Autism: a large unmet medical need and a complex research problem

Julia Gerlai, Robert Gerlai

Neuroscience Discovery Research, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285, USA

Received 4 April 2003; accepted 17 April 2003

Abstract

Autism has been becoming the focus of attention as its apparently increasing prevalence is better appreciated. According to some estimates, the frequency of children with autistic spectrum disorder (ASD) can be as high as 1 in 150. The diagnosis can be made as early as 2 years of age, and autistic patients often have a normal life span. Thus, in terms of the number of "patient years," ASD represents a market that is as large as that of the biggest neurological indication, Alzheimer’s disease. Despite the clear unmet medical need, no effective treatment is available. This may be because the mechanism of ASD is not understood. The aim of the present paper is to review recent advances in autism research and to discuss some of the most stressing problems mainly from a preclinical research standpoint. We hope to draw attention to the need to study this devastating disease that places an enormous burden on the society in general and the relatives and caregivers of autistic patients in particular.

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Keywords: Autism; Autistic spectrum disorder; Asperger syndrome; Mouse; Rat; Social behavior

1. Introduction

Understanding the neurobiology of social behavior has been in the focus of Dr. MacLean’s work [53]. He investigated the specific neural systems involved in social behavior. The questions he asked are highly relevant in the analysis of the mechanism of autism, a human brain disorder that manifests as abnormal social behaviors. Thus, it is our pleasure to contribute to this special issue devoted to honor Dr. MacLean’s research.

The last 10 years of the 20th century was proclaimed by U.S. President George Bush to be the decade of the brain. However, the 21st century is starting with a great embarrassment for neuroscientists: one of the biggest brain-related disorders, autism, remains a mystery. Its mechanism is not understood [2] and even diagnostic criteria with which patients may be identified are debated [31,87]. One thing is certain, however, this disease, which in the past was thought to represent a negligible problem affecting only a few unfortunate persons, is now recognized as a major problem with epidemic proportions [64,84]. Briefly, the need for effective medical intervention is enormous.

In this paper, we present a review of some of the advances of autism research and also discuss the most stressing problems. First, we attempt to describe autism and briefly discuss the diagnostic criteria of the disease. Second, we make the argument that the prevalence of autistic spectrum disorder (ASD) puts this disease on par with the biggest neurological disorder, Alzheimer’s disease. Thus, ASD should be a focus of major research efforts. Third, and most importantly, we describe some of the potential mechanisms of ASD that are under investigation and also describe animal models that have been suggested to mimic aspects of ASD. Fourth, we discuss the drug therapies presently employed and point out that they are only palliative. Lastly, we attempt to delineate what we would consider the most important future directions in the investigation of the mechanisms of ASD and in the preclinical research for the development of treatments for ASD.

2. What is autism? Definitions and diagnoses

Autism is a developmental brain disorder that is characterized by abnormal social behavior, reduced interests in communicating and interacting with others, language dis-
orders, repetitive and obsessive behaviors and rituals, and narrowly focused rigid interests (for a review, see Ref. [86]). Furthermore, autistic patients may also often suffer from a sensory overload and thus tend to avoid novel stimuli and situations [25,76]. Two main diagnostic instruments have been developed to identify autistic patients, the Autism Diagnostic Interview (ADI) [54] and the prelinguistic Autism Diagnostic Observation Schedule (PL-ADOS) [24]. These instruments, unlike categorical diagnoses, rely on rating scales and diagnostic checklists and offer evaluation of characteristics in three different areas of autistic symptoms: social reciprocity, communication, and repetitive or restricted behaviors. ADI was later revised (ADI-R) by Lord et al. [59] to allow the instrument to better discriminate autistic patients from nonautistic but mentally handicapped persons. ADOS has also been revised (ADOS-G) to make it better fit the tested person’s age and verbal abilities [58]. These diagnostic instruments now should allow clinicians to consistently rate patients for autism. However, despite their availability, they are not always used in research or in the clinic.

As information is accumulating on autistic patients, it has been becoming clear that autism is not an obviously demarcated disease but a spectrum of problems with differing combination and severity of symptoms [87]. Although ASD continues to be a frustrating conundrum, since the first description of autism by Kanner [47] and of Asperger syndrome by Hans Asperger (in Ref. [92]), much has been learned about these diseases. In 1981, it was realized that the two diseases described by Kanner and Asperger may in fact be different faces of the same disorder [91]. Nowadays, four main categories are recognized on the spectrum: (1) patients with known medical disorders, e.g., tuberous sclerosis, exhibiting most of the typical signs of autism; (2) classic autism of the Kanner type [1,47]; (3) patients classified as having “pervasive developmental disorders not otherwise specified” (PDD-NOS) who have a milder form of autism or exhibit only subsets of the symptom cluster; and (4) patients diagnosed as having Asperger syndrome [91,92] who have impaired social abilities but possess mainly unaltered and occasionally superior language skills and mental capacity.

Given the complicated nature of human social interaction, communication, and language processing and given the potentially large number of molecular mechanisms that subserve these complex behavioral phenomena, it is possible that the above four categories represent different disease mechanisms. Interestingly, however, family studies have revealed that several varieties of the autism spectrum may run, in an inherited manner, in the same family [33]. This finding thus raises the possibility that there may be a (set of) biological core mechanism(s) underlying the autism spectrum. Interaction of these mechanisms with other genetic and/or environmental factors may give rise to an epigenetically modified cluster of changes that manifest as more or less severe symptoms. However, the question of whether patients with different symptoms represent distinct disease categories or the symptoms actually vary along a continuum is hotly debated [87]. Clearly, we are faced with a catch 22: without the use of consistent diagnostic tools, it is difficult to separate different categories within the autism spectrum [9], and thus it is difficult to study possible underlying mechanisms of the disease. But without knowing the mechanisms, it is difficult to clearly identify the disease categories. This is a significant problem, especially because it is now being recognized that considering all the different forms of ASD, a potentially very large number of people are suffering from this disease.

3. Market size: is autism worth taking notice?

Once a neglected disease, ASD is now regarded an “epidemic” by the media [65] and by the scientific community [84]. The discovery that autism is not an infrequent disorder has sparked considerable interest in scientific and medical communities and pressure from frustrated parents has also invigorated research and its funding. Between the period of 1980 and 2000, the number of publications on autism (registered by MEDLINE) quadrupled and the trend is continuing. In 1997, the NIH started a 5-year US$42 million network of collaborative programs on autism. In 2001, the M.I.N.D. institute started the building of a 13,000 square meter state of the art clinic and research center at UC Davis that is solely devoted to the study of autism. In the same year, a large congressional (United States) caucus was formed for autism. Clearly, both governmental agencies and research communities have started to make significant efforts to address the problems associated with ASD.

The question whether the epidemic status of ASD is due to true increase of incidence of the disease or simply its better detection and diagnosis is debated. Nevertheless, according to a most recent report to the legislature on the principal findings from the epidemiology of autism in California, the M.I.N.D. institute has confirmed that the increase of incidence is real and cannot be attributed to changes in diagnostic criteria or misclassification [88]. Autism was estimated to have a frequency of more than 1 in 500 children [31], while more recent studies found its prevalence as high as 1 in 150 (for examples, see Ref. [84]; also see website http://www.cdc.gov/ncbddd/dd/aic/about/default.htm). Researchers, private (e.g., Alliance for Autism Research), and government (e.g., National Institutes of Health, USA) agencies have recognized the enormous need. As a result, funding for research has significantly increased. Surprisingly, however, autism is still not among the neurological or neuropsychiatric diseases onto which large pharmaceutical research companies traditionally focus. This is unfortunate as ASD represents a significant unmet medical need with an enormous market size (Fig. 1). Consider the following: ASD may be diagnosed as early as 2–3 years of age [78]. Some even argue that successful diagnosis may be
made at 8–12 months (http://www.nichd.nih.gov/autism/CPEAupdate.htm). Autistic persons can live a normal life span [14]. The market size can thus be calculated as follows:

\[ P_Y = P \times Y \]

where \( P_Y \) is the number of “patient years,” \( P \) is the number of patients and \( Y \) is the number of years for which patients live after diagnosis. Calculating with the conservative prevalence estimate of 1 in 500, there may be approximately 600,000 ASD patients in the USA alone. These persons may live for an average of 76 years. Using the conservative age span \( \text{Y} \) of 3 years for the time of diagnosis, \( P_Y \) may be calculated as follows: \( P_Y = 600,000 \times 73 = 43,800,000 \), i.e., almost 44 million patient years. To put this number into perspective, let us consider Alzheimer’s disease, a disorder that is considered to represent the largest market by big pharmaceutical research companies. Calculating with \( P = 9 \) million (say 15% of people above 65 years of age will have Alzheimer’s disease in the United States) and \( Y = 6 \) (Alzheimer’s disease patients live, on average, for 6 years after first diagnosis), \( P_Y = 54 \) million for Alzheimer’s disease. The actual numbers may slightly vary. The number of Alzheimer’s disease patients is actually smaller than 9 million but the disease may be diagnosed earlier and patients may live longer than for 6 years after diagnosis. On the other hand, the number of ASD patients can easily be twice or even three times higher than the presently estimated 600 thousand. Thus, it is clear ASD represents an unmet medical need that is comparable in order of magnitude to the largest neurological disease market, that of Alzheimer’s disease. Thus, ASD should be of major interest to pharmaceutical research companies even when the 17-year patent expiration rule is considered.

4. Mechanisms of the disease: a lot of candidates and a lot of unknowns!

Despite the concerted efforts, autism has remained an elusive target. The mechanism of the disease is still not understood. Family studies, however, are offering some hope. They have shown that the concordance rate in monozygotic twins is approximately 65%, whereas it is 0% in dizygotic twins [6]. Furthermore, the prevalence of autism is 100 times higher in families in which at least one autism case has been identified compared to the rate in the general population [79]. These quantitative genetic results have been taken as evidence that there may be only a few major genes that underlie this disease, perhaps as few as 4 or 5 but probably not more than 20 [84]. If indeed there are only a few major genes involved, there is hope that once they are identified, the molecular and neurobiological mechanisms of autism may be understood. It is also notable, however, that the concordance rate for monozygotic twins is significantly less than 100%, which implies that, although the disease is highly heritable, the effect of environmental factors is not negligible.

Numerous candidate loci or chromosomal regions carrying such loci have been identified (for a recent review of these genes, see Ref. [2], also see Table 1 for summary). Genes on the long arm of chromosome 7, e.g., on the 7q31 region, have been suggested on the basis of linkage studies. Among them, perhaps the most interesting ones are the SPCH1 locus (because it is known to be involved in an autosomal dominant language disorder; Ref. [32]), the CAGH44 gene (whose mutation was also found in a patient with speech and language disorder; Ref. [52]), the 5HT2A locus (because this gene encodes a serotonin receptor protein and the serotonin system has been implicated in autism; Refs. [10,18,62,88]), the Wnt2 gene (because it has been implicated in a genetic mouse model, Dvl1 null mutant, showing signs of social aberration; Ref. [57]), and the reelin locus (reelin protein has been shown to be reduced in autistic patients; Refs. [28,70]). Chromosomal abnormalities involving the 15q11-13 region and a segment of 13q have also been implicated in autism (for a review, see Ref. [2]). Among the numerous candidate genes, perhaps GABA_A receptor genes, e.g., \( \beta 3 \), may be worth mentioning as the protein products of these genes are known to be involved in inhibitory neurotransmission and can thus influence both developmental and functional plasticity of the brain.

In addition to linkage based results, single gene mutations have also been identified in patients with autism. The rare dominant genetic disease, Tuberous Sclerosis Complex (TSC) is due to mutations of two functionally related genes, TSC1 and TSC2 [16,66]. The rate of autism in TSC patients is very high (17–68%), but the rate of TSC patients among autistic persons is only 3%, indicating that TSC is not the main underlying mechanism in autism. Nevertheless, similarly to the situation in Alzheimer’s disease (e.g., the
discovery of mutations in the amyloid precursor protein gene), a relatively rare genetic alteration can still be a valuable tool with which molecular mechanisms of a disease may be untangled. Fragile X syndrome has also been implicated in autism as some fragile X patients exhibit symptoms compatible with the diagnostic criteria of autism [29]. Fragile X syndrome is due to the expansion of a polymorphic CGG repeat upstream of the coding region of the FMR1 gene, and the expansion blocks the expression of the gene product due to methylation of the FMR1 promoter [90]. As in TSC, fragile X may not represent the core mechanism of the disease, but its analysis may illuminate some possible mechanisms that contribute to autism.

It is also important to acknowledge the fact that autism is four to five times more prevalent among males [25]. Although the mechanism of this gender bias is not known, it is possible that hormonal differences leading to gender specific brain development and/or the protective effects of having an extra X chromosome in females may be the cause.

Two main neurotransmitter systems have also been implicated in autism, the serotonergic system and the glutamatergic system. Increased blood [18] and urine [39] serotonin levels have been detected in autistic patients; and paradoxically, serotonin reuptake inhibitors, which are thought to increase serotonergic tone, have been found to ameliorate some symptoms of autism [41]. Linkage and association studies, however, provided inconsistent results with regard to the role of serotonin receptors or serotonin transporters in autism (for reviews, see Refs. [2,89]). The second neurotransmitter system implicated in autism is the glutamate system [15]. Based on anatomic and imaging studies that indicate alterations in brain regions rich in glutamate-containing neurons in autistic individuals and on symptoms elicited in normal subjects by glutamate antagonists, it has been argued [15] that autism is a “hypoglutamatergic” disorder. To counteract the possibly reduced glutamatergic “tone” in autistic patients, clinical trials are ongoing with AMPAkines, small molecules that potentiate the effect of glutamate on AMPA-R, a ligand gated Ca\(^{2+}\) channel. Although plausible, the above hypotheses were questioned by the result of a recent study that investigated the density and distribution of hippocampal neurotransmitter receptors in autism [11]. The authors used autoradiographic techniques and surprisingly found no alterations in the serotoninergic, cholinergic, and glutamatergic neurotransmitter systems. However, they did detect significantly reduced binding of \(^{3}H\)-Flunitrazepam and \(^{3}H\)-Muscimol, indicating reduction of benzodiazepine binding sites and GABA\(_A\) receptor density in the hippocampus of autistic patients.

Numerous other mechanisms have also been suggested, but again their validity or importance in autism will need to be ascertained in the future. For example, a point mutation leading to adenosylsuccinate lyase (ADSL) deficiency was observed in a single Moroccan family whose members had mental retardation and autistic features [86]. Adenosine deaminase (ADA) allele 2 was found more than twice as frequently in autistic patients as in a control population in Italy [21,71]. ADA activity deficiency is known to lead to severe combined immunodeficiency (SCID). However, the frequency of the ADA\(_A\) allele was not increased in autistic patients of Caucasian North American origin (for a review, see Ref. [2]) suggesting that the association found in the Italian studies may be due to random founder effect or genetic drift. Other candidate molecules implicated in autism are oxytocin and vasopression, neuropeptides known to regulate social behavior [43]. For example, blood levels of oxytocin were found reduced in autistic patients [37,64]. Levels of the Brain Derived Neurotrophic Factor (BDNF) were also found different (elevated) in children with autism (in Ref. [84]) implying that perhaps analysis of abnormal
gene expression patterns using microarray technology or differential display would be a useful approach in autism research [77]. Finally, weak association between genetic markers of the locus of the engrailed 2 (En2) gene and autism was found [72], an intriguing result because En2 is known to regulate cerebellar development [44,63] and abnormal cerebellar structure has been reported for autistic patients [80].

Although the evidence for genetic factors in ASD is overwhelming, it is clear that the environment also contributes to the disease. As cited previously, the probability of one of a monozygotic twin pair to have autism is 65% if the other one has autism too [6]. This implies that a large proportion of variability (35%) is due to environmental factors. Thus, considerable amount of effort has been devoted to identifying environmental causes or risk factors that may be involved in ASD. Among these are injection of vaccines [22], exposure to toxins and infections [40], immunologic (autoimmune) problems [51], and metabolic problems [46,48]. Many of these factors are hotly debated by both scientists and the public [65], and the final word will have to wait until rigorous controlled studies are conducted. The reader is referred to the above-cited reviews for further discussion of these important topics. It must also be noted that although ASD is highly heritable, this does not imply that environmental intervention may not be employed to ameliorate symptoms of the disease. Genetic influence should not be equated with genetic determinism. Although evidence is mounting suggesting that autism is associated with abnormal development of brain structures leading to miswiring in the brain, given the fact that the brain is highly plastic and is expected to be able to make new synaptic connections and/or alter the strength of such connections throughout the entire life, the role of stimulation, practice, and behavioral training [38,60,83] in the treatment of autistic persons cannot be underestimated.

Anatomical and brain imaging studies have revealed numerous structural and functional alterations in the autistic brain. For example, significant neuronal abnormalities were found in the Purkinje cells of the cerebellum and limbic structures and paralimbic cortices [8]. Decreased neuronal size, increased packing density, and decreased complexity of dendritic arborization in the hippocampus and amygdala of autistic patients were also demonstrated [8], suggesting a developmental curtailment of the maturation of the brain. The volume of the amygdala and the hippocampus was found significantly reduced in adolescent boys with autism but without mental retardation compared to age-matched controls, and cerebellar hypoplasia was also reported [19,30,55,73]. Furthermore, a consistent finding has been increased cerebral volume in autistic patients, particularly affecting posterior but not frontal structures [5]. Increased volume of the basal ganglia was also reported in high-functioning autistic patients as compared to control persons using MRI [81]. Clearly, numerous developmental alterations have been detected but the question of how consistent and typical they are in autism and whether these alterations vary with the severity or particular composition of autistic symptoms will need to be addressed in the future.

Finally, functional neuroimaging techniques, including positron emission tomography (PET), single photon emission computed tomography (SPECT), and functional MRI (fMRI), have also contributed to the study of pathological brain functions in autism. These neuroimaging approaches have shown an abnormal pattern of cortical activation in autistic patients suggesting that different connections between particular cortical regions could exist in autism [13]. Perhaps the most intriguing among these findings are the results that show how autistic patients process socially relevant information: while normal subjects activated specialized brain areas, including the medial frontal and medial temporal cortices, people with autism relied on general association cortices (for a review, see Ref. [87]). Schultz et al. [82], using fMRI, showed that people with autism or Asperger syndrome activated the same areas of their brain, the temporal gyr, when shown faces or objects; however, normal subjects used their fusiform gyrus specifically only when shown human faces but not when shown objects. In the same vein, Baron-Cohen et al. [7] found that nonautistic adults showed increased activation in the amygdala, an area regarded to be the emotional center of the brain [20], when asked to judge the expression in the eye of a human face, whereas people with autism or Asperger syndrome did not exhibit such activation. These results are intriguing because they are consistent with the frequent observation that autistic people have difficulty understanding someone’s emotions and they treat humans as inanimate objects (for a review, see Ref. [87]).

5. Animal models of autism: limited successes

Although research is progressing, the above discussions demonstrate that the molecular and neurobiological mechanisms of ASD are still not clear. Creating an animal model without knowing mechanistic details about the disease is problematic especially if one considers that it is also unclear how “autism” should manifest in a rat or a mouse. Research, however, is usually an iterative process, and it is hoped that parallel preclinical (animal) and clinical (human) studies will strengthen each other and eventually will lead us towards the right answer.

Numerous attempts exist to model ASD in animals. Genetic models are as follows (see Table 2 for summary). The Eker rat [49] exhibits phenotypical abnormalities that could be rescued by transgenic overexpression of the TSC2 gene. The TSC2 transgenic mouse was generated in which a mutated TSC2 gene was introduced [68]. Unfortunately, however, no behavioral abnormalities were described in either model [16]. Fragile X syndrome was also modeled in mouse by silencing the FMR1 gene using gene targeting [50]. Although some functional protein may be expressed...
from the mutated gene, these mice exhibited some pheno-
typical characteristics similar to autistic humans, e.g., mac-
roorchidism and subtle learning deficits [23,50,67,69], as
well as abnormal dendritic spines of pyramidal neurons
suggesting maturation and pruning deficits [17]. Social
behavior has not been tested in these mice. A mouse
 carrying a null mutation at the En2 locus has been generated
[44] and these mice were found to exhibit subtle cerebellar
abnormalities at the structural level. Behavioral analysis of
these mice was focused on their motor function and a mild
motor learning deficit has been described [36]. Learning or
social behavior of these mice was not analyzed. Social
behavior was found altered, however, in a genetic rat model.
Spontaneous vasopressin deficiency has been found in a rat
strain, the Brattleboro rats, and these rats were found to
exhibit reduced social memory and other learning-related
deficits [26]. Reelin is a signaling glycoprotein that plays a pivotal part in the
migration of neuronal cells and in the establishment of
neuronal connections during brain development. The reeler
mouse exhibits cytoarchitectural abnormalities reminiscent
of those seen in autistic patients [72] and the reelin gene
maps to the region of 7q implicated in autism. Significant
linkage/association has been found between a polymorphic
triplet repeat located near the reelin gene and autism [72].

The utility of the reelin mutant for the analysis of pheno-
typical traits including learning and social interaction,
however, is hampered by the fact that these mice exhibit
severe ataxia, a reeling gait. Nevertheless, they may be
useful for investigating the effects of experimental, e.g.,
pharmacological or genetic, manipulations that may interact
with molecular mechanisms involved in the reelin pathway.

The last genetic model to be mentioned is the GS guinea
pig. These animals were found to exhibit abnormal explor-
atory behavior and sleep pattern, and they also possessed a
structural abnormality in their brain, hypopholiation of the
vermis and reduction of the number of granule and Purkinje
cells in their cerebellum [56] that were reminiscent of
abnormalities seen in autistic patients. Unfortunately, this
strain is not available any more due to reproductive prob-
lems of these animals (reviewed in Ref. [2]).

Table 2
Examples of genetic animal models of ASD

<table>
<thead>
<tr>
<th>Model</th>
<th>Molecular target</th>
<th>Phenotypical alteration</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eker rat</td>
<td>TSC2</td>
<td>Subtle learning deficits</td>
<td>[49]</td>
</tr>
<tr>
<td>TSC2 transgenic mouse</td>
<td>TSC2</td>
<td>Subtle cerebellar abnormalities, motor learning impairment</td>
<td>[68]</td>
</tr>
<tr>
<td>Fragile X mouse (KO)</td>
<td>FMR1 mutation</td>
<td>Subtle learning deficits</td>
<td>[23,50,67,69]</td>
</tr>
<tr>
<td>En2 KO mouse</td>
<td>En2</td>
<td>Subtle cerebellar abnormalities, motor learning impairment</td>
<td>[36,44]</td>
</tr>
<tr>
<td>Vasopressin deficient rat</td>
<td>Vasopressin</td>
<td>Reduced social memory and other learning deficits</td>
<td>[26]</td>
</tr>
<tr>
<td>Dvl1 KO mouse</td>
<td>Dvl1 and Wnt signaling pathway</td>
<td>Impaired social behavior and sensorimotor gating</td>
<td>[57]</td>
</tr>
<tr>
<td>Reelin mutant mouse</td>
<td>Reelin</td>
<td>Cytoarchitectural abnormalities similar to those of ASD, but severe ataxia</td>
<td>[27]</td>
</tr>
<tr>
<td>Oxytocin KO mouse</td>
<td>Oxytocin</td>
<td>Deficits in social behavior</td>
<td>[93]</td>
</tr>
<tr>
<td>GS guinea pig</td>
<td>Unknown</td>
<td>Abnormal exploratory behavior, sleep, hypopholiation of vermis, reduced number of cerebellar Purkinje cells</td>
<td>[56], also see Ref. [2] for a review</td>
</tr>
</tbody>
</table>

Table 3
Examples of nongenetic animal models of ASD

<table>
<thead>
<tr>
<th>Model</th>
<th>Manipulation</th>
<th>Phenotypical alteration</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bachevalier’s rhesus monkey</td>
<td>Bilateral amygdala lesion</td>
<td>Abnormal social behavior (only transient)</td>
<td>[4]</td>
</tr>
<tr>
<td>Rat amygdala model</td>
<td>Amygdala lesions</td>
<td>Abnormal social behavior</td>
<td>[94]</td>
</tr>
<tr>
<td>Rat cerebellar model</td>
<td>Lesion of vermis</td>
<td>Hyperactivity, increased perseveration, reduced attention</td>
<td>[12]</td>
</tr>
<tr>
<td>Valproic acid toxicity in rat</td>
<td>Embryonic exposure to valproate</td>
<td>Structural abnormalities in vermis</td>
<td>[42]</td>
</tr>
<tr>
<td>Borna virus rat model</td>
<td>Viral infection of newborn rat</td>
<td>Cerebellar abnormalities, reduced play behavior</td>
<td>[74]</td>
</tr>
</tbody>
</table>
Numerous nongenetic models also exist (for a summary, see Table 3) to mimic autism in animals. Again, their relevance to autism is debated. Given the structural and functional imaging results suggesting the role of amygdala in autism, it was hoped that disruption of this structure in animals will give rise to autism-like symptoms [3]. Bachevalier [4] induced bilateral amygdala lesions in newborn rhesus monkeys and observed abnormal social interactions, changes in body and facial expressions, and development of stereotypical behaviors. However, by the time the monkeys reached adulthood, the abnormalities disappeared diminishing the construct and predictive validity of the model. Amygdala lesions were also generated in rats and these manipulations have led to changes indicating potential alteration in social behaviors particularly in the group lesioned before puberty, i.e., at Day 7 after birth [94]. In order to model cerebellar abnormalities, Bobné et al. [12] lesioned midline structures (vermis) of the cerebellum of rats and investigated the behavioral effect of this manipulation. They found that lesioning at the age of postnatal day 10 led to hyperactivity, increased perseveration, and decreased attention. The authors argued that these features are autistic-like and that their model strengthens the argument that cerebellar developmental abnormalities play important roles in autism.

Finally, approaches that mimic toxin or infection-induced abnormalities must also be mentioned. In a population study, Strömeland et al. [85] observed that exposure to the toxin thalidomide at the time of neural tube formation during early pregnancy (20th–24th day) led to an incidence of autism of 5 out of 15 cases, an enormous increase compared to the usual incidence (1 in 500) seen in the population. In order to mimic the effects of this toxin, Ingram et al. [42] developed a rat model in which rat embryos were exposed to valproic acid, a teratogen, at prenatal days 11.5–12.5. The authors observed abnormalities in motor nuclei and in the vermis and argued that the structural changes resembled those seen in autism. In another model, cerebellar abnormalities were achieved by inoculating newborn rats with Borna virus [74]. The infection led to loss of cerebellar neurons and reduced play behavior in the rats, phenotypical alterations that resemble autistic features.

6. Pharmacotherapies for autism: do they help?

Briefly, numerous drugs have been tried and some were found to alleviate certain symptoms of autism. However, these drugs represent only palliative treatment as they affect only the secondary symptoms, e.g., aggression and anxiety. The core problems associated with abnormal communication and social behaviors remain unaffected. Prior to appropriate diagnostic criteria for ASD, antipsychotic drugs were widely used in autistic patients. Haloperidol, pimozide, and fluphenazine have been reported to have some ameliorative effects (for a review, see Ref. [41]), but these drugs are currently avoided because of their significant side effects. Clozapine and olanzapine have also been tried with limited or no success (reviewed in Ref. [41]). Risperidone has been investigated the most in recent studies and it has been found to treat moderate to severe behavior problems that are associated with autism [61]. Risperidone is similar to clozapine in that it has a similarly complex pharmacological profile. It is chemically unrelated to any other currently available antipsychotic drugs and is a potent 5HT2A, 5HT7, α1-, α2-adrenergic, and histamine H1 antagonist. It also acts as a weak antagonist at the D2 dopamine receptor. Risperidone treatment (reviewed in Ref. [41]; also see Ref. [61]) has been reported to improve sensorimotor functioning, effectual reactions, reduce repetitive behaviors, irritability and aggression, and improve the overall behavioral symptoms score. Impairments in social interaction and use of language remained unaltered in response to risperidone treatment. Buspirone, a partial agonist at the 5HT1A auto-receptor, has also been reported to reduce aggression, hyperactivity, and repetitive behaviors (for a review, see Ref. [41]). Naltrexone, an opioid antagonist, has been theorized to be beneficial [41] because it may reduce the effect of endogenous opioids and thus lead to reduction of self-absorbed “introvert” attitude. Clinical trials, however, have shown no or only very mild effect of this drug.

Based on observations suggesting hyperserotonemia, it may seem reasonable that drugs enhancing synaptic levels of 5HT, such as selective serotonin reuptake inhibitors (SSRI’s), would worsen autistic symptoms. However, fluoxetine (trade name Prozac), an SSRI that has been widely prescribed for depression indication, was found to ameliorate symptoms including repetitive, compulsive, and aggressive behaviors. Children on Prozac also tended to sleep better, make better eye contact, and retain more flexibility in their behavior (reviewed in Ref. [41]).

Secretin is a newly proposed treatment that has been in the spotlight of the lay public, the popular media, and the scientific community [46]. Secretins belong to a family of enterohormones that are involved in both the function of the gastrointestinal tract and the central nervous system [46]. Secretin receptors have been found in the cerebellum, and a secretin-like polypeptide has been found in the brain. Furthermore, secretin has been reported to lower plasma 5HT levels. Started from a chance observation, secretin has now been the subject of double-blind placebo-controlled studies. But unfortunately, these trials have not shown efficacy compared to placebo (reviewed in Ref. [75]). In summary, despite the large number of drugs tried, presently, drug therapy is only palliative at best.

7. Future directions

A large number of investigators, clinicians, and animal researchers are trying to solve the conundrum of autism.
Each may have a specific angle in their approach and each may view the priorities slightly differently. The list of directions we see as most important also represents our own view and biases and should not be regarded as absolute.

Given the heterogeneity of the manifestation and of the mechanisms of ASD, further development of detailed diagnostic criteria and testing/evaluation methods that will allow classification of ASD phenotypes and clustering of symptomatic sets will help both therapists and basic research scientists [9]. The former may be able to better tailor behavioral therapies according to the particular need of the patient, and the latter may be able to identify molecular and neurological mechanisms potentially idiosyncratic to particular subtypes of ASD. Good identification of autistic features in the population may also facilitate drug development, as the need for such drugs in milder forms of ASD will be better appreciated. It is also noteworthy that some of the features of ASD may be observed in other CNS disorders including schizophrenia, nonverbal learning disorders, communication disorders, and attention-deficit hyperactivity disorders [25]. Thus, drugs developed for ASD may ameliorate symptoms associated with these diseases and help a larger number of patients than what is estimated only based on the prevalence of ASD, an important consideration for market analysis at pharmaceutical research companies.

Given the fact that a large proportion of ASD cases are of heritable type, it should be possible to identify genetic markers that could be tested early. Early diagnostic markers would aid the development and implementation of intervention strategies. Early intervention is considered crucial as the best results have been achieved when the behavioral modification or training is started at the youngest possible age [60].

Linkage and association studies have facilitated the discovery of certain chromosomal regions within which may reside loci of genes involved in the disease, but some of the findings are controversial or not replicable. This may be due to numerous factors, one of them being sampling error. To avoid irreproducibility due to error variation, larger number of subjects will need to be included in future analyses. A large scale and properly randomized linkage or association analysis may also have the potential to identify populations or subgroups in which particular molecular mechanisms underlying ASD differ. Lastly, it is important to realize the limitations inherent in the way scientists are rewarded. Most often, publication of negative results, i.e., results showing no effect or finding no quantitative trait loci, is discouraged in scientific journals. Thus, what one can find in the literature may not contain all pieces of relevant information and the literature may represent a distorted view. To see clearly, it is advisable to publish all results, both positive and negative.

Detailed gene expression profiling using reverse transcriptase-based polymerase chain reaction (RT-PCR) microarray technology or variants of differential display will allow identification of genes that are differentially expressed in ASD [77]. These approaches may reveal novel molecular targets irrespective of whether a genetic marker close to the novel gene is known or not. Similar in principle to gene expression analysis is the analysis of expressed proteins, i.e., proteomics. It has also been suggested as a novel technology in the analysis of the molecular mechanisms of ASD [45].

Development of animal models continues to be an important priority for researchers. Presently available animal models have numerous promising features. However, some of the animal models are not yet fully characterized, or they mimic characteristics of ASD only partially. Clearly, generating proper animal models is very difficult but will be facilitated by human genetic studies. It is also noteworthy that although numerous behavioral tests have been used in the analysis of animal models of autism, no test battery has been developed that would specifically and consistently measure the main aspects of autistic-like features in animals. Focusing these batteries on intraspecific social behaviors, including social interaction, communication, and social learning while keeping the ethological characteristics of the species in mind [35], will be a step forward. Development of such a battery will aid the proper characterization of presently available animal models and will also help the generation of future ones. The question of test batteries, the need for standardization, and the problems associated with such batteries have been extensively discussed in recent articles suggesting that the difficulties are not specific for autism research (see review in Ref. [34]).

Research has been on-going in all of the above areas and more. Given the strong public pressure and the appreciation of the importance of finding effective treatment by academic and governmental agencies and scientists alike, it is hoped that the first decade of the 21st century will bring a breakthrough in autism research.

Acknowledgements

This paper is based on an oral presentation given by RG at the Satellite Symposium “The Triune Brain, a tribute to Paul MacLean: the neurobiological relevance of social behavior” of the International Behavioral Neuroscience Society in 2002, Capri, Italy. We would like to thank Dr. Kelly G. Lambert, organizer of the symposium, for the opportunity to present. We would also like to thank Leah Helvering (Indianapolis), our friend and colleague, for her help and comments on this paper.

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