Can a mouse model system be useful in elucidating the biology of autism?

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Heterogeneity of autism

• Etiology (cause)

• Pathophysiology
Autism is **not** this clearly defined

Mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene on chromosome 7

↓

Cystic fibrosis

↓

Cough, shortness of breath, alteration of breath sounds
Autism is currently defined by a cluster of symptoms—no diagnostic test available

To continue the pulmonary analogy:

• **Cough, shortness of breath, alteration in breath sounds**

  ↓

• Genetic syndromes (cystic fibrosis), infection (bacterial, viral, mycoplasma, fungal, tuberculosis), cancer, reactive airway disease (asthma), congestive heart failure, pulmonary embolus, exposure to fumes...
Heterogeneity of the autisms

Taken from Geschwind and Levitt 2007 Current Opinion in Neurobiology 17:103
Autism Spectrum Disorders: Definitions

• Neurodevelopmental disorders

• The defining features of autism spectrum disorders are impairment in the following:
  – Social interaction
  – Language, communication, and imaginative play
    • Language is less affected in Asperger syndrome
  – Restricted and repetitive patterns of interest or behavior
Brief History of Autism

• 1943 Leo Kanner, American psychiatrist.

• 1944 Hans Asperger, Austrian pediatrician

• 1967 Bruno Bettelheim “refrigerator mother” hypothesis—now discredited
  – Autism due to the impact of a threatening and unloving parent

• Growing evidence that autism is a set of neurodevelopmental disorders that are highly heritable
Pervasive Developmental Disorders (DSM-IV)

- **Autistic disorder** (Kanner autism, 1943)
  - Impaired social interaction, impaired communication, restricted/repetitive patterns of behavior and interests

- **Asperger syndrome** (Hans Asperger, 1944)
  - Impaired social interaction and restricted/repetitive/stereotyped behaviors and interests, but less difficulty with verbal communication (normal IQ and no clinically significant delay in language development)

- **Rett syndrome**
  - Only in girls, apparently normal development for the first 5 months of life, then deceleration of head growth, loss of previously acquired purposeful hand skills, development of stereotyped hand movements, decreased social interaction, mental retardation

- **Childhood disintegrative disorder**
  - Normal development in first 2 years of life, including normal language development, followed sometime between the ages of 2 and 10, by loss of skills in language, social behavior, bowel or bladder control, play, or motor skills

- **PDD not otherwise specified**
Symptom domain #1: Social Interaction

• Social interaction symptoms (early appearing, often starting as early as age 1-2 years)
  – Lack of attention to faces
  – Reduced eye contact
  – Deficits in joint attention
  – Reduction in social imitation or other social play
  – Lack of social or emotional reciprocity
  – Lack of spontaneous seeking to share enjoyment, interests (lack of showing, bringing, or pointing out objects of interest)
  – Difficulties in perceiving, responding appropriately to, and expressing emotions

• Problems in nonverbal social communication (later appearing)
  – Difficulty in perceiving and using body posture, gesture, eye contact, and facial expressions in nonverbal social communication
  – Failure to develop peer relationships appropriate to developmental level

• Social cognition symptoms (later appearing)
  – Deficits in “theory of mind”
  – Impaired ability to interpret facial expressions
  – Reduced facial recognition
Symptom Domain #2: Language, communication, and imaginative play

- Absence of language OR delayed language development
  - not accompanied by an attempt to compensate through alternative methods of communication, such as gesture

- Stereotyped and repetitive use of language or idiosyncratic language

- Lack of varied, spontaneous imaginative social play appropriate to developmental level in children

- In individuals with adequate speech (e.g. Asperger syndrome or high-functioning autism), impairment in the ability to initiate or sustain a conversation with others

- In individuals with adequate speech, deficits in prosody (tone of voice), “pragmatics” of language (tailoring language to social demands)
Symptom Domain #3: Restricted and Repetitive Behavior

- Stereotyped or repetitive motor mannerisms
  - Rocking, spinning, hand or finger flapping or twisting

- Restricted interests
  - Encompassing preoccupation with restricted interests that is abnormal in intensity or focus (e.g. train schedules)

- Inflexible (rigid) adherence to specific, nonfunctional routines or rituals

- Preoccupation with parts of objects
Associated Behavioral Phenotypes

- **Mental retardation**
  - Seen in up to 75% of patients with classical autism, or in 25-40% of individuals with autism spectrum disorders
  - By definition, no mental retardation in Asperger syndrome

- **Other more subtle cognitive features**
  - Executive dysfunction
    - Rigidity, perseveration, getting "stuck" in a particular task, deficits in planning
  - Central coherence (Happe et al)
    - Focus on parts rather than the whole
  - Complex information processing (Minshew et al)
    - Difficulties with complex sensory, motor, memory, language and concept formation

- **Seizures**
  - In up to 30% of patients with autism spectrum disorders
  - Bimodal peaks of onset of seizures in both early childhood and adolescence

- **Anxiety symptoms, depression**

- **Obsessive-compulsive symptoms**

- **Tantrums/aggressive behaviors and self-injurious behaviors**

- **Attention deficit and hyperactivity symptoms**

- **Motor incoordination**

- **Savant skills (10% of individuals with autism spectrum disorders)—outstanding ability in one narrow area, e.g. calendar calculation, musical or artistic ability**

- **Sensory sensitivities—to sound, sight, smell, taste or touch**
Associated Brain Phenotypes

• Abnormal acceleration in growth of brain in first few years of life. Larger, heavier brains, including forebrain (10% larger) in toddlerhood.

• Overgrowth of white matter, but underdeveloped interconnectivity in the brain (e.g. underdevelopment of corpus callosum)

• Reduced number of cerebellar Purkinje cells

• Small and densely packed neurons in limbic regions, including amygdala, hippocampus, and entorhinal cortex

• Decreased serotonin synthesis left frontal cortex and thalamus in childhood

• High blood serotonin
  – 25% of patients
  – 30-50% increase in platelet serotonin levels

• Decreased activation of fusiform gyrus ("face area") during facial recognition tasks
Prevalence and Male:Female Ratio of Autism Spectrum Disorders

- **Classical Autistic Disorder (narrow definition)**
  - Prevalence 0.1-0.2 %
  - Male:Female ratio 4:1

- **Autism Spectrum Disorders (broad definition)**
  - 1 in 150 children
  - Male:Female ratio is even higher (Male:Female ratio for Asperger syndrome is 8:1)

- **Is the incidence/prevalence increasing?**
  - Due to changes in diagnostic criteria and heightened awareness??
  - Due to true rising incidence of autism??
Concordance rates suggest high heritability and many susceptibility genes

- **Narrow phenotypic definition (classical autism)**
  - 60% concordance for monozygotic twins
  - 0% concordance for dizygotic twins

- **Broad phenotypic definition (including Asperger syndrome and other communication and social disorders)**
  - 92% concordance for monozygotic twins
  - 10% concordance in dizygotic twins

- **~5% sibling** (non-twin) recurrence rate

- Other family members have relatively high rates of social and communication difficulties and obsessive-compulsive traits ("broader autism phenotype")
Etiology of autism spectrum disorders

- **Very rare** causes of autism-like phenotype: particular environmental insults
  - Prenatal infections (e.g. rubella, cytomegalovirus, valproate)

- **Minority of cases** of autism (<10% of cases)—known etiology, relatively “simple” genetics
  - Single gene syndromes (e.g. Fragile X syndrome, Rett syndrome)
  - Chromosomal Translocations
  - Chromosomal region duplications and triplications (15q11-13 duplications)

- ** Majority of cases** of autism—unknown etiology, highly heritable, complex genetics, multiple genes are involved
  - Linkage studies
  - Association/Candidate gene studies
Why focus on social behavior phenotypes?

- Social behavior disruptions are arguably the core symptom cluster of autism
- Social behavior deficits are most specific for autism
- Social behavior deficits may have a somewhat distinct underlying genetics from the other two symptom domains…
Autism and Low Sociability

- Sociability = tendency to seek social interaction

- In autism, low sociability by age 1 or 2
  - Lack of attention to faces
  - Reduced eye contact
  - Not orienting when one’s name is called
  - Deficits in joint attention
  - Reduction in social imitation or other social play
  - Lack of social or emotional reciprocity
  - Lack of spontaneous seeking to share enjoyment

- Why?
  - Is social interaction less rewarding and/or more aversive?
  - Secondary to some attentional or cognitive deficits?
  - What is the underlying biology of the reduced sociability
Who needs an animal model??

• Animal models--a crucial component of biomedical research because of the experimental control that they afford
  
  – Ability to set up controlled breeding—very useful for identifying genes that affect brain and behavior
  
  – Availability of inbred strains—very useful for identifying genes
  
  – Ability to experimentally test effect of environmental factors and gene-environment interactions on brain development and behavior
  
  – Ease of studying brain and behavior across development, from prenatal period through adulthood
  
  – Access to brain tissue with no “artifacts” (no other disease states, no medications, no delay in access to tissue)
Quantitative assay of sociability in mouse model systems

Sociability = tendency to seek social interaction

**Phase 1:**
Test mouse habituation

**Phase 2:**
Stimulus mouse in cylinder on social side

**Phase 3:**
Free interaction between test and stimulus mouse
6 Inbred Mouse Strains—Prepubescent Female Test Mice

Stimulus mouse was prepubescent DBA/2J female for all groups

From Brodkin et al 2004 Brain Research 1002:151-157
C57BL/6J vs. BALB/cJ mice—Differences in Sociability

From Sankoorikal et al 2006 *Biological Psychiatry* 59:415-423
Replication of C57BL/6J vs. BALB/cJ social behavior difference

From Fairless et al 2008 Brain Research 1230:211-217
Social reward among juvenile mice

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Mammalian social relationships, such as mother–offspring attachments and pair bonds, can directly affect reproductive output. However, conspecífics approach one another in a comparatively broad range of contexts, so conceivably there are motivations for social congregation other than those underlying reproduction, parental care or territoriality. Here, we show that reward mediated by social contact is a fundamental aspect of juvenile mouse sociality. Employing a novel social conditioned place preference (SCPP) procedure, we demonstrate that social proximity is rewarding for juvenile mice from three inbred strains (A/J, C57BL/6J and DBA/2J), while mice from a fourth strain (BALB/cJ) are much less responsive to social contact. Importantly, this strain-dependent difference was not related to phenotypic variability in exploratory behavior or contextual learning nor influenced by the genetic background associated with maternal care or social conditioning. Furthermore, the SCPP phenotype was expressed early in development (postnatal day 25) and did not require a specific sex composition within the conditioning group. Finally, SCPP responses resulted from an interaction between two specifiable processes: one component of the interaction facilitated approach toward environments that were associated with social salience, whereas a second component mediated avoidance of environmental cues that predicted social isolation. We have thus identified

A breadth of social behavior takes place outside of the highly specific contexts that underlie monogamous pair bonds, mother–infant attachments and territoriality. Approach toward a conspecific, also referred to as social approach, is perhaps the most basic behavioral component of all social interactions, thereby providing a means through which specific relationships between individuals can be established, maintained and strengthened. In some situations, it is conceivable that social approach would be associated with a specific functional outcome (e.g. reproduction during the mating season), while in others the initial motivation to approach a conspecific may be independent of a specific benefit. Under the latter conditions, the likelihood and extent of social approach may thus constrain social experiences that occur only once a certain degree of spatial or temporal proximity exists between individuals. A critical biological problem, then, entails determining whether there are mechanisms and motivations in animals that can support social approach across a variety of situations, irrespective of the ensuing social context.

Classical theories of motivated behavior underscore a role for reward and punishment in essentially all forms of behavioral approach and withdrawal (Glickman & Schiff 1967; Schneirel 1959; Young 1959). Although reward is not necessary for social behavior to occur, reward processes have nevertheless been shown in social contexts that include mating (Agmo & Gomez 1993; Drewett 1973), monogamous pair-bonding (Young & Wang 2004), aggression (Fish et al. 2005), maternal–infant attachment (Insel 2003; Lee et al. 1999) and rough-and-tumble play among juveniles (Calcagnotto & Schenker 1992; Ikemoto & Panksepp 1992). However, many
Summary

• BALB/cJ mice showed lower levels of sociability than C57BL/6J mice overall in all measures

• This difference in sociability was not due to baseline strain differences in locomotor activity, olfaction, or other simple motor or sensory deficits
Literature Review:

Low social interaction in BALB/c mice

• **Low social grooming between males / low male-male interactions**
  - (Southwick and Clark, 1968)
  - (Sankoorikal et al 2006)

• **Low female-female interactions**
  - (Brodkin et al 2004)

• **Low sociability of BALB/cByJ male mice**
  - (Crawley and colleagues, 2004)

• **Social barbering (fur/whisker trimming) is completely absent in BALB/c mice, both in male-male and female-male interactions**
  - (Kalueff et al 2006)

• **Low male sexual behavior in BALB/c mice**
  - (long latency to mount, fewer mounts, move away from females more quickly following copulation)
  - Not attributable to low testosterone levels

• **Low maternal behaviors in BALB/cJ mothers**
  - Lick and groom pups less, less maternal arched back postures, less time spent in nest with pups, longer latency to retrieve pups
Other phenotypes of BALB/c mice that may be relevant to autism

- Moderately high levels of aggressive behaviors (intermale aggression)
- Large brain size and brain weight
- Absence or severe underdevelopment of corpus callosum (40% penetrance)
- Reduced brain synthesis of serotonin
  - (Tph2 gene polymorphism 1473G in BALB/c causes an amino acid change and lower brain serotonin levels)
On average, BALB/cJ mice show relatively low levels of sociability, but there is substantial within strain variability.

Within the BALB/cJ strain, there is variable development of the corpus callosum (40% of BALB/cJ mice show underdevelopment).

Mechanism of corpus callosum underdevelopment:
- A subset of BALB/cJ mice show delayed formation of an interhemispheric tissue bridge during prenatal brain development.
- Axons that fail to cross the midline form aberrant, functional synapses on the ipsilateral side of the brain, and myelinate...i.e. abnormal overconnectivity.
Autism and Abnormal Development of Brain Connectivity

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Key words: attention; cerebellum; development; EEG; electroencephalogram; frontal; imaging; immunity; network; autism; genetics

Autism spectrum disorders: developmental disconnection syndromes
Daniel H Geschwind¹ and Pat Levitt²

Mapping Corpus Callosum Deficits in Autism: An Index of Aberrant Cortical Connectivity
Christine N. Vidal, Rob Nicolson, Timothy J. DeVito, Kiralee M. Hayashi, Jennifer A. Geaga, Dick J. Drost,
Peter C. Williamson, Nagalingam Rajakumar, Yihong Sui, Rebecca A. Dutton, Arthur W. Toga,
and Paul M. Thompson
Corpus callosum at mid-sagittal section

C57BL/6J

BALB/cJ
Low sociability associated with underdeveloped corpus callosum

Cylinder sniffing change scores vs corpus callosum index of abnormality

Spearman rank correlation P = 0.02
Validity of BALB/c mice as a model relevant to autism

• **Face validity** — phenotypic resemblance of the model to the human disease
  – Reduced sociability, large brain, underdeveloped corpus callosum, positive correlation between sociability and corpus callosum size (connectivity)

• **Construct validity/Etiological validity** — same underlying cause (e.g. genes)
  – Underlying genes are unknown in humans

• **Predictive validity** — expected responses to treatments that are effective in humans
  – Essentially no treatments available
Future Directions

- **Genetic studies of sociability**
  - Studies of the effects of knockout of autism candidate genes in the mouse
  - Studies of brain pathways that may affect sociability

- **BALB/cJ mice**
  - Studies to determine the relationship between low social interaction and other brain and behavioral phenotypes
  - Studies to identify gene variants (alleles) that affect these phenotypes
  - Pharmacologic studies of sociability
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