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Research report

Autism-related behavioral abnormalities in synapsin knockout mice

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HIGHLIGHTS

- Deletion of Syn isoforms widely impairs social behavior.
- *SynII*$^{-/-}$ mice display impaired social interaction, novelty and recognition.
- *SynI*$^{-/-}$ and *SynII*$^{-/-}$ mice are characterized by increased social dominance.
- Young and adult *SynI*$^{-/-}$ and *SynIII*$^{-/-}$ mice exhibit deficits in social transmission of food preference.
- Social deficits in *SynI*$^{-/-}$ and *SynII*$^{-/-}$ mice appear before the onset of epilepsy.

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ABSTRACT

Several synaptic genes predisposing to autism-spectrum disorder (ASD) have been identified. Nonsense and missense mutations in the SYN1 gene encoding for Synapsin I have been identified in families segregating for idiopathic epilepsy and ASD and genetic mapping analyses have identified variations in the SYN2 gene as significantly contributing to epilepsy predisposition. Synapsins (Syn I/II/III) are a multigene family of synaptic vesicle-associated phosphoproteins playing multiple roles in synaptic development, transmission and plasticity. Lack of SynI and/or SynII triggers a strong epileptic phenotype in mice associated with mild cognitive impairments that are also present in the non-epileptic *SynIII*$^{-/-}$ mice. *SynII*$^{-/-}$ and *SynIII*$^{-/-}$ mice also display schizophrenia-like traits, suggesting that Syns could be involved in the regulation of social behavior. Here, we studied social interaction and novelty, social recognition and social dominance, social transmission of food preference and social memory in groups of male *SynI*$^{-/-}$, *SynII*$^{-/-}$ and *SynIII*$^{-/-}$ mice before and after the appearance of the epileptic phenotype and compared their performances with control mice. We found that deletion of Syn isoforms widely impairs social behaviors and repetitive behaviors, resulting in ASD-related phenotypes. SynI or SynIII deletion altered social behavior, whereas SynII deletion extensively impaired various aspects of social behavior and memory, altered exploration of a novel environment and increased self-grooming. Social impairments of *SynI*$^{-/-}$ and *SynII*$^{-/-}$ mice were evident also before the onset of seizures. The results demonstrate an involvement of Syns in generation of the behavioral traits of ASD and identify Syn knockout mice as a useful experimental model of ASD and epilepsy.

1. Introduction

Autism spectrum disorders (ASDs) are heterogeneous neurodevelopmental disorders characterized by deficits in social interaction and social communication, restricted interests and repetitive behaviors [1]. Abnormalities in language development, mental retardation and epilepsy are observed in autistic children [2,3]. Approximately 30% of autistic children display epilepsy [4] and, conversely, several forms of epilepsy also display ASD [5]. Several ASD candidate genes involved in synaptic plasticity, development and structure have been identified [6], including genes encoding for the postsynaptic proteins NLGN3/4, SHANK2/3 and IL1RAPL1 and the presynaptic proteins NRXN1 CNTNAP2 and RIMS3/NIM3. Although mutations in these genes account for a limited number of cases, a "*synaptic autism pathway*", in which dysfunctions of essential genes for synapse homeostasis and activity-dependent rearrangements can cause ASD, has been hypothesized [6–8]. Given the co-morbidity between ASD and

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epilepsy, the possibility of a common genetic basis for both diseases has been proposed [4,9,10].

Recently, nonsense mutations in SYN1 in families segregating epilepsy and/or ASD have been found. Initially, a W356X mutation in SYN1 was reported in a family segregating syndromic epilepsy associated with mental retardation, macrocephaly and behavioral disturbances (aggressive behavior and ASD-like phenotype) [11]. Moreover, another nonsense SYN1 mutation (Q555X) was identified in a French-Canadian family segregating epilepsy and ASD [12], indicating a causal role of SYN1 mutations in the pathogenesis of both diseases. In addition, genetic mapping analysis identified variations in the SYN2 gene as significantly contributing to epilepsy predisposition and a few SYN2 variants potentially associated with epilepsy and ASD [13,14].

Synapsins (Syns) are a family of synaptic vesicle (SV) phosphoproteins encoded by the *SynI*, *SynII* and *SynIII* genes [15]. The Syn gene family is good candidate for the synaptic epilepsy/ASD pathway, as Syns regulate synaptic transmission and plasticity with distinct roles in excitatory and inhibitory neurons [12,16–18]. Accordingly, mice lacking SynI, SynII, SynI/II or Syn I/II/III (but not SynIII; [19]) experience epileptic seizures starting at 2–3 months of age [20–25]. Moreover, Syn knockout (KO) mice display an array of mild cognitive impairments, including emotional and spatial memory deficits in *SynI*$^{-/-}$ and *SynII*$^{-/-}$ mice [26,27], schizophrenia-like phenotypes in *SynII*$^{-/-}$ and *SynIII*$^{-/-}$ mice [28–31] and impaired object recognition and fear memory in *SynIII*$^{-/-}$ mice [32]. However, the effects of the distinct Syn isoforms on the various aspects of social behavior have never been studied.

Here, we analyzed whether deletion of single Syn genes in mice causes core symptoms of ASD by affecting social behavior, social communication and repetitive behaviors. As autism predominantly affects males, only male mice were used in these studies. The results demonstrate a role of Syns in the behavioral traits of ASD and identify Syn KO mice as a good experimental model to define synaptic alterations involved in the pathogenesis of ASD and epilepsy.

2. Materials and methods

2.1. Animals

Previously generated *SynI*$^{-/-}$, *SynII*$^{-/-}$ and *SynIII*$^{-/-}$ mice [19,23,24] were backcrossed to a C57BL/6J background (Charles River Laboratories, Calco, Italy) through at least ten generations. Genotyping was performed as described [26]. During the experiments, the offspring of homozygous −/− mutant mice (Syn KO) and wild type mice from our C57BL/6J colony (C57) were housed in groups of 2–4 mice under the same temperature-controlled conditions (21 °C), with a 12:12 h light/dark cycle (light on 7:00 am–7:00 pm) and free access to food and water. Heterozygous male mice were found to be indistinguishable from C57 mice in preliminary experiments and were not analyzed. We decided to use a single group of C57 mice as a control for the three mutant lines instead of using littermates, although some limitations may apply because of the impact of the maternal environment on the offspring's behavioral phenotype [33]. Two month-old (young) and 6 month-old (adult) male mice of either genotype were used for the behavioral experiments. The experiments were conducted between 9 am–6 pm during the light phase. Mice were placed in food restriction for 24 h for the buried food olfactory test and the social transfer of food preference test, as described below. One hour before behavioral experiments, mice were habituated to the experimental room, whose illumination was maintained between 150– and 180 lx throughout behavioral testing. In the event mice displayed seizures immediately before or during the behavioral test (adult *SynI*$^{-/-}$, *SynII*$^{-/-}$ mice only) data were excluded from the analyses. In all the other cases, behavioral tests in adult *SynI*$^{-/-}$ and *SynII*$^{-/-}$ mice were performed at least 2 h after the end of occasional seizures. All the procedures involving animals and their care were carried out in accordance with the guidelines established by the European Community Council (Directive 2010/63/EU of September 22, 2010) and were approved by the Italian Ministry of Health.

2.2. Behavioral experiments

2.2.1. Open field

Exploratory activity in a novel environment was assessed by a 5-min session in an open field chamber (44 cm *L* × 44 cm *W* × 44 cm *H*), constructed of grey Plexiglas. Locomotor activity in the central and external part of open field was measured by a video camera and analyzed using the ANYmaze program (Ugo Basile, Varese, Italy).

2.2.2. Light–dark test

The test was conducted in a Plexiglas box divided by an open door in a small dark (20 cm *L* × 22 cm *W*) and in a large light sector (32 cm *L* × 22 cm *W*). Mice were placed in the dark chamber to explore for 5 min. The number of transitions and the time spent in the light chamber were measured by a video camera.

2.2.3. Test for sociability and social novelty preference

Mice were tested for sociability and social novelty preference as described elsewhere [34]. The social testing apparatus was a rectangular clear Plexiglas box (20 cm *L*, 40.5 cm *W*, and 22 cm *H*). During the habituation phase, tested mice were placed in the box for a 10 min session. Afterwards, an unfamiliar stranger mouse (stranger 1) was placed in a round wire cup at the corner of the box. The wire cup (7 cm *L* × 10 cm *H*; pencil box, Ikea) with small holes allowed nose contacts between mice, but prevented fighting. At the opposite corner, a second empty wire cup was located. The animals used as strangers were male C57BL/6J mice, previously habituated to placement in the cup. The location of strangers in the left *vs* right side cups was counterbalanced between mice and trials. In the first phase of the test (sociability test), the subject mouse was placed in the box and allowed to explore for 10 min. The time spent exploring the wire cups was evaluated. Sociability indicates the preference for the cup containing a novel mouse compared to the novel empty cup. At the end of the first 10 min, each mouse was tested in a second 10 min session to evaluate the social preference for a new stranger. A second, unfamiliar C57BL/6J mouse (stranger 2) was placed in the empty cup. Measures of the amount of time spent in approaches of cups were taken during the second 10 min session. Social novelty indicates the preference for cup containing an unfamiliar mouse compared to familiar one.

2.2.4. Social recognition test

The experimental mouse was habituated for 30 min to a clean large cage (42.5 cm *L* × 26.6 cm *W* × 15.5 cm *H*) before an unfamiliar mouse was introduced into the cage for 2 min. The unfamiliar animal (C57BL/6J) was removed and then reintroduced to the same experimental mouse 10 min later. The procedure was repeated four times. At the fifth session, a novel unfamiliar animal was placed with the experimental mouse for 2 min. The last trial was used to rule out the possibility that reduced investigation was due to fatigue or habituation. The time spent in social interaction (contact, sniffing and close approaches) during all the sessions was measured.

2.2.5. Tube test for social dominance

The test employed two start areas, a two-section tube and one neutral area between the two-sections. The apparatus (30 cm long × 3.5 cm diameter) was made of clear Plexiglas material. Gates at the end of each section of the tube allow olfactory, but not physical contact. To carry out the test, two age- and weight-matched mice (one C57 control mouse and one KO mouse) were placed at the opposite ends of the tube. Both mice began to explore in a forward direction. Gates were removed as the mice advanced, allowing the two mice to approach each other. If one mouse was dominant and the other was subordinate, the dominant mouse approached, while the subordinate backed away, quickly forcing the subordinate out of the tube. The matches were repeated an average of five times. Each mouse was engaged in two or three matches per day with an unfamiliar mouse.

2.2.6. Resident-intruder test

Previously group-housed males were separated and housed individually for 1–2 months before testing. A group-housed male mouse (C57BL/6J) of the same age was used as intruder mouse and was introduced into the cage of the experimental mouse. The test was stopped immediately after the first attack (an attack being defined as a bite) and lasted up to 5 min if no attack occurred. The number of animals engaged in aggressive and non-aggressive social behavior (contact, sniffing, close approaches) was recorded.

2.2.7. Social transfer of food preference test

All mice were food deprived for up to 24 h before the task. In the first day of the task, mice familiarized with two cups (6 cm × 6 cm) containing standard pellets (Test Diet, 5TUL-1811142, 20 mg) on the top [35]. The next day, one mouse per cage (demonstrator) was introduced in a cage containing a cup with flavored-pellets (cued food) for 1 h. The amount of cued food that was eaten was evaluated; mice that consumed less than 0.2 g of the novel food in 1 h were excluded from the task. Afterwards, demonstrator mice were introduced to their home cages and allowed to socially interact with cage-mates (observers) for 30 min, before being removed and placed alone in another cage until the end of experiment. One hour later, observer mice were placed into individual cages containing the cued food, previously eaten by the cage-mate demonstrator, and a novel flavored food (novel food). The cued and novel food consisted of chocolate (Test Diet, 5TUL-1811223, 20 mg) or banana-flavored pellets (Test Diet, 5TUL-1813985, 20 mg) that were counterbalanced between animals and condition of the task. Mice were assessed for food preference by determining the percentage of novel and cued food consumed in 1 h.