Coloboma mouse mutant as an animal model of hyperkinesis and attention deficit hyperactivity disorder

Michael C. Wilson*

Department of Neurosciences, University of New Mexico, Health Sciences Center, 915 Camino de Salud, Albuquerque, NM 87131-5223, USA

Abstract

Hyperkinesis and developmental behavioral deficiencies are cardinal signs of attention-deficit hyperactivity disorder. In mice, the mutation coloboma (*Cm*) corresponds to a contiguous gene defect that results in phenotypic abnormalities including spontaneous hyperactivity, head-bobbing, and ocular dysmorphology. In addition, coloboma mutant mice exhibit delays in achieving complex neonatal motor abilities and deficits in hippocampal physiology, which may contribute to learning deficiencies. The hyperkinesis is ameliorated by low doses of the psychostimulant D-amphetamine and can be rescued genetically by a transgene encoding SNAP-25, located within the *Cm* deletion. Together with syntaxin and synaptobrevin/VAMP, SNAP-25 constitutes a core protein complex integral to synaptic vesicle fusion and neurotransmitter release. Despite the ubiquitous role of SNAP-25 in synaptic transmission, and uniformly decreased expression in the mutants, coloboma mice show marked deficits in Ca^{2+}-dependent dopamine release selectively in dorsal but not ventral striatum. This suggests that haploinsufficiency of SNAP-25 reveals a specific vulnerability of the nigrostriatal pathway which regulates motor activity and may provide a model for impaired striatal input into executive functions encoded by the prefrontal cortex associated with ADHD. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Hyperkinesis; ADHD; Dopamine; SNAP-25; Coloboma; Striatum; Ampetamine; Mouse mutant; Genetic complementation; Pair-pulse inhibition; Nigra-striatal pathway

1. Introduction

Attention deficit hyperactivity disorder (ADHD) is a major pediatric neuropsychiatric disorder affecting about 5% of school-aged children [1]. The three major symptoms of inattention, excess impulsivity, and uncontrolled hyperactivity that define ADHD reflect a wide constellation of age-inappropriate behaviors which tend to cluster, leading to the assignment of two subtypes of ADHD, inattention and hyperactivity/impulsivity. While affected children may independently present either subtype, the combined phenotype of inattention, impulsivity and hyperactivity appears to be most common. The behavioral impairments associated with ADHD are typically exhibited early in childhood, before the age of seven. Although the severity of these deficits, particularly hyperactivity, generally wane at adolescence, elements of these impairments persist in 50–80% of adolescents and 30–50% of adults [2,3]. Considerable evidence from family studies, and twin and adoption studies has strongly suggested that ADHD is heritable (for review see [4]), although penetrance is clearly not complete.

Taken together these observations suggest that as other complex neuropsychiatric disorders such as schizophrenia, ADHD is a multifaceted, likely heterogeneous disorder that arises from a variety of genetic interactions contributed by different gene loci. The genetic loci with contribute to the variable impairments of ADHD are thus likely to represent a collection of susceptibility or modulatory genes that initially influence development and consequently impact on information processing in the mature brain.

Despite compelling evidence for genetic heritance ADHD, few animal models of ADHD have emerged. However, characteristics of ADHD drawn from patient populations can be used to help identify certain elements of the disorder that may be modeled in animals, in particular rodents. For example, the observation that indirect dopamine agonists, particularly methylphenidate and D-amphetamine, effectively improve both behavioral deficits of hyperactivity and in classroom performance, together with the altered levels of dopamine metabolites in ADHD affected individuals (for example, see [5]) provides support for the notion that dopaminergic pathways are particularly affected in ADHD. Morphometric studies based on magnetic resonance imaging have, in fact, provided additional evidence for the involvement of prefrontal

* Tel.: +1-505-272-8451; fax: +1-505-272-8082.
E-mail address: mwilson@salud.unm.edu (M.C. Wilson).

Contiguous Gene Deletions in Syntenic
Mouse/Human Chromosomal Regions
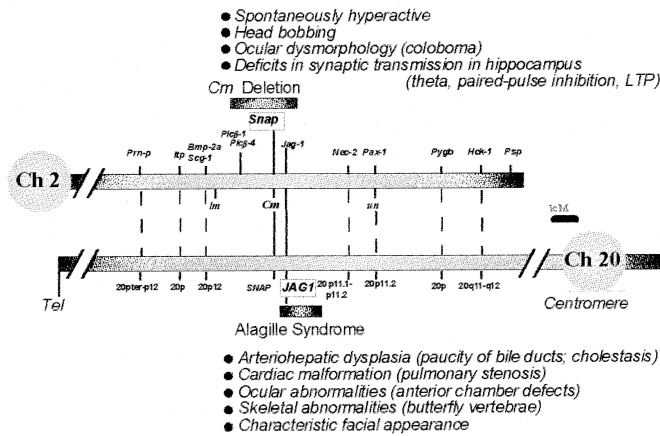
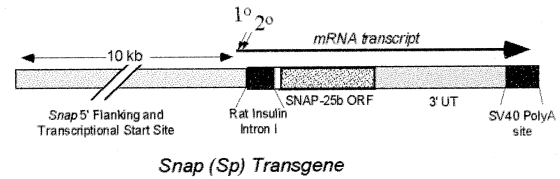Complementation of Coloboma Gene Defect
via SNAP-25 Mini-Transgene

Fig. 1. Left Panel. Genetic maps of syntenic regions of mouse Ch 2 and human Ch 20 depicting relative positions of the coloboma and Alagille's deletion loci, respectively. Positions of known genes are indicated with relative cytogenetic map positions for the human gene sequences. The major phenotypic abnormalities associated with each mutation are indicated. Whereas the hyperactivity produced by the coloboma mutation can be attributed to the gene Snap encoding SNAP-25, the critical gene for Alagille's is JAG1 (see text for references). Right Panel. A SNAP-25 minigene was constructed using promoter sequences 5′ of the mouse gene sequence conferring neural specific expression and transcriptional start sites and a cDNA encoding the predominant SNAP-25b isoform expressed in adult brain. The rat insulin intron and SV40 polyadenylation sites were inserted for post-transcriptional processing and to promote effective expression. The expression of the transgene was assessed by in situ hybridization and found to be predominantly neuronal and consistent with the pattern of expression seen for the endogenous gene (for additional detail see Hess et al. [17]).

cortex-striatal systems, which receive strong dopaminergic input [6]. Recently, Barkley has proposed that the principal deficit in ADHD lies in executive functions performed by the prefrontal cortex to control the initiation of motor responses leading to impulsivity and hyperactivity [7]. Since prefrontal cortex receives modulatory input from the striatum, this again suggests a link between the circuitry of striatal and prefrontal dopaminergic pathways and behavioral deficits in ADHD. Studies examining the co-inheritance of polymorphic alleles of the dopamine reuptake transporter DAT1 and the dopamine D4 receptor DRD4 have identified specific alleles of these genes that may provide increased risk for ADHD (reviewed in Ref. [8]). While these findings still appear controversial ([9], and see Ref. [8]), they have provided specific gene targets to evaluate for a direct role in ADHD. It is of interest, therefore, that mice homozygous for the null DAT1 alleles, generated by homologous recombination gene "knockout" technology, are spontaneously hyperactive. However, in the case of the DAT1 polymorphism associated with ADHD, this mutation lies outside the protein-coding region and within the 3′ untranslated portion of the mRNA. Thus it remains unclear how this might affect dopamine reuptake function in ADHD and correspond to the lack of dopamine transporter function in DAT1 knockout mice.

Several rat models displaying characteristics of ADHD have been described, including the genetic models based on the spontaneously hypertensive rat (SHR) [10] and Naples-Excitability (NHE) [11] lines, as well as neonatal lesions of the dopaminergic input to the striatum [12]. Behavioral and pharmacological studies have shown that such genetic and lesion models do faithfully reflect ADHD-associated deficits including, although not limited to, hyperkinesis (for example, [10,13,14]). However these models have been limited by the lack of precise mapping of genetic defects to trace in molecular mechanisms underlying brain dysfunctions relevant to ADHD. In contrast, studies in the mouse have benefited enormously from the vast collection of genetic information and great number of genetically defined mutants, which when coupled with the advance of transgenic technology, and more recently targeted gene disruption methods, provide an effective means to identify genes that influence behavior. Based on this genetic groundwork, investigations in the mouse that complement the pharmacological and behavioral insight gained in rat models can begin to unravel the complexities of gene expression and development that underlie neuropsychiatric diseases, such as ADHD.

2. The coloboma mutant mouse

Coloboma mutant mice display a variety of behavioral, neurophysiological and developmental deficits, of which a subset may be compared with those, presented by ADHD children. Although the *Cm* mutation is early embryonic lethal when homozygous [15], adult heterozygote mice (genotype, *Cm/+*) are viable but exhibit pronounced spontaneous hyperactive locomotor behavior, originally termed "circling," as well as head bobbing, and a distinctive ocular dysmorphology leading to sunken and often closed eyes [16]. As described below, the hyperkinesis of these mutant