Chapter 20
An Expanding Spectrum of Autism Models

From Fixed Developmental Defects to Reversible Functional Impairments

Martha R. Herbert and Matthew P. Anderson

Abstract In this review, we contrast previous models of autism pathogenesis with newer models inspired by some recently appreciated and previously minimally considered pathological and clinical features of the disease. Autism has conventionally been viewed as an incurable behavioral disorder resulting solely from genetic defects impacting brain development. However, emerging evidence suggests that autism affects many organ systems beyond the brain and that some neuropathological and somatic pathophysiological processes are active even into adulthood. We incorporate these newer observations into a model of how this systemic and persistent disease process might impact brain *function* and ultimately impair behavior through potentially reversible mechanisms. In particular, observations of substantial transient and sometimes enduring increases in function and even losses of the diagnosis challenge researchers to identify pathophysiological mechanisms consistent with this dynamic course and potential plasticity. This broadened appreciation of the disease phenomenology and prognosis of autism calls for mechanistic models that encompass the full range of its features. To this end, our review contrasts several models of autism pathophysiology and lays out their differing underlying assumptions regarding mechanisms. First, we compare models of autism that are based on different underlying biological and experimental perspectives to addressing the question of autism pathogenesis. We contrast a "bottom-up, modular, genes–brain–behavior" model with a more inclusive "middle-out, multi-system biology" model. We then contrast different models that consider autism's development over time. Beginning with a purely genetic prenatal brain development model, we expand the framework to include early environmental influences, epigenetics, later and ongoing environmental influences, and features consistent with chronic encephalopathy. The implications of these models, particularly the last, are spelled out through a discussion of the functional

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consequences of one prominent chronic feature, persistent immune activation, and its impact on neural–glial interactions. The implications of these newer models on potential treatments are also discussed.

Keywords Autism · models · pathogenesis · function · brain · plasticity · epigenetics · environment

Introduction

For many years, autism was considered to be an incurable behavioral syndrome resulting from genetically determined in utero alterations in brain development. There is now a growing shift to an appreciation of autism as a heterogeneous whole-body, multi-system set of conditions that may begin during development, but whose pathophysiologic features may remain active and impact brain function well into adulthood [1]. These interrelated dimensions of reconceptualization are necessary responses to emerging evidence in clinical, pathophysiological, and epidemiological fields that challenge older models. Clinically, the high prevalence of gastrointestinal and immune symptomatology challenges the idea that autism, or what many are coming to call "*autisms*," is purely "brain and behavior" disorders [1, 2, 3, 4, 5, 6, 7, 8, 9, 10]. The description of pathophysiological features such as persistent immune responses, oxidative stress, and mitochondrial dysfunction in multiple tissues suggests systemic rather than brain-specific perturbation [11, 12, 13, 14]. A further clinical dimension is the emerging appreciation that these persistent components of autisms, when present, may be medically treatable. Etiologically, there is growing evidence that both genetics and environmental factors contribute [15]. Epidemiologically, the increased incidence of autisms may be due in part to increased awareness or broadening of diagnostic criteria; but those do not exclude the possible impact of environmental factors such as the growing numbers of and complex interactions among new-to-nature chemical agents present in the environment [16]. Finally, prognostically, substantial improvements and even loss of rigorously ascertained autism diagnoses are being reported in some autistic individuals [17, 18]; this observation is becoming an object of study, because validation of this phenomenon would challenge both the presumption of incurability and the neurobiological models based on that presumption.

Reconceptualizing autisms has provoked a re-review of the research findings and clinical phenomenology with fresh eyes. It is leading to a shift in the perceptual frames within which evidence is considered and according to which figure and ground are distinguished from each other. It is also reorganizing the models and narratives that organize research programs and clinical approaches. In this article, we will review some of the conceptual reorganization that is occurring. We will discuss schematized versions of how these models are being

reconstructed, and will show how many emerging findings, when viewed from this new framework, point toward novel but plausible pathophysiologic mechanisms that may have major clinical and treatment implications. We will especially focus on immune activation as a theme with which to illustrate the points of our argument; this choice is based on our belief that the identification of immune, autoimmune, and inflammatory processes in autism are of fundamental importance to modeling the disease process. A similar discussion could be constructed around the metabolic or energetic disturbances that are also prevalent in the disorder, which may in many cases be at least equally important. We must remember that autism is defined by a specific set of behavioral abnormalities in the DSM manual, but just like motor impairments, abdominal pain, and even social phobias, autism is not a single disease, but instead a condition caused by multiple etiologies. Consequently, although we lay out a set of general arguments that we hope help frame how we think about the complexity of autisms, the examples we choose in our discussions below might only be relevant to a subset of all possible autism etiologies and pathophysiologies.

This conceptual reorganization is being fleshed out both up and down the biological hierarchy and across the lifespan along the temporal axis. Identification of immune activation and other emerging pathophysiological findings inspires us to consider new levels of the biological hierarchy in addition to genes, brain, and behavior. The persistent postnatal and chronic changes and processes suggest the disease process is dynamic over time. The increasing number of affected individuals and substantial intra-individual variability in behavioral symptoms in at least some people with autism suggest a neuromodulatory control of the phenotype. The reports of improvement with fever and cases of remission suggest the disease might at least in part be contingent and potentially partly or even completely reversible if the pathophysiologic processes are inhibited or overcome. Depending on the mechanisms that come to more comprehensively explain the various autisms, the significance of these mechanisms may relate to other "neurodevelopmental disorders" beyond autism (e.g., schizophrenia).

Viewing the behavioral impairments that define autism as reflecting an *ongoing effect* of biological dysregulations rather than a fixed neurodevelopmental defect carries the implication that autism includes more than the traditional "triad" of deficits that define it in the psychiatric DSM-IV manual [19]. From the broader whole-body vantage point, approaches that define autism in purely behavioral terms can be seen as limiting treatment targets, and is vulnerable to the criticism of some members of the autism community who see treatment as focusing upon those symptoms that are troubling to caregivers (e.g., behaviors seen as "inappropriate" by neurotypical individuals), whereas a whole-body approach can address a further range of symptoms (as well as their underlying mechanisms) that are uncomfortable, painful, or troublesome to those with the diagnosis.

Reflection on Autism Models

In what follows, we will present and contrast two pairs of models of autism:

1. In the first pair of models (Fig. 20.1), we focus on contrasting formulations of the biological hierarchy. This involves comparing an older genetic reductionist model in which the biological hierarchy is populated by a "bottom-up" sequence leading from causal genes through brain to behavior on the one hand with a newer and more inclusive "middle-out" model that shifts the focus toward dysregulated immunologic and biochemical processes and their impairment of neural circuit function, and grounds the investigations of upstream gene/environment/epigenetic contributors and downstream behaviors in an underlying dysregulated cellular biologic process, which is a "middle level" from which causes and consequences emerge.

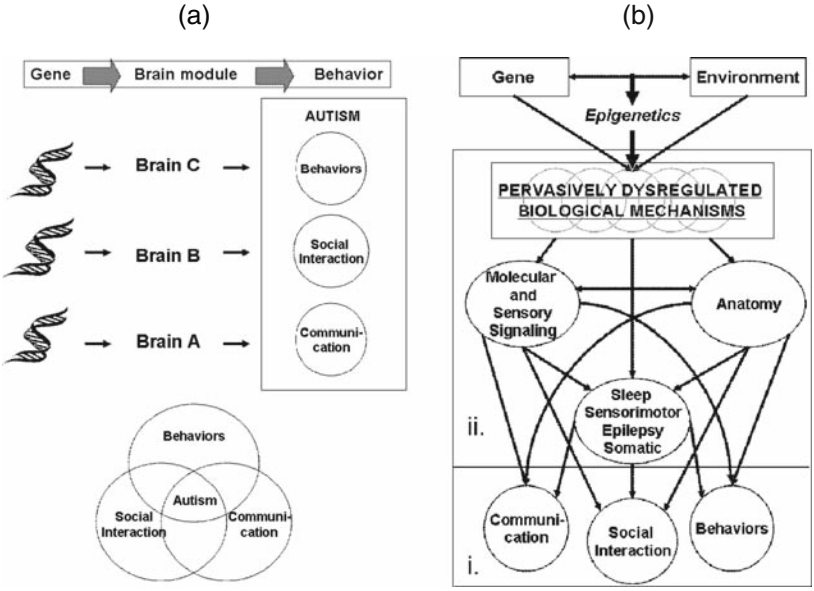

Fig. 20.1 Modular and Systems Approaches to Autism. **(a)** *Modular Approach to Autism*. This figure illustrates the "modular, gene–brain–behavior" model of autism, which is a "bottom-up" approach in which genes change the brain, and these brain alterations yield behavioral deficits. In this version of the model, each behavioral deficit is the result of its own genetically driven brain abnormality. The combination of three "gene–brain–behavior" modules yields the behavioral syndrome we call autism. Individuals with one or two of these modules would share features with autism but would not meet full criteria for autism disorder. **(b)** *Systems Biology Approach to Autism*. This figure depicts a "middle-out" approach with a set of interacting pervasively dysregulated biological mechanisms at the core, driven from upstream by a variety of gene–environment–epigenetic interactions, and cascading downstream into altered signaling, anatomic, neural and somatic functions, and behaviors. Figure 20.1b-i indicates the conventional behavioral characterization, whereas Fig. 20.1b-ii indicates the underlying biological abnormalities that may in the future provide the basis for biology- and signaling-based nosologies that use pathophysiological mechanisms to parse autism's heterogeneity

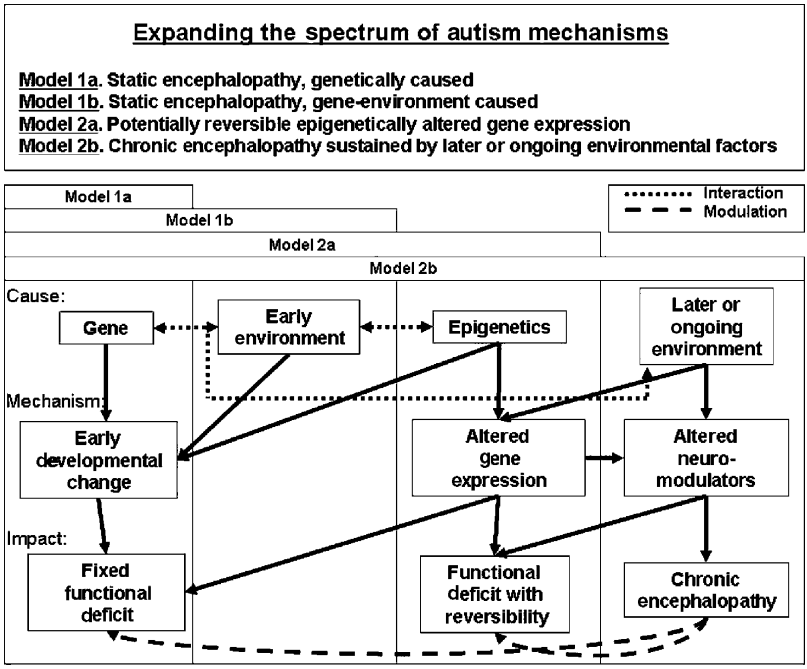

Fig. 20.2 Expanding the temporal axis of autism. This figure depicts a series of temporal models of autism, each of which adds to the one before at one or more of the cause, mechanism, and impact levels sketched in this schematic. Model 1a: *Static encephalopathy, genetically caused:* In this model, autism is a genetically determined disorder of early brain development with fixed functional deficits. Model 1b: *Static encephalopathy, caused by gene–environment interactions:* In this model, autism results from gene–environment interactions, with early environmental exposures contributing to developmental injury or alteration that leads to fixed functional deficits. Model 2a: *Potentially reversible epigenetically altered gene expression:* In this model, autism results from gene–environment–epigenetic–timing interactions, and involves a variable combination of early developmental injury or alteration with persistent but conceivably reversible alterations of gene expression. Model 2b: *Chronic encephalopathy sustained by later or ongoing environmental factors:* In this model, autism results from gene–environment–epigenetic–timing interactions, but environmental influences now include later and ongoing as well as early influences. These may affect not only gene expression but may also chronically dysregulate signaling and chemistry factors that may serve as neuromodulators, and may in this fashion impact function to create a modest or substantial component of a chronic encephalopathy. This raises the possibility that alterations of cellular structure, brain volume, and brain tissue parameters may be downstream consequences of persistent, chronic pathophysiologic abnormalities

2. In the second set of models (Fig. 20.2), we focus on the dimension of time in autism, contrasting an exclusively prenatal model with a model that adds and emphasizes the potentially fragile postnatal period when major synaptic remodeling occurs and an additional model in which ongoing chronic and dynamic physiological changes of brain circuit function are triggered by persistent disturbances in immunity and metabolism.

Although the multiple biological levels of integration are separable from the axis of developmental time at a conceptual level, these two axes are intertwined in the autism phenomenology that is driving this reconceptualization. In particular, several interrelated features that stretch the temporal axis well past the prenatal period raise compelling questions that need to be addressed in a systems biological fashion that integrates across levels of biological hierarchy. That is, we are seeing physical and functional changes over the life course in at least some of the autisms and not just prenatally, including postnatal rapid brain enlargement [20, 21], ongoing neuroinflammation and systemic immune dysregulation [22, 23], persistent systemic redox chemistry abnormalities [11, 12, 24, 25, 26, 27, 28], and fluctuating changes in brain functional connectivity [29]. These changes all have features that cannot be explained within a gene–brain–behavior model but instead demand a more comprehensive research agenda that features metabolic and glial–neuronal interactions in the disease pathophysiology [30].

As an example, one of the most consistent anatomical changes in autism is the larger than average brain size in young autistic individuals (with 20% over the 97th percentile, and most above average—although some quite small, supporting heterogeneity) that occurs during early postnatal periods—maximally during the first 2 years of life, a period of maximum axosynaptic remodeling [20, 21]. This observation raises the possibility that brain circuitry may be altered during postnatal rather than exclusively during the prenatal periods as previously assumed. More recent work has identified the presence of immune activation and signs of oxidative stress in the brain tissue of autistic individuals [14, 31, 32], and in particular involving glial alterations in a distribution that appears to follow the distribution of white matter enlargement, with recent evidence suggesting that astroglial hypertophy may preferentially occur in the later-myelinating subcortical (radiate) white matter where macroscopic volumetric enlargement has been documented but not in the earlier-myelinating deep or bridging white matter where this macroscopic white matter enlargement does not occur (Pardo C, 2006, personal communication) [33]. Glial reactions were however also observed in gray matter such as cerebellum and cingulate cortex. Immune activation was also observed to be substantially more pronounced in brain tissue from a 3-year old than in brains from autistic individual ages 5–44 years (Pardo C, 2006, personal communication) . These observations suggest the brain and white matter volume increases may spatially and temporally co-localize with the immune activation.

Do these tissue changes have functional consequences pertinent to the behavioral syndrome we call autism? Altered interregional coordination or functional connectivity has been measured in a widespread distribution [29, 30, 34, 35]. Placing the tissue, volume and connectivity findings side by side, the question arises whether the connectivity abnormalities are a manifestation at the functional level of the same underlying pathophysiological mechanisms as the physical changes. If so, it becomes central to address at the cellular and molecular level the mechanisms by which these tissue reactions might impact

functional connectivity. The confluence of brain developmental trajectories, brain tissue changes, functional decrements, and symptom evolution timetables points toward an integrated model of autism pathophysiologies crossing multiple levels of biological hierarchy and tracking far along the temporal axis of brain maturation. The implications of this confluence are the motivation for this discourse; the underlying details will be described in the following analysis of the evolution of concepts in the field.

From Behavioral to Multi-Leveled Nosology

A Modular "Bottom-Up" Approach: Genes–Brain–Behavior

Figure 20.1 addresses the question of biological hierarchy, illustrating the differences in structure between the commonly held "modular, gene–brain–behavior" models and the emerging more inclusive "systems biology model" of autism. In the conventional "modular" approach (Fig. 20.1a), the mechanisms by which genes affect the brain are often not of central interest—instead this model, which focuses on gene–behavior correlations, moves the underlying biology into the background. In one variant of this model, the three impaired behavioral domains (communication, social interaction, restrictive-repetitive behavior) are assumed to be distinct and to relate to disturbances in distinctive neural systems that are each controlled by distinct genes. This leads to models in which the disturbances in the three behavioral domains are considered to be independent but co-occurring; autism is thus thought to result from multiple separate gene defects coexisting in the same individual. Disorders that share a subset of autism's behavioral features (e.g., repetitive or obsessive behaviors) are thought to share a subset of autism's gene–brain–behavior alterations. This version of the modular model presumes a congruence or continuity—i.e., a tight coupling—across the gene–brain–behavior levels within each module. Findings that behavioral features of autism can travel separately [36] are cited to support this line of reasoning. Critics would, however, argue that behavioral fractionation does not prove a unique gene–behavior link, because not only genes but also non-genetic features (such as in utero exposure to maternal autoantibodies, infectious agents, or environmental toxins affecting gene products at both pre- and post-transcription levels) may affect specific behaviorally pertinent brain regions and cells during vulnerable time periods.

A less modular variant of this gene–brain–behavior view is that genetic mechanisms are valuable to identify not because they are applicable across the entire autistic population, but instead because even rare genetic variants can point toward a pathway that contributes even in differently inherited or non-inherited forms of the condition. Identifying these biologic pathways may then permit rational treatment design. This version of a gene–brain–behavior model begins to consider neurobiological mechanisms, but is still often heavily

weighted toward genetics and would typically put biological factors outside the nervous system into background.

The Critical Nature of Models and Diversity in Scientific Investigation

This conventional modular model biases research programs toward presuming that the brain and behavior phenotype data will provide sufficient correlates for researchers to identify the genes that are currently presumed to cause autism. The agenda it sets for researchers is to improve the characterization of behaviors and to obtain ever larger samples of subjects characterized across the levels of genetics, brain, and behavior so that sample size can be sufficiently large to permit correlates across the three levels. However, if this model of autism pathophysiology is incorrect or incomplete, it sets an agenda that is bound to fail. We cannot stress enough how critical it is for experimentalists to work under accurate mechanistic models. One author's experiences in cystic fibrosis research are instructive. When the mutated gene in this disease was first identified, a viewpoint announced in *Nature* argued that the cystic fibrosis gene cannot encode a chloride channel, but instead must encode a transporter of some metabolite that secondarily regulates channels to impair apical membrane chloride permeability and lung fluid transport [37]. Although this public statement may have at first seemed innocuous, surprisingly, most laboratories pursuing cystic fibrosis research abandoned their ongoing research programs that had evolved through careful evaluation of the literature in the field and their own focused thoughts on potential mechanisms to pursue this "novel" hypothesis. Unfortunately, this shift led to unproductive research, failed research training experiences, and financial losses. The hypothesis announced in *Nature* was later shown to be incorrect [38], but not until after many laboratories had abandoned their prior agendas and fully invested into the new idea [39]. Three major lessons in research emerge from these experiences: (1) carefully examine and re-examine all evidence in the field before holding fast to any single model; (2) as an individual, be cautious in blindly following "novel" hypotheses made by others in the field that ignore existing information; and (3) as a funding agency, maintain a diverse portfolio in a research field; do not let individuals or small groups dominate the research agenda for the whole field. These rules may be difficult to follow during a period of heavy financial constraints and intense competition for research funding, but in times of scarce resources it is all the more important to invest wisely.

Limits of a "Gene–Brain–Behavior" Model

The gene–brain–behavior model setting the current autism research agenda is incomplete in that it does not encompass the other known system-wide

endophenotypes or the potential additional utility of metabolic endophenotyping methods to characterize its subjects. The hope of this traditional model is that this gene–brain–behavior correlation process will identify causative or risk promoting genes, and then once such genes are identified, treatments can be developed. The shadow of this hope is the idea that without such gene-based mechanisms rational treatments of autism will not be possible; in the eyes of some proponents of this model, treatment should therefore be deferred until these genes and their associated mechanisms are identified.

Non-Neurological Features of Autism

One problem with this conventional autism model is that many prominent and common signs, symptoms, and pathophysiologies occur outside the confines of the central nervous system in probably the majority of individuals with autism. Interestingly, these somatic and systemic symptoms of autism are seen by some as recent and perhaps inappropriate interlopers in the discussion of this "neuropsychiatric" disorder. But we argue that characterizing autism as a purely behavioral disorder was probably overly restrictive from the start. The initial article by Leo Kanner that first identified the phenomenon of autism characterized it as a behavioral syndrome, and years of subsequent effort have continued to focus on refining its behavioral criteria. Yet, even in this very first case series, one finds that almost every individual manifested some symptoms of abnormal feeding, vomiting, constipation, diarrhea or nutritional malabsorption, and/or immune dysregulation. Many cases also included recurrent infections [40, 41]. Belief in the idea that autism is a pure brain disorder and neglect of these systemic findings may have persisted in part because of the general misperception that the blood-brain barrier is impermeable, in part due to the lack of broad knowledge of the now accumulating evidence for brain-immune-gastrointestinal cross-communication, and in part from the long-standing placement of somatic findings into background rather than foreground by most key opinion leaders in the field of autism pathogenesis and in psychiatry more broadly.

Epidemiology and Non-Genetic Contributors

A second problem with the "modular, gene–brain–behavior" formulation is that epidemiological evidence points away from autism being a purely genetic disorder. Twin studies in autism are intrinsically confounded, because twins share not only genetic material but also an intrauterine as well as family environment. One could even argue that the unusually high concordance rate of autism reported in identical twins results from a double contingency of a genetic susceptibility concurrent with an intrauterine environmental insult. In fact, there is no intrinsic reason why genetic and environmental influences need to be considered mutually exclusive. A monozygotic concordance rate of 60% can coexist with a 60% or even a 100% role for environmental contributors—these

numbers do not have to add up to 100% (Hertz-Picciotto I, 2007, personal communication). In other words, a particular genotype or set of genotypes can be a precondition for environmental susceptibility, but without that environmental influence the disease phenotype may not manifest. If the environmental trigger is common, there could be substantial overlap of genetic and environmental factors across a population. Thus, high recurrence rates even within individual families could result from autoimmune mechanisms or environmental factors (chemical, infectious or other) rather than pure genetics. However, in our formulation of the pathogenesis of at least some autisms, genetic alleles that influence immunologic, metabolic, or even growth responses are likely to strongly influence the expression and severity of an environmentally induced autism disorder. Increasing autism rates may drive increased awareness as well as be driven by them, and there is no rigorous proof that the tenfold increase in autism rate is purely artifactual or solely because of broadened diagnostic criteria or diagnostic substitution [42]. On this account, the autism research and treatment agendas need to face the possibility that new environmental stressors are contributing, potentially strongly so, to the increasing number of autism cases.

Non-Genetic Brain Pathophysiology

A third problem with a modular gene–brain–behavior approach is the shortcomings of the idea that one need only consider genes in accounting for neurobiological abnormalities. This is not proving to be the most useful way to approach autism. First, the overwhelming majority of brains of autistic individuals that have been examined *by* neuroimaging or neuropathology do not show signs of migrational or other classically described developmental abnormalities, nor do they except for a small minority (e.g., tuberous sclerosis) show signs of previously described neurogenetic disorders. When examined by magnetic resonance imaging (MRI), most brains of autistic individuals look clinically normal. Quantitative volumetric methodologies have been required to identify more subtle abnormalities that, aside from frequently being inconsistent, cannot be assumed to derive from genetic causes [43]. However, when specific techniques are used, a different and very prominent neuropathological process becomes apparent: marked immune activation (see chapter 15 by C. Pardo). Brain samples from a series of autistic individuals showed activation of microglia and astroglia along with increased levels of numerous chemokines and cytokines in the brain tissues and cerebrospinal fluid [14]. Surprisingly, these changes were found even in the brains of older individuals with autism diagnoses. Chronic inflammatory reactions are also found in the gastrointestinal system of at least some individuals with autism, along with a variety of immune system abnormalities, which may reflect a system-wide inflammatory response and immune dysregulatory process [22, 23, 44].

Immune activation is found in the central nervous system (CNS) even in strongly genetic neurodegenerative disorders such as Huntington's disease in

the focal regions of neuronal cell death and in Alzheimer's disease where neuritic plagues have formed. Neuroinflammatory activation of astroglia and microglia is also well known to occur as a consequence of assorted environmental (infectious and toxic) exposures, autoimmune conditions (e.g., Rasmussen's encephalitis), and in response to traumatic and ischemic/hypoxic injuries. Consequently, this finding does not point to a specific etiologic agent responsible for autism, but it does undermine the assumption that autism pathogenesis must involve a static defect of brain development, because these disturbances of glial–neuronal interactions are ongoing and could underlie disturbances in brain function that could contribute to the autism behavioral syndrome. Not all of these brain conditions are known to be associated with the autism-like behavioral condition, but a few pro-inflammatory factors (e.g., maternal anti-brain antibodies, pre- or perinatal Cytomegalovirus (CMV) infection) have been well documented in cases of infantile autism. These observations suggest that either differences in the *distribution* (cell types or brain regions affected) or the *timing* (in utero, postnatal developmental periods, or adulthood) of the pro-inflammatory challenge might account for some of the heterogeneity of clinical presentation seen amongst individuals in the autism spectrum as well as amongst other conditions involving immune activation.

Brain-Body Cross-Talk

Until recently the brain was considered immune-privileged, so that inflammatory events occurring within the body would be considered irrelevant to local environment and ongoing functions of the brain. Recent studies, however, have identified ongoing cross-talk between the body and the brain. To illustrate with just a few examples, adipocytes (fat cells) synthesize the peptide hormone leptin, which travels through the blood stream into the brain to regulate neurons within the forebrain, hypothalamus, and brainstem that control food-seeking behavior, cellular metabolism, and autonomic nervous system activity [45]. The regulation of activities within these brain regions provides feedback signaling to peripheral organs to control blood flow and energy consumption with the goal of achieving energy homeostasis. Similarly, inflammation in the body signals the brain to promote not only the fever response, but also increased cardiac output and an immune response, and suppressed social interaction [46]. Thus, we see that there can be mutual influence between peripheral and brain immune responses.

Furthermore, the rationale for dismissing peripheral influences on the brain because of the belief that the brain is protected from the events that impinge on the peripheral organs is being progressively undermined. We now know that "barrier" is an over-statement regarding the blood–brain barrier, because it does not mature until substantially after birth, and its permeability is significantly modulated even in adulthood by factors such as fever and circulating cytokines.

This brings the question of the neurobehavioral pertinence of somatic infections and inflammation into the foreground. Placed alongside the documentation of immune activation in postmortem autistic brain tissue and in CSF, the relationship between peripheral and central immune dysregulation becomes an important area of investigation. Thus, the accumulating literature on the substantial prevalence of gastrointestinal and immune symptoms and disease processes in autism is moving from a parallel and subordinate track—a study of coincidental "comorbidities"—to an integrated study of the condition that takes into account each of these diverse manifestations in order to provide a common or at least interacting set of underlying pathophysiological mechanisms, as depicted in Fig. 20.1b.

A Systems Pathophysiology "Middle-Out" Approach: Dysregulated Biology

In light of the systemic pathophysiology that is being repeatedly documented in brain and peripheral tissues, it is now imperative that any model of autism pathogenesis incorporate mechanisms at additional physiological and signaling levels to explain the many findings that do not fit into the simpler "genes–brain–behavior" paradigm. Fig. 20.1b illustrates a "systems biology" or "systems pathophysiology" approach, which expands the dimensions under consideration beyond those that are highlighted in the "modular" approach. It also shifts the focus. The core of this model is a widely distributed dysregulated cell biology that goes beyond just the neuron. Beginning with the level of dysregulated biology represents a "*middle-out*" approach, where one works upstream to pathogenesis and downstream to a cascade of phenotypic manifestations that includes behavior but also much more.

- Upstream pathogenesis includes not only genes but also environmental factors and epigenetics. Even within genetics, this model calls for moving from the current narrow focus only on genes that specifically regulate neural development to a broader approach that also includes genes that regulate the metabolic and immune responses to environmental (infectious and xenobiotic) insults and the autoimmune response. These immunologic and metabolic perturbations would be upstream of impaired neural circuit and neural system functions in this model [15]. Additionally many genetic mutations may well target a substantially smaller set of cell, molecular, and biochemical mechanisms.
- Downstream of the core dysregulated biology are the components of the various autism phenotypes at the levels of biology, information processing, somatic symptoms, and behavior. Rather than assuming that each distinct behavioral domain has its own genetic basis, this model allows for the more probable interactions among systems and especially of emergent systems properties [47, 48], with multiple disease and behavioral manifestations

that *appear* distinct, but in fact, arise from common underlying pathophysiological mechanisms. Autism's substantial heterogeneity would result at least in part from differences in the severity, distribution, and timing of these systemic events, potentially relating to specific physical properties of the insult and the distinct biological responses to these insults.

Because phenotypic studies so far have been largely behaviorally oriented and correlated not to "mechanism," but rather to epidemiological data, and a limited number of "biomarkers," a fresh research agenda is needed to pursue the implications of this model.

Tight coupling between specific metabolic endophenotypes and specific behaviors might exist. This is an area badly in need of further study, and at this point one can only guess which potential mechanisms connect the pervasive metabolic, biochemical, and signaling dysregulation to the changes in function of specific target organs, brain regions, neuromodulatory systems, and behaviors, given that some kind of preferential targeting would need to be involved for locally enhanced effects to occur. However, we should note that the need to study underlying pathophysiology at the level of metabolism, biochemistry, immunology, and neural circuit levels is strongly defensible independent of the precise impact of these perturbations on the specific components of the autism behavioral syndrome.

It is in relation to how the levels in Fig. 20.1b relate to each other that the "systems model" also raises the question of the nature of autism. Autism has been defined behaviorally, i.e., in terms of the communication, social interaction, and behavioral domains included in box "i." But if autism rests on underlying biological factors, i.e., the components in box "ii", one aim of research might be to parse out subgroups within the larger population of individuals (i) who meet behavioral criteria and (ii) who are further characterized by having distinctive biological features. Such subgroups might be better defined, understood, and treated based on their specific underlying biological dysregulation rather than based on their behavioral deficits.

The "systems biology" model more sharply brings to the forefront critical questions of heterogeneity and of "final common pathways" regarding multiple mechanisms that may eventuate in common outcomes [49]. It also raises the question of where the bottleneck may be—i.e., at what level there may be the least heterogeneity. That is, are there few or many things *sufficient* to cause the autistic syndrome? And are there any things at all that are always *necessary*? Given the likely roles of epigenetics and environment, we can no longer assume that genes are the sole causal agents. Consequently, the question of the levels at which heterogeneity is both greatest and most restricted becomes pertinent, and poses a challenge to the research community in developing coordinated research strategies explicitly aimed at answering this class of questions. For example, how do we collaborate to determine whether and how multiple genes, environmental, and epigenetic factors may contribute to only one or a few types of dysregulated biology? Do all autistic individuals have immune activation? And if some do not, what does their biology look like and by what mechanisms do these non-

inflammatory forms lead to autistic behaviors? Is there convergence between the genetic targets in the rare familial cases and the inflammatory targets that may exist in the more prevalent forms of autism? Do all autistic individuals have reduced or otherwise altered functional connectivity? And if some do not, how can their processing patterns be characterized? Overall, what is it that different mechanisms leading to autistic behaviors have in common?

Analogies with multiple sclerosis

Concerning the issue of heterogeneity of etiologies and mechanisms in autism, another inflammatory neurologic disease with clear heterogeneity is multiple sclerosis. In this disease, demyelinating plaques affect different regions of the white matter in each individual. Although autism differs from multiple sclerosis in the type of immune reaction—innate vs. adaptive, respectively—it may still be similar in showing variable distribution and intensity from case to case and over the time course of the disease. Importantly, in MS, despite many studies, no correlation has been found between CNS [cerebrospinal fluid (CSF)] and peripheral immune markers, suggesting that it may be equally difficult to make such correlations between behavioral deficits and immune responses in autism. The heterogeneity in autisms, as in MS, may not result from the level of CSF (or systemic) immune markers, but instead from the brain regions targeted by the immune cells. Addressing this idea will require methods of labeling immune cells with radioisotopes combined with high-resolution imaging of these markers across different gray matter structures within the brain. The development of behavioral assays with quantifiable end points in humans will also be important in the analysis of these relationships. Ultimately, such methods will be necessary to monitor therapeutic responses to drugs. Embracing a systems model of autism with variable etiologies and disease penetrance will improve experimental design aimed at understanding the disease in these fashions.

Links Between Expanded Biological Hierarchy and Expanded Temporal Axis

The addition of environmental and epigenetic factors to the level of pathogenesis brings to the foreground the question of temporality. Although temporality in genetics may be implicit in that many genetically based conditions may not become symptomatic until substantially after birth, the time dimension is much more explicit regarding epigenetic mechanisms, which are by definition not inborn in the same way as are genes. Moreover, environmental exposures, whether chemical, infectious, or other, may influence critical periods of brain and somatic development *by* epigenetic or pretranscriptional mechanisms (e.g., may act as teratogens), but they may also accumulate or occur at subsequent points and yet still have an impact on brain *function*. This means that

rather than taking for granted an exclusively early genetic determination of the autism brain wiring diagram, it is now becoming necessary to additionally consider contributors that may enter the system later and/or influence it in an ongoing fashion.

Presumptions about exclusively prenatal determination have been challenged by the phenomenon of what is called "autistic regression"—i.e., loss of acquired milestones, by the presence of chronic pathophysiology as discussed, and more recently also by the phenomenon of improvement, loss of diagnosis, and even reports of full recovery from autism. These challenges support revising the temporal narrative about autism.

Delayed Onset of Autism

Not so long ago, parental reports of later onset autism (beyond 2 years of age) were considered to be based on the inability of these parents to recognize early signs of autism. However, in recent years, the phenomenon of later onset autism or "regressive autism" has been validated, particularly by retrospective analysis of videotapes of children for signs of autism before the clear emergence of autism syndrome features [50]. Currently, it is estimated that upwards of 25% of autistic children develop the condition during the second year of postnatal life, with subtle or even absent signs of prior abnormality.

Neurodegeneration

The question of a neurodegenerative component in autism is being discussed, based on the identification of pathophysiological features such as ongoing systemic and CNS redox abnormalities and inflammation, and the evidence of brain volume [51] and neuron [52] loss, albeit modest, that are features of neurodegenerative conditions. Although there are no clear clinical correlates of continued decline in function as might be expected in a progressive neurodegenerative process (with the caveat that adult autism is poorly studied), it is commonly thought that early intervention is more effective than intervention even in mid to late childhood, suggesting a reduction of potential plasticity over time. Whether this preferential early responsiveness to therapy reflects the time course of the natural critical periods of human neurologic development or whether it identifies early postnatal development as a uniquely fragile period remains an open question for future research.

Improvement and Recovery

Finally, if current reports on the internet, in film, and in popular books and magazines chronicling loss of diagnosis and recovery [53] can be rigorously validated, the question arises whether and how at least some underlying pathophysiological mechanisms may at least partly be reversible. If even some cases of substantial recovery are validated, this is another important direction for future research. Moreover, reports of improvement and recovery have involved a variety of interventions ranging from standard behavioral therapies to treatment of somatic symptoms. This suggests either (a) a heterogeneity in potential treatment targets, (b) the possibility that the pathophysiology is a systems problem held in place by multiple simultaneous reinforcers, so that a potential may exist for self-correction even when only a subset of stressors are reduced, or (c) both.

From a Static Defect in Early Circuit Development to a Chronic Ongoing Disturbance of Circuit Remodeling and Function

A Model of Static Encephalopathy (Fig. 20.2)

The sequence in Fig. 20.2 addresses the contrast between models framing autism as a fixed, static encephalopathy with those including chronic and potentially reversible features. We elaborate the expansion of the temporal dimension in autism to include other key etiological levels and timetables of pathophysiology beyond a fixed deficit genetically caused entirely in utero.

Genetic Static Encephalopathy (Fig. 20.2, Model 1a)

In Fig. 20.2, Model 1a schematizes the standard narrative about autism cause and mechanisms. In this model, genes cause alterations in early brain development that permanently alter brain function. This model temporally instantiates the gene–brain–behavior version of the biological hierarchy. Along the temporal axis, autism in this model results from a genetically determined disturbance of early brain development, and autism is a static encephalopathy, i.e., a fixed trait.

Static Encephalopathy Caused by Gene-Environment Interactions (Fig. 20.2, Model 1b)

In Model 1b, environmental and in utero immunologic factors enter the picture, and these, along with genes interacting with them, lead to a disturbance of early brain development that causes autism. But although the causal matrix is more complex, autism is still a static encephalopathy, a fixed trait. This model has been dominant in developmental neurotoxicology. Various chemicals, cytokines, and other substances have been shown to target morphogenetic signals, transduction pathways, and developmental events critical for multiple brain development processes [54]. The impact of insults at this level is arguably indelible and permanent. Immune activation and neuroinflammation could have such indelible effects, as reflected in a growing body of literature demonstrating immune influences on cellular proliferation and vulnerability as well as other features of brain development [55].

A Model of Autism as a Chronic Condition (Fig. 20.2, Model 2)

Epigenetic Contributors and Potential Plasticity (Fig. 20.2, Model 2a)

In Model 2a, one considers that not only might there be changes in the structural architecture of the brain's neural circuitry and glial cell distribution and numbers, but there may also be changes in nuclear histone-DNA architectures that dictate the levels and patterns of gene expression that must also be carefully adjusted to create a biologic system where all cells provide the appropriate quantities of molecular machineries (synaptic signaling, synapse-action potential coupling, oligodendroglial myelination, and glutamate transport) that cooperate to achieve a finely tuned neural circuit that transmits signals with high fidelity and precision. As an example, the use of valproate to treat epilepsy during pregnancy is associated with an increased risk of an autism spectrum disorder in the offspring [56]. This compound is a potent histone deacetylase inhibitor [57] and could therefore produce a lasting disturbance to the nuclear architecture that is critical to the tuning of neural circuit function [58]. Deficiencies of the methyl–CpG binding protein, MECP2, in Rett syndrome leads to a disturbance in the expression of a variety of proteins (e.g., decreased brain-derived neurotrophic factor (BDNF) [59] and increased Dlx5 [60]), suppressed layer V pyramidal neuron activity because of an increased inhibitory to excitatory synaptic input ratio [61], and behavioral deficits resembling autism. These observations suggest that disturbing the nuclear DNA architecture during development might be sufficient to produce autistic behavioral deficits. Importantly, some of the behavioral defects of the mouse model of Rett syndrome are reversible after development through conditional genetic rescue experiments [62]. Recent work also implicates alterations in chromatin remodeling in memory formation, recovery following

neuronal damage, and a variety of inherited neurodevelopmental disorders (e.g., Rubinstein–Taybi syndrome, Rett syndrome) [63, 64, 65, 66]. We speculate that inflammation in utero or during early postnatal development, whether because of autoimmune mechanisms, xenobiotics, or infection, may also disturb nuclear DNA architecture to produce long-term effects on gene expression, some of which may be reversible but not easily so.

Ongoing Environmental Contributors to Chronic Encephalopathy (Fig. 20.2, Model 2b)

Model 2b formulates a hypothetical model in which the possible ongoing impact of accumulating environmental factors extends beyond the period of early development. Toxicants, autoantibodies, and infectious agents may not go away after the exposures during critical periods of development, and furthermore may continue to accumulate, or even arrive in the early postnatal period for the first time. For example, heavy metals such as lead, cadmium, mercury, or other neurotoxicants (such as polychlorinated biphenyls, pesticides, and air pollutants) can penetrate the nervous system. Once there, it is possible for them, by various mechanisms (e.g., organic mercurials that are de-ethylated or demethylated in microglia in the brain, yielding inorganic mercury) [67, 68, 69, 70, 71, 72, 73], to promote an oxidative response in these cells to stimulate a cytokine/chemokine inflammatory response within the brain. This effect may be long lasting if the toxin also impairs the function of cells (macrophages or microglial) that would normally try to clear the toxin. Various metals and persistent organic pollutants also accumulate in a number of body compartments, such as fatty tissue and liver, where they can have chronic metabolic impact such as inhibition of mitochondrial or hepatic enzymes. Viral infections might also contribute to chronic metabolic alterations [74]. The later or ongoing presence of such environmental influences can lead to a chronically dysregulated neuroglial environment, even if brain development is already substantially complete in all but the most subtle respects, and such dysregulation may not always require a prenatal initiating process.

Autism as Trait vs. Autism as Trait Modulated by State vs. Autism as State

The spectrum of mechanisms we are describing here are not mutually exclusive, as is illustrated in Fig. 20.2 in which each successive model includes additional features beyond the prior one but still includes the simpler model. It is likely that the heterogeneity of autism is substantially related to variability in several or all of the parameters involved in the gene–environment–epigenetics–timing interactions [75]. Although autism has been considered a “trait”—i.e., a fixed condition, it may also have elements of a “state”—i.e., contingent condition.

Autism as Trait Modulated by State

Autism in some cases may derive from a disturbance of early brain development whose impact is worsened by chronic or intermittent environmental influences (e.g., the child who gets worse with exposure to certain allergens or dietary peptides). In this model, autism is a static encephalopathy aggravated chronically or intermittently by disturbed intermediary metabolism and a dimension of metabolic encephalopathy—so it is still a trait, but has some malleability at the level of state changes that may lead to variability of severity within an individual. Support for this model of exacerbations of the neuroinflammatory state with systemic inflammatory challenge is supported by recent work showing that there is a magnified central pro-inflammatory cytokine response to a systemic inflammation trigger (lipopolysaccharide) when there is a long-standing weak stimulus of the brain's innate immune system [76]. Even a brief systemic proinflammatory insult to a 2-month-old rodent was shown to cause a persistent central elevation of the pro-inflammatory agent TNF-α 10 months later [77]. We propose that these observations suggest that an inflammatory insult in utero (e.g., maternal infection) might be a set-up for a marked central inflammatory response to a peripheral pro-inflammatory stimulant during early childhood (e.g., immunizations or viral illness) [78, 79, 80, 81]—that is, a low level of early onset immune activation may be the primary developmental condition that sets up a child for further injury later on (See also Patterson et al., Chapter 13).

Autism as Predominantly State Rather than Trait

For other autistic individuals, the interaction of gene–environment–epigenetics–timing may be not so much to *cause* autism as to increase vulnerability to subsequent contingent environmental stressors whose impacts are predominantly functional. In these individuals the tipping point into chronic immune activation, redox or energetic abnormalities, for example, may be reached more easily than in other individuals, leading to a chronically and multiply reinforced disturbance of intermediary metabolism, a state more than a trait—which can on occasion be reversed. Examples of such reversal include improvement on allergy medication; the striking amelioration of autistic features that has been anecdotally observed by various clinicians and families in some children who are on clear fluids or total parenteral nutrition, with a reversion to autism when enteral feeding is resumed; the transient but sometimes marked amelioration of autistic features in association with fever [82]; and the intermittent character of autistic features in some children diagnosed with mitochondrial disorders, whose autistic behaviors appear during fatigued but are absent when energetic (Korson M, 2007, personal communication). Further examples include instances of loss of diagnosis mentioned previously [17], which are poorly documented academically because of, among other things, the lack of inclusion until recently of useful indicators of improvement and recovery in

outcomes research, presumably because of the assumption of autism as "trait," i.e., of incurability [83].

Mistaking State for Trait

It is important to note that chronicity may masquerade as incurability because the pathophysiology of encephalopathy secondary to disturbed intermediary metabolism in many cases may be quite complex. Inflammation may interact with redox abnormalities, recurrent and/or chronic infection, allergies, malabsorption and/or self-restricted diet leading to nutrient insufficiencies, disordered sleep and heightened stress responses to create a highly mutually reinforcing set of pathological feedback loops. The resultant physiological "gridlock," observed by some metabolically oriented clinicians (and much in need of more systematic study) may surface as a range of somatic and systemic symptoms that are just now moving from background to foreground in autism research and clinical practice. Because clinical phenotyping, which to date has skipped over these levels of autism phenomenology, is finally starting to move beyond behavior to include whole-body features (Fein D, 2008, personal communication) [1], the nature and prevalence of these features as well as the model that diverse somatic features may be linked by a shared set of cellular, signaling, and metabolic features can finally be explored and tested. If these chronic somatic and systemic features are core components of autism pathophysiology rather than secondary accompaniments, then their treatment and reversal may lead to reversal at the level of brain and behavior as well. Although it may require great ingenuity to unlock such chronicity, and although without such ingenuity and persistence this chronicity may be for all intents and purposes "incurable," the recalcitrance of such a state is not sufficient proof that the condition arises exclusively from early developmental mechanisms (although those may also be present at the same time as ongoing chronic effects in some or many individuals). With newer perspectives on previous findings in autism research [84] and the newer findings of a chronic ongoing pathophysiologic process characterized by inflammation and disturbed metabolism, autism should now be viewed as possibly resulting from a dynamic metabolic and physiologic disturbance rather than simply an incurable developmental injury.

Chronic Mechanisms in Functional Impairment in Autism

Considering the astroglial and microglial immune response now shown to be present in brains of autistic children [14], in this section we discuss potential mechanisms whereby activated glia could drive brain malfunction in autism. We must emphasize that this model is erected solely to guide experimental design, and that much of the evidence must still be acquired. The models will surely evolve as data are accumulated. We focus on mechanisms related to

chronicity, i.e., to Model 2b in Fig. 20.2 as described just above. We will confine ourselves to this model not because it is the only possibility but rather because it has received the least attention to date, even though it includes plausible mechanisms that can be tested experimentally and that would have broad implications if they were to be identified as operating in autism.

Disturbances of Astroglial Function

Environmental xenobiotics may alter glial–neuronal interactions through effects on astrocyte electrolyte homeostasis. One effect of neurotoxins is astroglial swelling, which may be caused by inhibiting mitochondrial respiration, by altering passive chloride or cation conductances, or by impairing active transport of ions. Astrocyte swelling leads to amino acid efflux. This solute efflux in part serves to recover normal astrocyte volume, but more importantly, can produce major effects on neuronal synaptic transmission and intrinsic excitability as described below. Beyond these functional effects, glutamate and glycine release can promote excitotoxic electrolyte disturbances (swelling and calcium overload) in the neurons, which may ultimately lead to loss of axonal or dendritic processes or cause outright cell death. Some xenobiotics may block anion channels, which could reduce excitotoxicity at the expense of prolonging the astroglial swelling. Although not a major focus of current research programs, toxicity to astroglia themselves (process collapse or cell death) may also be a critical component of the neural circuit dysfunction because these cells play critical roles in promoting the formation and function of synapses [85, 86]. Another poorly appreciated effect of astroglial toxicity is the glial–vascular signaling that enables changes in neuron metabolism to couple to changes in cerebral vascular blood flow [87, 88]. From a purely structural perspective, astroglial swelling could also cause a substantial decrease in average capillary lumen volume, impeding blood flow that could cause ischemia in neurons possibly analogous to the cognitive defects that can arise in hypertensive or severe diabetic cerebrovascular disease. This may be a mechanism pertinent to multiple published reports of reduced cerebral perfusion in autism [89, 90, 91, 92]. If so, ameliorating some of the physiological reinforcers of this reduced perfusion could plausibly improve the level of functioning in autism. Additionally, astroglia provide the major energy source to neurons in the form of lactate, and therefore, impaired astroglial cell metabolism can have major secondary effects on neuron energy metabolism [93]. Astroglial foot processes also create the functional blood–brain barrier, and defective astroglia could impair this barrier, leading to increases of extracellular potassium or other metabolites that could impair neuron functions [94]. A potential link between defects in the blood–brain barrier and autism is inspired by the observations that neuroligin 3 is mutated in a family with X-linked autism [95] and that the Drosophila homolog of neuroligin 3, gliotactin, plays a critical role in formation of the

blood–brain barrier [96]. Interestingly, this same protein may also contribute to formation of epithelial barriers based on its effects in Drosophila suggesting a possible link to the gastrointestinal problems and inflammation found in autism [2, 3, 4, 5, 6, 7, 8, 9, 97]. Finally, impaired diffusion of substrates and waste products between the intravascular compartment and the neuron could have major consequences for neuronal and glial metabolic efficiency [98].

Neuronal and Neural Circuit Changes from Astroglial Metabolic and Structural Changes Deriving from Chronic Inflammation

Model 2b in Fig. 20.2 regarding ongoing contributors to chronic encephalopathy, described above, may significantly describe the impact of immune activation and inflammation on autism. There are a variety of potential mechanisms. Components of the inflammatory response may normally reduce neuronal metabolic activity to protect neurons during self-limited disease (e.g., viral infection) and to promote neural circuit growth and repair after disease-induced damage. In autism, however, the condition is no longer self-limited; neural circuit silencing appears to be too widespread and too persistent, perhaps related to the chronicity of the immune activation. Although resting astroglia promote synaptic signaling, activated glia may impact synaptic signaling and neuronal functioning differently. Little is currently known about the effects of activated glia on neuronal circuit function during a chronic innate immune response as observed in autism. However, a sequence of changes can be proposed for investigation. It appears likely that chronic immune activation leads to metabolic changes in astroglia, which in turn might inhibit neuronal and neural circuit functioning in a series of ways. Primary impairments in glial metabolism could readily impair neural circuitry through their failure to provide metabolic support to neurons (i.e., lactate) and to clear glutamate from the synaptic cleft. Glia could *release* inhibitory neuromodulators, *retract* glial processes supporting synaptic or axonal transmission, or *dismantle* synapses, and even *collapse* dendrites.

Release of Inhibitory and Excitatory Neuromodulators

The release of inhibitory neuromodulators such as ATP, lipid metabolites, chemokines, cytokines, and growth factors by glia could lead to acute changes in neuronal function. Astroglia also release glutamate, GABA, taurine, and D-serine [99, 100, 101]. These latter agents might produce acute excitation or inhibition through group III metabotropic glutamate receptors, tonic GABA A receptors, glycinergic receptors, or NMDA receptors (on GABA interneurons for example), respectively.

A further potential mechanism of inhibition is the release of lipids such as *arachidonate metabolites* by astroglia [102, 103, 104, 105, 106, 107, 108]. Lipoxygenase metabolites inhibit synaptic transmission [106, 107, 108]. In the lateral amygdala, they also inhibit action potential firing by activating α-dendrotoxin-sensitive, low-threshold K^+ channels [108].

Inhibition at this level could impact neural systems functioning. Purinergic agonist (ATPγS) stimulation of glia in the retina causes them to release *ATP that is metabolized into adenosine* to inhibit neurons *by* adenosine R1 receptors [109]. We speculate that similar mechanisms might occur in the brains of individuals with autism because of innate immune activation. Chronic immune activation in autism might cause glia to release ATP to inhibit neural activity. In the retina, acute stimulation of glia causes them to release ATP that is sequentially metabolized into adenosine by ecto-ATPase and ectonucleotidases. Adenosine binds to adenosine A1 receptors to activate G-protein-coupled inwardly rectifying K^+ channels (GIRK) and inhibit action potential firing of retinal ganglion cells [109]. The thalamus contains a very high density of adenosine A1 receptor A_1R [110]. Adenosine activates adenosine A1 receptors to hyperpolarize thalamic relay neurons of lateral geniculate nucleus, converting the action potential firing mode from tonic to burst [111]. Burst firing by thalamic neurons is suspected to help achieve a stable sleep state [112]; consequently, inflammation-induced activation of burst firing in the thalamus of an individual who is awake might put affected regions of the thalamus into their sleep mode of firing and prevent normal sensory transmission, which is thought to utilize the tonic firing mode. Hyperpolarization and inhibition of tonic firing is explained by the GIRK K^+ currents activated by adenosine A1 receptors [111, 113].

Haznedar et al. [114] reported severely reduced [18F]-fluorodeoxyglucose uptake in the thalamus, striatum, and frontal cortex in autism. We suspect that effects of the innate immune activation on neural circuit function may be responsible. These effects could be due to depressed glutamatergic synaptic transmission, enhanced GABAergic synaptic transmission, or impaired neuronal excitability, all of which could be induced by neuromodulators released during chronic innate immune activation. The effects could occur within the local circuits or they could result from defects in neuromodulatory systems projecting to these circuits (e.g., the basal forebrain cholinergic system or the hypothalamic preoptic area that inhibits monoaminergic systems to initiate the sleep state). Alternatively, the suppressed activity measured by Haznedar's group might result from impaired synchronization of firing within populations of neurons that project to these regions. For example, BDNF is released by activated microglia. BDNF decreases the KCC2 K^+-Cl-cotransporter, causing the high intracellular chloride gradient to collapse. This could result in defective GABAergic synaptic transmission. The basal forebrain GABAergic system generates a synchronous GABAergic synaptic input at theta frequencies, which temporally synchronizes firing across broad regions of the cerebral cortex and hippocampus. Loss of inhibitory post-synaptic potentials in cortical

pyramidal neurons because of BDNF down-regulation of KCC2 could impair pyramidal neuron synchronization and consequently lead to a failure to synchronously excite down-stream target sites. Synchrony is achieved in part through the broadly projecting GABAergic systems of the basal forebrain that temporally couple the firing of large populations of neurons within the theta and gamma frequency bands. These mechanisms of circuit suppression could also interact with defects in synaptic connections because of developmentally altered axonal targeting, growth, or pruning. An outcome of these mechanisms could be the underconnectivity measured through fMRI or an alteration of coherence as measured by EEG in autism [30, 34, 35].

Retraction of Glial Processes

Persistent innate immune activation could progress from a neuromodulatory and neurophysiological to a neurostructural level of impact depending on the duration or intensity of the inflammation and its temporal relationship to postnatal developmental time windows. *Retraction of glial processes* supporting synaptic or axonal signal transmission in neurons has been implicated in the supraoptic nucleus of the hypothalamus during lactation [99]. This has functional consequences: as the glial covering of a synapse is lost, glutamate released at the synapse that is usually taken up by the glial transporters now escapes into the extrasynaptic space where it can bind to presynaptic metabotropic glutamate receptors that will inhibit synaptic transmission. Similar mechanisms may be at work during autism-associated inflammation in the thalamus and elsewhere.

Dismantling and Collapse of Synapses and Dendrites

Dismantling and collapse of synapses and dendrites could silence neuron activity during chronic inflammation. Lehnardt et al. in 2006 [115] showed that bacterial meningitis causes a neurodegenerative process through toll-like receptor 2 (TLR2) possibly mediated by microglia. Although collapse would seem to be a pathologic process, it might also be adaptive because it preserves the neuron soma and might permit reconnection of the circuit later once the inflammatory condition has been cleared. This possibility of reversible impairments even in structural connectivity is highly pertinent to interpreting and pursuing reports of improvement and recovery phenomena in autism, which, to be adequately studied, require longitudinal repeated measures and a model of autism that is dynamic rather than static. Endotoxin, which activates TLR4, exacerbates neurodegeneration [76]. It may be pertinent to the trajectory of mild volume loss that appears to occur in adolescence and adulthood, after the volume increase seen in early childhood [20, 51].

Glial Calcium Oscillations

Inflammatory glial activation might have a more direct neurophysiological impact. One possible consequence is a strengthening of glial–neuronal coupling. Parri and Crunelli [116, 117] found that a minor subset of resting astroglia (4%) in the thalamus undergo spontaneous *calcium oscillations* (approximately 1/60 s). Although it has not yet been investigated, we hypothesize that immune activation might increase the number of astrocytes undergoing calcium oscillations. Increases of intracellular calcium during each cycle of the oscillation could promote pulsatile release of cytokines and chemokines.

We further speculate that intracellular calcium oscillations and waves could be promoted by the combination of gap junction coupling between astrocytes and calcium-induced calcium release mechanisms through ryanodine receptors. Under baseline conditions retinal *glial–neuronal inhibition* is relatively weak [109]: amongst retinal ganglion cells, 35.5% showed little or no adenosine-mediated slow hyperpolarization events (<0.2 mV), 52.2% showed moderate hyperpolarizations (0.2–5 mV), and only 12.3% showed large hyperpolarizations (>5 mV). We speculate that immune activation might increase the number or incidence of large hyperpolarizing events because of either more frequent large calcium oscillations or altered ATP release mechanisms, or altered adenosine responses of the neuron. These are all important issues we will investigate to gain a better understanding of the potential mechanisms of immune activation-induced alteration of neuronal activity in the brain of autism patients.

Sustaining Inflammation

What sustains the immune activation? The presence of *neuroglial synapses* has only recently been recognized [118], and their function remains a complete mystery. We speculate that neuroglial synapses might couple neural activity to glial release of inhibitory neuromodulators under conditions of immune activation. This type of mechanism could target the glial release of chemokines and cytokines to neuronal activity, providing feedback inhibition only when and where neuronal activity is occurring. Such a mechanism could conserve metabolic energy consumed by the immune-activated glia. We further speculate that this mechanism might promote greater inflammatory changes in resting-state or default-mode regions of the brain in autism (cingulate cortex, anterior thalamus) because these are the regions with the highest activity during the resting state [119].

Summary of Glial Impacts: The Foregoing as Examples of New Classes of Mechanisms to Pursue

The above speculations arise from the need for models to guide experiments that address the ongoing effects of immune activation on neuroglial interactions and on circuit dysfunction. Mechanisms such as we sketch in discussing Model 2b of Fig. 20.2 bear consideration as possible explanations for the reports of improvement and loss of diagnosis that have emerged in recent years and are recently coming to be studied. These mechanisms are not meant to be an exhaustive survey of possibilities, but rather to illustrate some levels at which investigation could profitably be pursued. Although it is also important to investigate how immune activation might additionally disrupt the normal pattern of neural circuit remodeling that occurs during early postnatal development (as in the Models 1a and 1b in Fig. 20.2), our focus has been on the later and chronic functional impacts of chronic innate immune activation, because these mechanisms of disruptioning circuit function have received almost no attention in autism research and might represent reversible defects that could be targeted by therapies.

Implications for Heterogeneity, Reversibility, Autism Symptoms, and Development–Chronicity Interactions

Heterogeneity of autisms may map to differences in the relative weightings of links in this set of causal chains, as mentioned above. There may also be heterogeneity in reversibility because among the various ongoing mechanisms, some can apparently reverse quickly (e.g., improvement during fever or abstinence from solid foods as mentioned earlier) although others, such as the dismantling and collapse of synapses and dendrites mentioned above, may take longer to reverse. Identifying such potentially reversible mechanisms needs to become an important part of the autism research agenda. Reversibility, particularly short-term marked improvement, raises the further question of whether recovery involves gains of skills or loss of inhibition (or some of both), because even transient markedly improved capabilities suggests more underlying soundness of circuitry than previously suspected.

It is also worth noting that anecdotal reports from individuals with autism suggest that many "autistic behaviors" result from sensory dysregulation and represent ways of coping with problems such as sensory overload. Such behaviors include self-injurious and so-called self-stimulatory behaviors and compulsions in addition to sometimes crippling anxiety [120]. Insofar as such sensory dysregulation is a consequence of functional impairment of neuroglial functioning through mechanisms such as those described above, such sensory

overload could be lowered by reducing the chronic reinforcers of this impaired neuroglial functioning, thereby reversing behaviors [121, 122].

The alteration of neuronal functioning in Model 2 of Fig. 20.2 cannot be described as "developmental" in the classic sense, because it may occur at any point in the life course, even remote from the period of exuberant brain development in early life. Constructing the autism state may depend on an interaction of these chronic mechanisms with specific windows of developmental vulnerability. This may account for the apparent contradiction between the substantial overlap in dysregulated biology between autism and a variety of other multisystem conditions (such as autoimmune diseases, metabolic syndromes, and other neurodegenerative disorders) on the one hand and the uniquely autistic neurobehavioral profile on the other. Thus, the timing parameter in genes–environment–timing interactions may be exquisitely important.

The Challenge of Generic Mechanisms in a Specifically Described Disorder

Nevertheless, although understanding autism's uniqueness may be of great interest, it should be remembered that substantial advances in the well-being of individuals with autism may be achieved by addressing more mundane and even generic features of the condition's dysregulated biology, such as immune dysregulation, inflammation, and oxidative stress. What makes autism unique may be upstream or, perhaps more likely, downstream of pathophysiological mechanisms that may be more generic but that nevertheless may provide more leveraged treatment targets. Moreover, the specific features of the disease at the behavioral level may simply be a function of neural systems that are impacted by these mechanisms. The instigating mechanisms could be quite varied and yet lead to a common set of neural system defects related to common features of the pathological response (e.g., immune cell activation, cytokine release).

Summary and Conclusion

The foregoing discussion has sketched the structure of a reconceptualization of autism from an incurable genetically determined brain disorder into a heterogeneous whole-body, gene-environment, complex, and dynamic multisystem condition with multiple potential treatment possibilities. Our exposition of a range of autism models illustrating various forms and stages of this reconceptualization is meant to bring into full view the underlying structures of argument that motivate hypothesis formation and research agendas in autism. Such assumptions often go unstated. To articulate unstated assumptions is to allow them to be subjected to systematic and deliberate reflection. This

allows us to move beyond the confusion generated when unstated assumptions are left merely implicit.

We have argued that we are moving to a systems approach that incorporates the complexities of many levels of the biological hierarchy in addition to genes, brain, and behavior, particularly including dysregulated biology and signaling. And we have argued that we are moving to an expanded temporal model of the autism syndrome as a dynamic condition with ongoing as well as developmental environmental modulation. Within this framework, it is possible that by correcting dysregulated biology and signaling we can ameliorate the severity of the condition, sometimes substantially. These systems and expanded temporal models of autism have several critical implications for the autism research agenda.

1. The logical outcome of the systems approach is that research in autism at any level needs to aim for some linkage to the underlying dysregulated biology and cellular signaling pathways.
2. The logical outcome of the expanded temporal approach is that the autism phenotype may be more fruitfully addressed as a modulated condition rather than an inborn defect with genetic and environmental determinants. A concept is proposed that the disease state can represent an ongoing mutually reinforcing physiological and signaling gridlock that may look incurable but that actually may be approached as a puzzle (as opposed to a mystery) to be untangled and addressed stepwise and systematically.
3. The logical outcome of integrating the systems and the expanded temporal models is that change is possible in autism, and that we will be most successful at optimizing opportunities and outcomes if we move beyond exclusively considering just genes, brain and behavior to inclusively considering all levels and interactions in the biological hierarchy. We posit that autism research and treatment need to address genes, brain, and behavior in relation to dysregulated biology, somatic symptoms, and clinical pathophysiology. The "black-box" approach to empirical therapy may be helpful, but it is reasonable to suggest that a more thorough and whole-body systems understanding of the disease and therapeutic responses will lead in the future to greater treatment efficacy.
4. The nature of systems dynamics and the idea that behavioral deficits may be emergent properties of perturbed systems function suggest that reducing dysfunction or stress at any level may have effects that cascade through multiple levels of the system. Thus, an analytic approach to identifying treatment targets, however mundane or seemingly removed from the "autism" as behaviorally defined, may improve quality of life and reduce the aspect of autism that involves suffering. Moreover, an expanded, more inclusive modeling of autism will help us to articulate precisely what aspects of autism involve suffering for the affected individual, which will enable practitioners to more effectively address the concerns of those members of the autism community who feel that their autism gives them many strengths and do not

wish to be subjected to treatments they feel are aimed at making them neurotypically "normal."

The models discussed herein have substantial implications for the research agenda in autism. The field needs to substantially upgrade attention and resources allocated to measuring the dysregulated intermediary biology, which to date has been sorely understudied in comparison with the efforts invested in genetics. It needs to consider autism dynamically, which means focusing more centrally upon change and in particular on multidisciplinary physiological measures in the analysis of treatment response and on what such data can teach us about subgroups and mechanisms. It means developing methodologies for analyzing complex datasets derived from subjects who are in many respects heterogeneous. It means looking for different underlying biological mechanisms in responders versus non-responders in treatment trials rather than averaging away the significance of treatments that may be effective for biologically distinct subgroups. And it means testing models as well as hypotheses, and welcoming a test of fresh models proactively. These implications, taken as goals, all follow from pursuing treatment, improvement, and recovery with thoroughness and expectation of success.

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