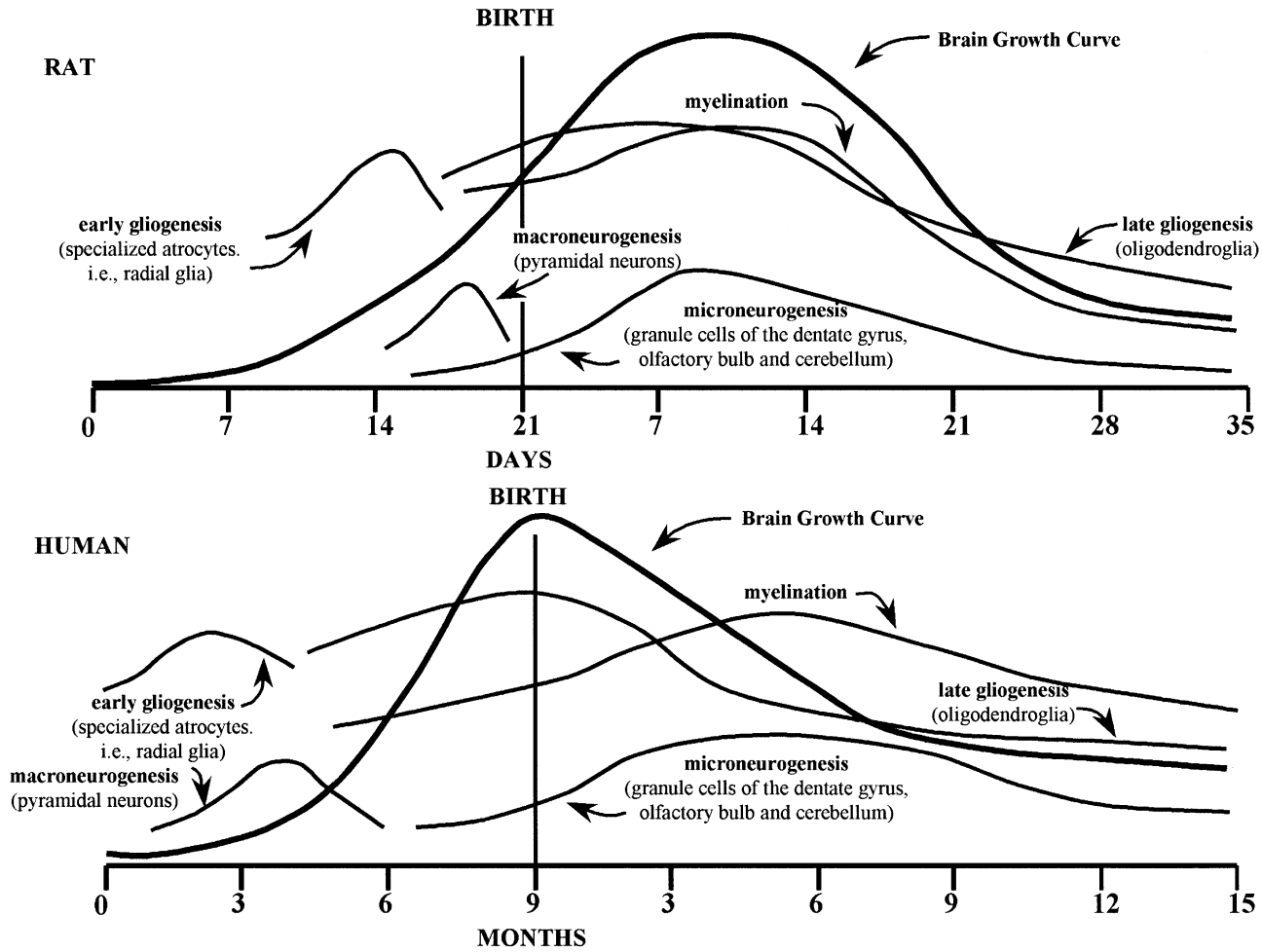

Fig. 2. Velocity curves comparing the relative rates, duration, and timing of specific developmental processes in the rat and human brains. The brain growth spurt curves (rates of brain weight changes) are superimposed (bold curve) in relation to underlying developmental events in the brain. Note the early genesis of astroglia and pyramidal cells in the human, resulting in the acquisition of approximately 27% of adult brain weight at the time of birth, compared to the approximately 12% of adult brain weight seen in rats at birth. The so-called brain growth spurt curve in the rat is shifted to the right compared to the human.

cross the placenta in significant amounts. The nutrient value of proteins in the diet resides essentially in the individual amino acids that have been absorbed in the maternal digestive system. It appears that all the amino acids that are known to be essential for the mother are likewise essential for the fetus. Therefore, omission of any single amino acid from the maternal diet also hinders protein synthesis by the fetus. The omission of a single essential amino acid, e.g. methionine, phenylalanine, arginine, lysine or tryptophan, from the maternal diet can have deleterious effects on fetal brain development similar to those produced by the omission of proteins as a whole [54].

Nutrition is arguably the single greatest environmental influence on the fetus and neonate. An appropriate supply of essential nutrients is required for the maintenance of growth as well as for the normal development of all physiological functions. It now appears clear that the single-most significant non-genetic contributor to mental retardation or permanent alterations of fetal brain development is ultimately nutritional inadequacy in one form or another [28]. There is little question that maternal malnutrition, including placental insufficiency, is a principal cause of perturbed development of the fetal brain. All nutrients to a certain extent have some influence on brain maturation, but protein appears to be the component most critical to development of neurological functions. Various amino acids are precursors of neurotransmitters or, in many cases, are themselves neurotransmitters. Amino acids are the precursors of the structural proteins that are essential for growth of body tissues, including the brain. They are also direct precursors of enzymes, peptide hormones and peptide neurotransmitters. Therefore, it is obvious that the broad involvement of amino acids in the functions of the central nervous system go considerably beyond their simple roles in protein synthetic function.

4. Critical periods

The critical period concept pervades discussions of the

anatomic, physiological, biochemical and psychological maturation of the brain in relation to insults and deprivations [17,40,41]. The substance of the critical period concept is that maturation of the nervous system is, by no means, a linear process. A critical period represents a once-only developmental window that cannot be reversed or repeated at a later time. It is based on the finding that organizational processes are modified or disrupted most easily during those times when they are proceeding most rapidly. An organizational process cannot have a critical period if it proceeds at a uniform rate since, under such circumstances, it can be modified just as easily at one time as another. It is clear that developmental, as opposed to maintenance, processes do not and cannot proceed at uniform rates. Accordingly, all developmental processes must exhibit critical periods whose duration and importance will depend on the rates of change in these processes. In the embryonic brain many critical processes accelerate their activity for short periods of time and then cease completely. Or, a process may initially proceed rapidly and then fall off to a low level, but never entirely cease. The concept of vulnerability implies that the organizing process is labile to perturbations, which either slow it down or otherwise distort it. Relating the above to malnutrition and the developing central nervous system, interference with the fixed developmental progression of morphological, physiological and biochemical processes of brain growth and maturation results in irreversible changes, i.e. if a process does not occur at its programmed time, there will be a permanent functional deficit. We emphasize again, as we did in our reviews of Morgane et al. [40] and Galler et al. [30], that both the age at which malnutrition occurs and the duration and severity of the insult are critical factors relating to how and to what extent this insult affects the brain.

Though there are differences among species in the timing of the rapid phase of brain weight gain relative to birth (termed the brain growth spurt by Dobbing's group [20]). This is only a small component of the critical period and does not fully reflect the multiple cellular and organizational processes that are particularly vulnerable to insult during early developmental periods (Fig. 2). It has become pervasive in the malnutrition literature to give undue emphasis to the so-called growth spurt period based on total brain weight. Rather it reflects size increases and differentiation of individual cellular elements (neurons and glial cells), and, especially, myelinization. We prefer to emphasize that rapid changes in the rate of brain weight do not correspond to fundamental organizational periods of the brain, particularly with respect to neurogenesis, neuron migration and orientation and alignment of cells necessary to form proper synapses and, particularly, neuronal circuits.

With regard to the key concept of developmental plasticity (see later), it is important to stress that after a true critical period, only limited plasticity may remain in the system. It is this residual level of developmental plasticity that may allow environmental enrichment to exert some ameliorative effects following malnutrition insult. For example, environmental experience generates neural activity, which, in turn, modifies existing neural circuitry resulting in elaboration of new circuitry during the course of brain development, thereby markedly influencing information processing and storage. In reality, the entire developmental period appears to be critical for the final outcome of the mature brain since it contains multiple overlapping and separate critical periods. Assuming that alterations of brain development have long-term functional significance, the study of the effects of nutritional inadequacy on such development serves two primary purposes: (1) the prevention of harmful effects on early brain organization and function, and (2) the optimizing of environmental conditions which may improve compromised brain function.

5. Animal models in malnutrition studies

The nutritional model used in our laboratories is detailed for the nutritional, mating and fostering procedures employed in earlier papers [40,47,52]. Briefly, nulliparous female rats are obtained five weeks prior to mating. Females are allowed ad lib access to one of two isocaloric diets that are formulated to be of adequate (25% casein) or low (6% casein) content. The low protein diet is supplemented with additional methionine. Males obtained from the same source are acclimated to the same diet as the females for one week prior to mating. Two females and one male are put into a single cage for one week for mating. Following parturition, all litters were culled to eight pups (two female, six male) and cross-fostered to lactating, well-nourished (25% casein diet) mothers, which have given birth within the previous 24 h. After weaning at 21 days, all offspring are given ad lib access to Purina rat chow (Formula 5001). Litters are of normal size in both groups. Malnourished pups are initially low in weight but catch up by 90 days of age.

Most concepts basic to nutritional investigations have been derived from animal studies. However, if one looks only at absolute time schedules, no single animal species is an ideal model for human brain development. Nevertheless, all mammals studied have similar sequences in which neurons and glia are born (neurogenesis and gliogenesis). It is also important to emphasize that the degree of functional maturity at birth differs greatly from species to species. One must examine comparative ages in different animals in terms of brain maturational event (Fig. 2). Thus, a clear understanding of the effects of malnutrition must necessarily include consideration of the time of malnutrition in relation to the schedule of brain development in each species. It should be stressed that postnatal brain development is markedly influenced by prenatal nutritional status, and, further, that gestational development is greatly influenced by the pregestational nutritional status of the mother. Generally, undernutrition or malnutrition will have

different effects on the proliferation of different types of neurons (macro and microneurons) or glial cells (astroglia and oligodendroglia) depending on whether the nutritional insult occurs during the embryonic, fetal, early postnatal or late postnatal period (Fig. 2). For example, undernutrition or malnutrition before birth will result in a deficit in cell numbers at birth, mostly macroneurons, and, therefore, a permanent neuron deficiency throughout postnatal life. This is of special interest since, as noted, nutritional deficiencies ultimately impose limitations on the complexity of neuronal circuits.

Relative to the issue of so-called brain 'sparing,' emphasized widely in the malnutrition literature, it is known in humans that macroneuronal proliferation terminates in mid-fetal life (Fig. 2). This has previously been interpreted by the Dobbing group [20–22] to indicate that, in humans, the proliferation of macroneurons is not markedly affected by prenatal malnutrition since early in pregnancy the nutrient requirements of the fetus are still negligible (the so-called 'highly protected second trimester'). Our group, as well as Zamenhof [54,55] and others, disagree with the interpretation that early and middle pregnancy is a highly protected period for the brain. As an example, there is evidence that rat embryos are usually resorbed a few days after implantation if the maternal diet lacks protein. This occurs even though the rat embryo requires comparatively even less protein at this initial stage than a human fetus in the second trimester. Embryos that do survive exhibit deficient placental and brain development.

The brain growth spurt is an arbitrarily defined period of development when the brain is increasing its weight particularly rapidly (Fig. 2). The anatomical and neurochemical events underlying the growth spurt have not only all the extreme complexities of the adult brain, but also all those additional ones resulting from ongoing dynamic developmental processes [45,46]. Each small brain region and each regional collection of histological units must follow a carefully and intricately programmed developmental sequence. Thus, although each small region, tract, or pathway is passing through a similar order of processes within itself, it must arrange its own timetable in relation to that of other interrelated regions so that the finished product arrives intact and fits usefully into the mature structure. Any quantitative description of the whole brain growth spurt is a simplification derived by ignoring the complex interrelationships of the timetables followed by the sub-regions of the developing brain. Developing neuronal circuits in the central nervous system depend on the proliferation, migration, and differentiation of nerve cells being closely coordinated in both time and space. Normal development also depends on the formation and differentiation of both nerve and glial cells occurring in an interrelated fashion. A major task of the developing brain is to produce the correct pattern of connections during a sharply limited period of time, the so-called window of opportunity.

6. The hippocampal neuronal model

In order to study the effects of malnutrition and other insults on the brain, it is essential that a satisfactory neuronal model system be used. In recent years, one widely used brain model system that others and we have studied is the hippocampal formation, particularly the dentate gyrus [40,41]. The hippocampal formation is an essential component of the limbic system of the brain. Among its functional roles is the processing of information derived from the polymodal association cortices. As such, it influences cortical and subcortical areas that modulate complex behavioral processes. The primary circuitry of the hippocampal formation is based on a series of sequential synapses that relay information in the following principal direction: entorhinal cortex → granule cells of dentate gyrus → CA3 pyramidal cells → CA1 pyramidal cells which, in turn, project back to the entorhinal area. This forms what is termed the hippocampal trisynaptic circuit, which along with GABA projection neurons and local circuit interneurons, appears to represent the basic processing module in the hippocampal formation. Interneurons within the hippocampal formation, which include the inhibitory basket and many other types of GABAergic interneurons [25], serve to modulate synaptic transmission within this structure. Importantly, within the hippocampal formation there is a delicate interplay between excitatory and inhibitory mechanisms, which integrate and process the complex algebra of its synaptic inputs.

An especially important property of the hippocampal circuitry, that has been widely used to study malnutrition and other insults to the brain, is that synaptic efficacy or strength can be enhanced or diminished through repeated activation of afferent inputs. Thus, the hippocampal formation has figured prominently in studies of both synaptic plasticity and memory processes. These two phenomena are thought to be closely related and it is now widely accepted that fundamental aspects of these phenomena are perturbed by perinatal insults such as malnutrition. Behavioral studies in animals, as well as neuropathological studies in humans, validate the essential role of the hippocampal formation in consolidating information into enduring memory. The hippocampal formation, however, is not an 'archive' (storage facility) but a modifiable 'gateway' and shunting mechanism with several 'gatekeepers' interposed along its circuitry. The memory trace is not conceived as a 'stored copy' but rather as a whole set of changes occurring in the strength of synapses, i.e. memory 'resides' in the long-term strength of neuronal connections. A candidate neurophysiological mechanism thought to underlie memory formation is the phenomenon of long-term potentiation, an activity dependent (use-induced) increase in synaptic efficacy produced by brief repetitive activation of afferents to and within the internal hippocampal circuitry. This selective increase in synaptic strength remains the predominant model for a cellular mechanism underlying learning and memory. Recent studies in this field indicate that many insults, including malnutrition, disrupt hippocampal function, by

enhancing inhibition, resulting in limitations of hippocampal plasticity, either by direct actions on the hippocampal formation itself (particularly on the GABA interneurons) or, indirectly, by affecting sub-cortical inputs to the hippocampal formation [3,4,41,51]. These latter function to modulate activity of a wide number of inhibitory interneurons creating fine-tuning processes for gating of information and stabilization of basic hippocampal processes.

There are several advantages of the hippocampal model system in malnutrition studies. In choosing a neuronal model system for studying malnutrition insult to the brain it is important to select one in which many of the basic aspects of anatomy, physiology and behavioral functions are known or, at least, hypothesized and in which behavioral studies can be designed. Its internal geometry makes the hippocampal formation ideal for studies of neuronal organization, circuit and cellular functions, and neuroplasticity. The precise lamination of terminal inputs is a feature of special interest since individual input systems can be manipulated to affect activity in precise laminar areas in the hippocampal formation. The laminar distribution of dendritic trees of principal cells and interneurons predicts the source of afferent inputs. The axon arborizations provides in formation on postsynaptic target selection. Due to its relatively well-defined structure and its special position at the crossroads between cortico-cortical pathways and cortico-subcortical circuits, the hippocampal formation best serves as a model of higher cortical functions. The position of the hippocampal dentate gyrus, functioning as a sequential processor stage in the neocortex–hippocampus–neocortex circuit, underlies its importance for extra-, inter and intrahippocampal biasing of information throughput. Polymorphic interneurons in the hilar region of the dentate are proven target cells for extrinsic regulation of information flow through the hippocampal component of this circuit. The excitability of dentate granule cells is largely regulated by feed-forward and local feedback neurons (basket cells and chandelier cells) providing specialized GABA-mediated inhibition. The dentate gyrus is innervated by cholinergic and GABAergic fibers from the medial septum and by afferents from the dorsal and median raphé, locus coeruleus, and supramammillary nucleus. It is now widely accepted that input from these sub-cortical regions acts to modulate the pattern of information flow through the neocortex–hippocampus–neocortex circuitry largely via the intermediary of various families of GABAergic interneurons as reviewed by Freund et al. [27], Freund [23], Freund and Buzsaki [25], and Gulyas et al. [31]. Since these extra-hippocampal inputs play a major role in hippocampal plasticity, including vigilance-state plasticity, these are presently under intense study in malnutrition and other insult experiments.

7. Extra-hippocampal inputs

Newer studies in our laboratory and others point to the importance of extra-hippocampal pathways in regulating the plasticity of the hippocampal formation. This is of particular interest since prenatal malnutrition enhances inhibitory activity in the hippocampal formation. This appears to result from altered activity of extra-hippocampal inputs, particularly serotonergic inputs to interneurons in the hippocampal formation. Studies are also in progress to examine how malnutrition affects noradrenergic and dopaminergic as well as GABAergic and cholinergic systems. Many of these neurotransmitter systems, when active, may be facilitatory (via interneurons) rather than obligatory to neuroplastic processes such as long-term potentiation. Especially exciting in this framework are the ultrastructural and immunocytochemical studies of Freund and his group [24,26,27,36] regarding the disinhibitory circuits by which relatively sparse sub-cortical inputs to the hippocampal formation can profoundly affect its activity. Importantly, gating of information flow (gated information transfer) into and through the hippocampal formation is powerfully modulated by most of these extrinsic inputs. Recent studies of the effects of malnutrition point to altered functions in these extrinsic hippocampal input systems that likely affect their modulation of long- and short-term plasticity.

Of particular interest is the serotonergic innervation to the hippocampal formation. Studies by our group [9] have shown, that in malnourished animals, there is a consistent decrease in serotonergic fibers innervating the hippocampal formation (which arise primarily from the median raphé nucleus), as well as persistent increases in the basal level of serotonin released in the raphé and hippocampus in conscious, behaving animals using in vivo microdialysis. These serotonergic fibers terminate primarily on various classes of GABAergic inhibitory interneurons in selective regions of the hippocampus (Fig. 3). This serotonergic innervation, though largely inhibitory on GABA interneurons, may be excitatory on inhibitory select interneurons through 5-HT_3 receptors, thus enhancing GABAergic inhibition in the hippocampus. This would thus lead to enhanced inhibition, which has been seen in terms of deficits in induction and maintenance of long-term potentiation and learning deficits in malnourished animals. Long-term potentiation of inhibitory circuits has been described by Korn et al. [33] and de Jonge and Racine [19]. This is a key factor in enhanced inhibition. Our present view is that the primary pathology in malnutrition involves alterations in select types of inhibition interneurons, the resultant being enhanced inhibition in the hippocampal formation. This, then, results in altered information flow into and through the internal hippocampal circuits.

8. GABAergic interneurons; general organization in the hippocampal formation and implications for understanding how malnutrition insults impact the hippocampal formation

Neuronal activity in the CNS is regulated by a balance

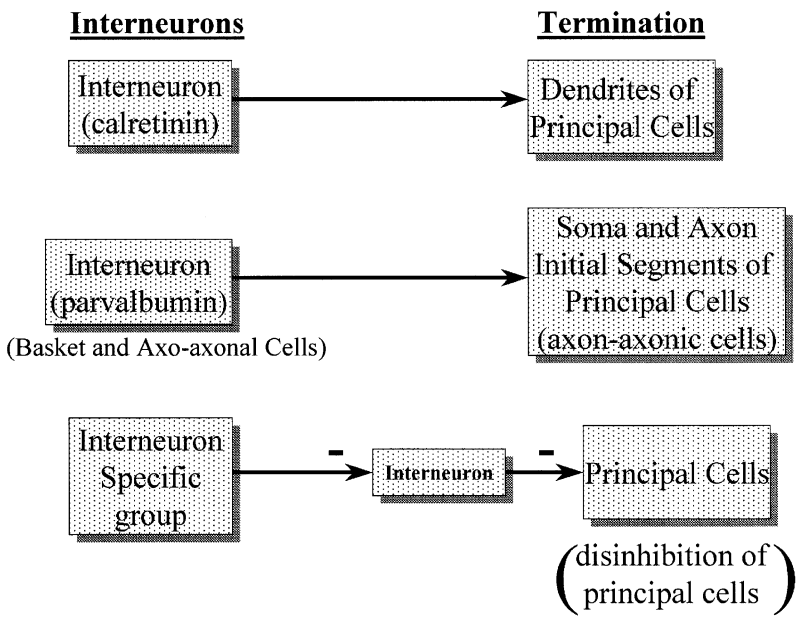

Fig. 3. Schema showing three types of interneurons acting, respectively, on dendrites and soma and axon initial segment of granule cells. The third type of interneurons acts only on other interneurons resulting in disinhibition of dentate granule cells.

between excitatory and inhibitory influences [7,42]. Two general types of inhibition are seen in all cortical structures, including the hippocampal formation: feedback and feed-forward inhibition [5] (Fig. 4). In the feed-forward regulatory system, afferent volleys directly activate the inhibitory neuron, which reduces probability of firing of principal cells. In the feedback system, an excitatory input discharges the principal cells, whose excitatory output is fed back to the inhibitory cells through recurrent axon collaterals. Some interneuron sub-types are innervated exclusively by extra-hippocampal afferents and therefore are a part of the feed-forward mechanism only. When interneurons are serially connected, it is assumed that increased activity of the primary interneurons will lead to increased firing of the target of the secondary interneuron through a process generally referred to as 'disinhibition' [25].

These well-characterized feedback and feed-forward inhibitory circuits modulate the powerful excitatory actions

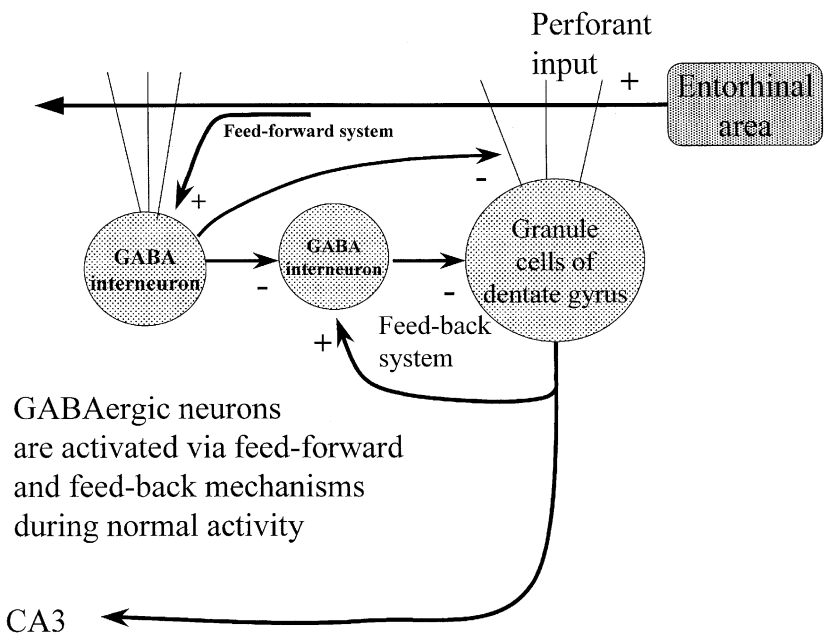

Fig. 4. Schema showing feedback and feed-forward circuits in the dentate gyrus of the hippocampal formation. In this representation we show feed-forward systems acting on one GABAergic interneuron while the feedback system acts on a second type of GABAergic interneuron. The feed-forward system is active on a GABAergic calbindin-containing interneuron which projects largely to dendrites of the granule cells. The feedback system activates a type of interneuron containing parvalbumin that act primarily on the soma and axon initial segments of the granule cells.

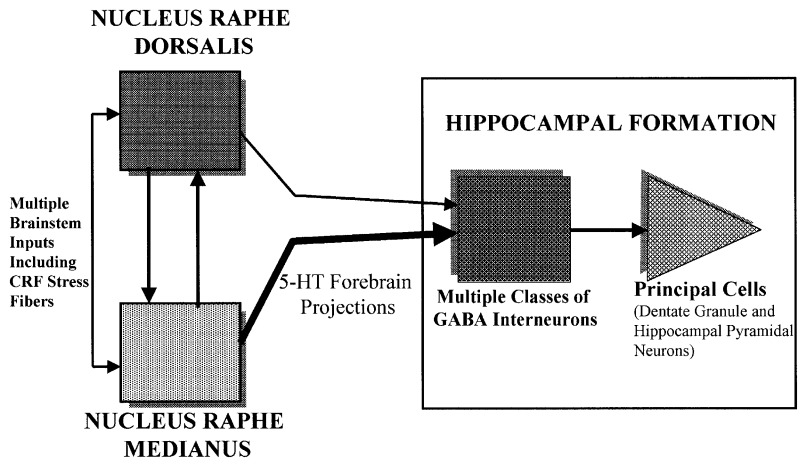

Fig. 5. Schema of raphé projections onto multiple classes of GABA interneurons in the hippocampal formation. Heavier line from nucleus raphé medianus indicates more dominant projection from that nucleus especially to the dorsal hippocampal formation. Corticotrophin releasing factor neurons (stress system) heavily enervate the dorsal and median raphé.

of pyramidal neurons. Thus, inhibitory interneurons provide stability to the activity of principal cell populations by both feedback and feed-forward inhibition [25]. The GABA neuronal networks in the hippocampal formation are especially capable of ordering, controlling, coordinating and synchronizing the activity of hippocampal principal cells, including dentate granule cells [4,8,10,12–14,34,40,41]. Ultimately answers to hippocampal network function lies in understanding interactions between various types of interneurons and principal cells, both in the hippocampal formation and raphé nuclei. We put particular stress on interneuron organization since we feel, on the basis of a variety of our studies that this is the system most impacted by malnutrition insult. Overall, we also look at the larger 'system'; particularly the raphé 5-HT input to the hippocampal formation as well as other neurotransmitter afferents (Fig. 5). There is good reason to speculate that interneurons represent a key to the understanding of hippocampal network function. This also involves consideration of receptors governing 5-HT release in the hippocampal formation. Interneurons are excited or inhibited by a great number of modulators [43]. The afferent and efferent connections of interneurons also show great variation thereby enabling them to carry out multiple tasks. Inhibition is critical in shaping response properties in single neurons and in assisting co-operativity in large cell populations. Overall, of particular importance are the roles of the various types of interneurons in the control of network patterns associated with plasticity and memory formation.

GABAergic neurons take various forms, the different types targeting different sub-sets of principal cells and, particularly, different parts of the cells [16,25]. Most importantly, GABAergic inhibition in the hippocampal formation is subject to considerable regulation by sub-cortical neurotransmitters, particularly from the medial septum, midbrain raphé nuclei and locus coeruleus [25]. Various GABA interneurons, approximately 50% in some layers, co-localize neuroactive peptides such as somatostatin, cholesystokinin and neuropeptide Y, as well as calcium-binding proteins. GABA interneurons display heterogeneous firing characteristics typical of various classes of cells [25]. Various subtypes of GABAergic neurons have also been classified based on localization of synaptic connections, electrophysiological properties, and the content of neuropeptides and calcium binding proteins. GABAergic interneurons provide both inhibitory and disinhibitory modulation of hippocampal circuits and play key roles in generation of oscillatory activity, information processing and 'gating' of sensory information [2,49].

To better understand operational principles of the hippocampal network we need a reliable taxonomy of interneuron types and information on their termination on principal cells and other interneurons (Fig. 3). Perisomatic inhibitory cells influences firing (output) of target principal cells. Activity of dendritic inhibitory cells may have to be synchronized to modulate dendritic conductance efficiently. Inhibition in the dendritic region controls dendritic calcium spikes and is associated with synaptic plasticity in dendrites [35,37]. Information processing and transfer in the hippocampal formation is represented by coherently firing neuronal assemblies. It appears that the anatomical substrates for maintaining synchrony are the GABAergic assembly of neurons [6,8]. Increases in inhibition results in lowered synchrony. Hence, examination of inhibitory circuits and their modulation by sub-cortical afferents is an important part of understanding how pathological inhibition in malnutrition may perturb learning and behavior.

Studies by Miles et al. [37] indicate that distinct actions of dendritic and perisomatic inhibition may allow differential regulation of both input and output of hippocampal pyramidal cells. Perisomatic inhibitory cells limit repetitive discharge of Na^+-dependent spikes and, thereby, efferent signaling. On the other hand, dendritic inhibition may

control the efficiency of afferent inputs both by suppressing generation of dendritic Ca^{++} spikes and by limiting depolarization due to excitatory synaptic events. Different sub-sets of inhibitory neurons express receptors for and receive innervation from fibers that release different modulating neurotransmitters. 5-HT median raphé fibers [27] and GABA septal afferents [24] innervate distinct subsets of inhibitory cells. Further, different groups of inhibitory interneurons also express 5-HT receptors. From this it appears that modulations of activity in subgroups of inhibitory cells with different functional roles may moderate behavioral control of hippocampal informational processing and plasticity. Our data indicate that alterations in both information processing and plasticity are perturbations seen in malnourished animals and that such changes clearly relate to enhanced inhibition as shown in paired-pulse studies in kindled animals [4,12,13,15,40,41].

All intrahippocampal and hippocampopetal afferents that terminate on principal cells also innervate interneurons [25]. As noted earlier, different inhibitory cell types target precisely specified areas of principal cell membranes. Dendritic inhibition could also selectively regulate the efficacy of NMDA receptors and prevent plasticity simply due to the dendritic localization of NMDA receptors. The rich interconnectivity of interneurons and their hypothesized role in synaptic plasticity provide the possibility that the different interneuronal types may selectively influence the efficacy of afferent inputs [25]. To understand the roles of well-defined groups of interneurons it is necessary to first determine the nature of their afferent synaptic drive, both excitatory and inhibitory. Interneurons of the hippocampal formation are excited or inhibited by a great number of combinations of modulators [43]. Contacts between, for example, parvalbumin-containing basket cells and principal cells establish a functional link between networks of interneurons and networks of principal cells. This allows interneurons to participate in various oscillatory phenomena thought to be involved in higher brain functions. It is clear that the large suites of interacting interneurons afford an enormous array of regulatory combinations. From this, it should now be understood that interneurons are not merely providers of inhibition but play key roles in a wide variety of brain functions [16].

Characterization of alterations in specific subgroups of GABAergic interneurons will likely shed light on how insults to the brain result in defects in GABAergic neurotransmission resulting in enhanced inhibition, which, in turns, alters basic activity of the hippocampal system. Examination of perturbations in subclasses of interneurons in malnutrition is one major goal of our molecular, receptor, sub-unit studies in GABA interneurons. Additionally, electrophysiology of GABA cells in slice preparations and in vivo studies utilizing dual probe microdialysis studies are further elaborating the functional significance of the GABA system. GABAergic interneurons are essential to the organization of complex behavior and defects in their functioning gives rise to various dysfunctions in cognitive development and behavior. Based on our physiological findings [3,4,11–14,40,41], this may be one critical type of 'deficit' seen in prenatal proteins malnutrition insult.

9. Studies of altered neuroplasticity in malnourished animals

One of the principal hypotheses in the malnutrition field relates to the issue that decreases in neurophysiological plasticity may be a critical brain mechanism underlying learning deficits observed as a result of nutritional insults to the developing brain. As mentioned earlier, long-term potentiation is a synaptic model of memory representing an enduring alteration in synaptic efficacy following afferent activation. This long-term potentiation is a use-dependent synaptic strengthening that can be induced by seconds or less of stimulation and has been shown to last for hours, days or even months, hence the term 'long-term' potentiation. Various features of long-term potentiation have caused it to become the leading candidate synaptic substrate for certain adaptive functions (plasticity) of hippocampal and other neural networks. Overall, current interest in long-term potentiation within the field of malnutrition research is related to its use in evaluating both long- and short-term plasticity of synaptic transmission in the central nervous system. We have shown earlier that in malnourished brains it is difficult to induce and maintain LTP. On other hand, long-term depression presents a potentiation of inhibition. Paired-pulse studies monitor inhibitory events in the hippocampal formation and in malnutrition studies we have shown marked enhancement of inhibition [4,11–13]. This appears to be one principal pathology in prenatal malnutrition and is reflected in the diminished plasticity we have observed in malnutrition insult and is reflected in the diminished plasticity we have observed in malnutrition insult [3,4].

Importantly, plasticity contributes to the long-term impact of nutritional imbalances on the developing brain. This refers particularly to the restorative plasticity, which occurs as a result of nutritional rehabilitation. Our group has, by a variety of techniques, shown marked diminishment of hippocampal plasticity following malnutrition insult and has related this to significant increases in inhibition such that information inflow and transmission through the hippocampal formation are diminished. Considerable research is presently underway to determine the extent to which rehabilitation modifies plastic capacities for structural and functional rearrangement, thus contributing to the remodeling and rearranging the brain's connectivity and strengthening of individual synapses. Such changes are thought to contribute to reducing the altered plasticity and ameliorating long-term deficits in learning and memory [44], though by no means fully reversing them.

10. Studies of the behaving animal

The use of intact animals remains the ultimate basis for understanding integrative mechanisms in the pathophysiology of disease as well as the development of new treatments. In studies of malnutrition and other insults using the hippocampal model system it is of importance that the behaving, chronically recorded animal be used. This is especially so since the intact brain with all its afferent inputs is best examined across all behavioral (vigilance) states (waking, slow-wave sleep, REM sleep). Hippocampal activity goes 'on-line' and 'off-line' across vigilance states (vigilance state plasticity). Thus, these states, via afferent inputs, 'gate' information flow into and through the hippocampal formation. Further, they alter the amount of inhibition in this hippocampal network. One of the major advantages to using the hippocampal formation as a model system resides in the fact that relatively large, stable field potentials can be recorded from each of the sub-regions of the structure. These population responses provide both synaptic and cellular information that can be reliably measured over long intervals in the behaving animal. Thus, quantitative evaluation of the impact of insults on specific aspects of neuronal transmission can be made in a manner not possible using intracellular recordings in behaving animals. Chronic recording of field potentials from freely moving animals provides a promising middle ground from which the functional relationships between altered neuronal activity and behavioral deficits following nutritional insults are being established.

Another technique that is of significant value in examining the brain is in vivo microdialysis. This allows for real time analysis of the release of neurotransmitters in the extracellular matrix of the brain in the active, behaving animal. Microdialysis has been used to evaluate the release of 5-HT in multiple brain areas simultaneously during various vigilance states. 'Reverse' dialysis is also used to deliver drugs locally to further evaluate the functions of brain circuits and receptor sensitivity. Studies of this type provide valuable information on release of neurotransmitters in the hippocampal formation in behaving rats [39]. We have studied the release of serotonin in the raphé nuclei and the hippocampal formation of malnourished rats [38]. The median raphé nucleus is one primary nucleus of serotonin neurons, which projects predominately to the septum and the hippocampal formation [36,40]. We have shown in microdialysis studies that the hippocampal formation of prenatal protein malnourished rats revealed an enhanced release of serotonin as compared to well-nourished controls. Electrical stimulation of the median raphé nucleus resulted in a decrease in the release of serotonin in the hippocampal formation in malnourished and well-nourished rats with malnourished showing a greater decline in release following stimulation [38]. Further studies in our laboratory are investigating the mechanisms of this enhanced release using 'dual' probe microdialysis with probes into the median raphé nucleus and the hippocampal formation of the same behaving rat and the use of reverse dialysis to examine receptor sensitivity in the median raphé. These studies may reflect changes in serotonin release across vigilance states [18] and during long-term potentiation and long-term inhibition.

11. Discussion and conclusions

In this summary, we have stressed that during the entire period of brain development various rate-limiting events are altered by exogenous factors, in particular nutrition. Thus, the entire developmental period of the brain has sub-critical periods, each of which may be perturbed by insults or deficiencies and, thereby, affect the overall maturational schedule and serial order of organization of the brain. The critical period, defined only in terms of the brain growth spurt hypothesis, represents only a fraction of the developmental events crucial to brain development. Hence, in examining the effects of malnutrition, it is essential to understand the major developmental sequences as they occur in their overlapping stages in order to interpret final outcome. Studies of nutritional effects on brain maturation relate to the prevention of long-term harmful effects on brain function and behavior as well as to considerations of how brain function may be improved once an insult has occurred. Of special importance of long-term value for society is that patterns of behavior can be altered by malnutrition such that they often continue to be expressed by succeeding generations.

Nutritional deprivation in humans appears to be associated with varying degrees of intellectual disturbance such as cognitive impairments and attention deficit disorders. In terms of studying the developing nervous system, it is of value to make correlations between nutritional deprivation and specific parameters of neuronal and glial development as outlined above in order to derive information concerning the underlying mechanisms of neuronal insult. A long-term goal in this field is to relate quantitative anatomical, physiological and neurochemical data to integrated cognitive functions such as information processing, memory and judgment that produce the complex behaviors that we describe as intelligence. Using the hippocampal formation to examine aspects of altered neuroplasticity, we are now beginning to identify some of the highly specific brain processes, particularly involving different classes of inhibitory interneurons that are altered by nutritional insults.

We also point out that, in terms of the insult to the developing brain, distortions in the normal pattern of development, including neurotransmitter imbalances, are frequently seen. These include alterations in the coordinated development of neurotransmitter systems such as the disinhibitory circuits by which relatively sparse sub-cortical inputs to the hippocampus can profoundly affect its activity. In view of the importance of chronological developmental

programming in relation to the ultimate integrative organization of the central nervous system, such neurochemical imbalances, rather than overt focal lesions, contribute most significantly to later functional impairments.

In analyzing the effects of malnutrition on the developing brain we need, in particular, to examine adaptive neuroplastic mechanisms rather than 'curtailments' that have so often been emphasized in the past. We should also emphasize that developmental changes in brain potential are related not only to structural maturation of the various neural elements and their connections within the brain, but also to the emergence of organizational patterns that depend, to a large extent, on the individual's interaction with the environment. Considerable evidence now indicates that synapses in the malnourished brain are less capable of supporting plasticity [14,40,41]. Altered plasticity of inhibitory interneurons is seemingly a key part of this picture. Diminished plasticity is one result of enhanced inhibition, which we consider the primary pathology in the hippocampal formation associated with malnutrition. Although it is possible that distortions in the coordinated maturation of various differentiating functions may have lasting effects on ultimate performance, the developmental and restorative plasticity of the central nervous system offers special advantages for the implementation of positive rehabilitation programs.

It is clear that both nutritional and environmental factors affect the central nervous system's capability and performance in a variety of ways, including: (1) abnormalities of morphological, biochemical and physiological characteristics which may alter brain function so as to reduce learning abilities; (2) impairment of developmental processes by decreased exposure and responsiveness to enriching environmental stimuli during critical periods when essential sequences of experience must be acquired to provide for continued orderly development of the brain; and (3) disruption of the learning process by adverse changes in personality, emotionality and behavior. These changes can markedly interfere with the interpersonal relationships that are necessary for learning experiences. Thus, malnutrition among persons in regular social contact with the malnourished individual make it impossible for them to provide an adequate learning environment, thus further diminishing chances for rehabilitation.

We also point out that it is necessary to distinguish between retarded normal developments as opposed to abnormal development such as long-term mental retardation. The term 'catch-up' growth in the malnutrition field implies that development taking place in malnourished animals is normal development that has only been slowed. We maintain that malnutrition results in considerable abnormal development, including neurotransmitter imbalances, and not merely in a retardation of normal development. Also of interest is the fact that a failure of optimal development due to nutritional insult does not necessarily lead to immediate brain dysfunction. Most biological phenomena operate on a principle of redundancy with substrate levels well in excess of those required to achieve maximum rates or to maintain biochemical equilibrium conditions. Thus, the consequences of an abnormal limitation during a particular stage of brain development can remain hidden, or may only manifest themselves as predispositions until such time that the system is stressed by unusual emotional or environmental circumstances. In other words, additional stress breaks down an already tottering system.

Finally, we re-emphasize that human malnutrition has biological, psychological, and sociological aspects. These various dispositions and behavioral practices associated with malnutrition tend to be passed from generation to generation. This appears to be a true 'cumulated record'. The extent to which such effects become perpetuated and even cumulative over several generations argues that combined educational, economic, behavioral and nutritional rehabilitation extending beyond a single generation will be needed to mitigate long-term residual effects of multigenerational malnutrition.

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