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 SynII −/− 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 SynII −/− mice. SynII −/− and SynIIII −/− 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 SynIIII −/− mice both 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 SynII 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.

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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
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 disturbance (aggressive behavior and autism-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 SYN1, SYN2 and SYN3 genes [15]. The SYN gene family is good candidates 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 SYN1, SYN2/III or SYN I/II/III (but not SYN3; [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 SYN−/− and SYN−/+ mice [26,27], schizophrenia-like phenotypes in SYN−/− and SYN−/−/− mice [28–31] and impaired object recognition and fear memory in SYN−/− 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 SYN−/−, SYN−/−/− and SYN−/−/− 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 ± 2°C) with a 12:12 h light/dark cycle (light on 7:00 am–7:00 pm) and free access to food and water. Homozygous 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 littersmates, 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 housed bird olfactory test and the social transfer of food preference test. One hour before behavioral experiments, mice were habituated to the experimental room, whose illumination was maintained between 150–180 lx throughout behavioral testing. In the event mice displayed seizures immediately before or during the behavioral test (adult SYN−/−, SYN−/−/− mice only) data were excluded from the analyses. In all the other cases, behavioral tests in adult SYN−/− and SYN−/− 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, [box]) 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. During testing, 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. Mice were offered banana-flavored water in each section of the tube, 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, STUL-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 experimental group. Afterwards, demonstration-like procedures were introduced to the 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, STUL-1811223, 20 mg) or banana-flavored pellets (Test Diet, STUL-18113965, 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.
2.2.8. Buried food olfactory test

The day before the task, mice were food deprived for 24 h. During the task, each mouse was placed in a clean rat cage with 4 cm deep bedding containing a hidden piece of food pellet (0.2 g). The time needed to find the buried food pellet (up to 5 min) was measured.

2.2.9. Marble burying test and self-grooming behavior

Each mouse was placed for 45 min into a clean rat cage with 4 cm deep bedding and 12 glass marbles placed on the surface. The number of marbles that were buried by the mouse was measured. To evaluate the role of novelty, the experiment was repeated in a new group of mice after they became familiar with 6 marbles placed in their home-cages for 24 h before test. The day after, each mouse was placed in a clean rat cage with familiar marbles for 45 min and the number of buried marbles was evaluated.

For the evaluation of the self-grooming behavior, mice were habituated for 10 min in an empty cage without bedding. Afterwards, grooming of all body regions was observed for 10 min by a trained observer with a stopwatch.

2.2.10. Statistical analysis

Statistical analysis was performed with STATVIEW program (SAS Institute, Cary, NC). Two-way ANOVA with genotype and age as factors was used to analyze the open field test, the light–dark test, the buried food olfactory test and the grooming behavior. For the Sociability and Social Novelty tests, social interaction was analyzed by repeated-measures ANOVA with genotype as between-subject factor and condition of the task (familiar/unfamiliar) as within-subject factors. For the Social Recognition test, social interaction was analyzed by repeated-measures ANOVA with genotype as between-subject factor and sessions within-subject factors. For the Social Transmission of Food Preference test, food preference was analyzed by repeated-measures ANOVA with genotype as between-subject factor and food (cued/unfamiliar) as within-subject factors. The number of buried marbles and the extent of social interaction in the resident-intruder test were analyzed using a one-way ANOVA. Post hoc multiple comparisons were made using Fisher’s PLSD test as appropriate. The percentage of winning in the tube test for social dominance was analyzed with a binomial test between C57 and Syn KO mice.

3. Results

To investigate whether mutation of Syn genes is associated with an ASD-like endophenotype, we analyzed social behavior and repetitive behaviors in SynII−/−, SynIII−/− and SynII−/− mice. In order to clarify the relationships between autistic and epileptic phenotypes, behavioral studies were conducted in independent cohorts of Syn KO mice before and after the onset of seizures in SynII−/− and SynII−/− mice (young and adult mice, respectively). Prior to testing of ASD-related behaviors, we first evaluated whether mutant mice had motor alterations, olfactory impairments or anxiety-related behaviors that may impact social behavior. No major differences in the distance covered and in the number of internal entries in the open field were found among genotypes and age (Fig. 1A and B). When anxiety-related behavior was analyzed, neither young nor adult Syn KO mice significantly differed from control mice in the number of central entries (Fig. 1B). In line with the results of the open field test, Syn KO and C57 control mice displayed a similar number of transitions between light–dark compartments (Fig. 1C) and spent the same amount of time in the light chamber (Fig. 1D). These data are consistent with the normal anxiety-related behavior previously described for SynI−/−, SynII−/− and SynII−/− mice [26,32] and suggest that Syn KO mice are amenable for further analysis of their social behavior. To rule out a role of olfactory impairments on social deficits, we performed the buried food olfactory test, by evaluating the olfactory ability of mice to find a buried piece of food. No differences were found between C57 and Syn KO mice in the latency to find buried food (two-way ANOVA; genotype F3,80 = 0.81, P = 0.49; age F1,80 = 0.006, P = 0.94; genotype × age F3,80 = 0.49, P = 0.69), excluding the presence of olfactory deficits.
3.1. Syn knockout mice display abnormalities in the sociability and social novelty tests

To evaluate animals for their voluntary initiation of social interaction and their ability to discriminate social novelty, we used a modified version of a three-chamber social approach test. In the sociability phase of test (Fig. 2A and B), repeated measures ANOVA indicated significant differences in the time spent in exploration of the empty cup vs the social stimulus for both young (F1,36 = 137.26, P < 0.0001) and adult mice (F1,36 = 157.35, P < 0.0001). In fact, young and adult control mice investigated the social stimulus (unfamiliar stranger mouse) for a longer time than the novel empty cup (post hoc Fisher’s PLSD test: young C57, P = 0.0001; adult C57, P < 0.0001). Similarly, Syn KO mice explored the cup containing the stranger mouse for a longer time than the empty cup, showing normal sociability (Syn I−/−: young P = 0.0007, adult P = 0.0160; Syn II−/−: young P = 0.0005, adult P < 0.0001; Syn III−/−: young P < 0.0001, adult P < 0.0001). Nevertheless, repeated-measures ANOVA indicated a significant effect of genotype in the time spent in social and non-social interactions (young mice: F3,36 = 5.24, P = 0.004; adult mice: F3,36 = 3.50, P = 0.04). In particular, post hoc analysis revealed that young Syn II−/− mice had reduced interest for both cups than C57 control mice (stranger mouse cup: P = 0.005; empty cup: P = 0.01).

In the social novelty phase of the test, normal preference for social novelty was evaluated (Fig. 2C and D). Both young and adult C57 control mice were found to explore more the cup containing a novel mouse than the cup containing the familiar one (young C57: P = 0.003; adult C57: P = 0.007). However, significant genotype-related differences in the social novelty were found for Syn KO mice (young F1,36 = 9.015, P = 0.005; adult F1,36 = 18.02, P = 0.0001). While Syn I−/− mice behaved similarly to C57 control mice, Syn II−/− and young Syn III−/− did not discriminate between familiar and novel mice (young Syn II−/−: P = 0.91; adult Syn II−/−: P = 0.07; young Syn III−/−: P = 0.57). Overall, these data indicate that social preference was preserved in Syn mutant mice, although the time spent in social interaction was decreased in Syn II−/− mice and the social novelty preference was significantly altered in both Syn II−/− and Syn III−/− mice.

3.2. Syn II−/− mice display deficits in short-term social recognition

Social recognition test is based on the natural tendency of rodents to investigate novel animals and allows evaluating short-term, social recognition abilities [36]. Typically, experimental C57 control mice, either young (Fig. 3A) or adult (Fig. 3B), progressively decreased the time spent exploring an intruder mouse over the sessions (from 1 to 4 sessions). Repeated-measures ANOVA indicated that there was a significant effect of sessions for both ages (young C57: F4,12 = 20.7, P < 0.0001; adult C57: F4,12 = 6.85, P < 0.0001). The comparison between the first and the fourth session indicated that experimental C57 mice recognized the intruder mouse explored in the preceding sessions (young P = 0.0048; adult P = 0.0085). When “habituated” experimental C57 mice were exposed to a novel mouse on the fifth session, they suddenly increased the exploration time as they did on the first session (session 4 vs session 5: young C57, P = 0.010; adult C57, P = 0.011). While Syn I−/− and Syn II−/− mice behaved very similarly to C57 control mice (session 1 vs session 4: young Syn I−/−, P = 0.045; adult Syn I−/−, P = 0.036; young Syn II−/−, P = 0.0002; adult Syn II−/−, P = 0.029; session 4 vs session 5: young Syn I−/−, P = 0.028; adult Syn I−/−, P = 0.049; young Syn II−/−, P = 0.001; adult Syn II−/−, P = 0.029), adult Syn II−/− mice spent a similar time in exploring the intruder mouse during all sessions (session 1 vs session 4: Syn II−/−, P = 0.341) and both young and adult Syn II−/− mice did not increase the exploration of the novel intruder mouse on the fifth session (session 4 vs session 5: young Syn II−/−, P = 0.57).
To evaluate the ability of mice to learn and communicate social information, we tested the animals in the social transmission of food preference task [35]. One hour after social interaction with the demonstrator cagemate, repeated measures ANOVA demonstrated a preference for the cued food over the novel food both in young (F(1,36) = 5.46, P = 0.025) and adult (F(1,34) = 6.90, P = 0.01) mice. However, the preference was significant in C57 control mice (post hoc Fisher’s PLSD test for young P = 0.0017 and adult P = 0.015) and SynII−/− (young P = 0.038; adult P = 0.035), but was virtually absent in both young and adult SynI−/− (young P = 0.114; adult P = 0.634) and SynIII−/− (young P = 0.699; adult P = 0.823) mice (Fig. 5A and B). Notably, these differences were observed in the absence of significant effects in the time spent in social interactions between the demonstrator and the observer mice (Fig. 5C and D) and in the amount of food eaten by demonstrator mice (Fig. 5E and F) across age and genotype.

3.4. SynI−/− and SynIII−/− mice display an impaired social transmission of food preference

To measure social approach-avoidance behavior and dominance hierarchies, without allowing the mice to injure each other in a real fight, we employed the social dominance tube test [37,38]. In this test, adult SynI−/− and SynII−/− mice displayed more dominant behavior than C57 mice, while SynIII−/− mice were more submissive than C57 mice (Fig. 4A). In fact, SynI−/− and SynII−/− mice won 62.7 ± 11.3% and 76.5 ± 8.4% of the matches against control mice, respectively (binomial test: SynI−/−, P = 0.02; SynII−/−, P = 0.0004). By contrast, SynIII−/− mice lost the 85.7 ± 4.7% of the matches against control mice (binomial test: SynIII−/−, P < 0.0001).

Because Syn KO mice showed an altered dominance in the tube test, we hypothesized the existence of a genotype-dependent aggressive behavior. To assess the aggressive behavior and evaluate inter-male social interaction, we used the resident-intruder test in adult mice of the various genotypes (Fig. 4B). After 1–2 months of social isolation, experimental C57 and Syn KO mice did not show a marked aggressive behavior against the intruder mouse (not shown), likely for the low propensity of the C57BL/6J strain to be aggressive [39,40]. However, experimental SynI−/− and SynII−/− mice showed reduced social interaction with the intruder mice (one-way ANOVA, F(3,30) = 3.08, P = 0.042; SynI−/−, P = 0.04, SynII−/−, P = 0.02, SynIII−/−, P = 0.01; PLSD Fisher’s test, Fig. 4B). These results show that, when challenged by prolonged isolation, Syn KO mice display social impairments. Social impairment of SynIII−/− mice was also associated with digging behavior (data not shown), consistent with the submissive behavior observed in the tube test.
Syn II−/− mice were associated with reduced time spent in digging with respect to control mice, whereas the rearing behavior was unchanged (data not shown). The reduced time spent in digging by of Syn I−/− and Syn II−/− mice could also be contributed by an increase in the time spent in grooming (see below). Several studies indicated that marble burying depends on both interest for the environment and stress novelty. Thus, to evaluate whether an altered response to novelty can elicit impairments in marble burying, a second group of mice was tested after habituation to marbles. As shown in Fig. 6B, adult Syn KO mice buried less marbles than C57 mice also after habituation to marbles (one-way ANOVA, F3.31 = 3.668, P = 0.023; Syn I−/− P = 0.015, Syn II−/− P = 0.008, Syn III−/− P = 0.015; LSD Fisher’s test), suggesting that the changes in marble burying are not due an altered response to novelty, but rather to a decreased interest toward the environment.

3.6. Syn II−/− mice display increased repetitive self-grooming behaviors

Self-grooming and motor stereotyped behaviors in mice mimic several aspect of repetitive behavior in autistic children [41–43] and Syn III−/− have been reported to experience increased thigmotaxis [32]. The evaluation of grooming behavior revealed the presence of a significant effect of genotype in both young and adult mice (two-way ANOVA young: F3.45 = 2.91, P = 0.045; adult: F3.53 = 3.72, P = 0.017). In particular, while Syn I−/− and Syn III−/− mice were similar to C57 mice (young Syn I−/− P = 0.631; adult Syn I−/− P = 0.127; young Syn III−/− P = 0.469; adult Syn III−/− P = 0.669), both young and adult Syn II−/− mice displayed a strongly increased self-grooming (young P = 0.005; adult P = 0.021; Fig. 6C).

4. Discussion

Abnormalities in presynaptic function affecting neurotransmitter release and short-term plasticity have been identified at the basis of epilepsy and cognitive disorders such as ASD [7,8,44]. One family of such presynaptic proteins, whose deletion or loss-of-function mutations generates an epileptic phenotype in mouse and man, are the synapsins [11–13]. Mice lacking Syn I, Syn II, or Syn I/II/III are all prone to epileptic seizures starting at an age of 2–3 months [22,24,45], a period of intense synapse maturation and refinement in which Syns I/II have already reached high expression levels [46,47]. A similar onset during childhood or adolescence is
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3–5 months old) animals. Moreover, hyperactivity of SynI−/−, SynII−/− and SynIII−/− mice was observed in sex–mixed group of mice [30,32], while in this study only male mice were used.

Since mutations of SYN1 in humans have been linked to ASD and epilepsy and proposed to be a common basis for both diseases [11,12], we examined whether single deletions of the three Syn genes are associated with specific autism-related phenotypes. To this aim, we evaluated the core symptoms of ASD such as altered social behavior, poor communication skills and repetitive behaviors. Our findings revealed complex social behavioral alterations in SynI−/− and SynII−/− and SynIII−/− mice (Table 1), whereas motor and olfactory abilities as well as anxiety-related behaviors were not affected. In particular, SynII−/− mice displayed the strongest phenotype characterized by impaired social interaction, social novelty and social recognition and increased social dominance, while SynI−/− and SynIII−/− mice showed deficits in adult social interaction and social communication.

It has been shown that SynII−/− mice, or rats with SynII knockdown in the prefrontal cortex display deficits in social interaction [28,29]. Nevertheless, the amount of sniffing the cup containing the unfamiliar mouse of SynII−/− vs the empty cup (as shown in Fig. 2A and B) was not impaired in the modified three-chamber test. One difference could be that, while in the resident-intruder test mice can engage in direct social behavior, in the modified three-chamber test direct contacts between the subject and the stranger mice are prevented, allowing only for olfactory, visual and auditory communication. These results suggest that social impairments in genetic mouse models of ASD can be paradigm-dependent, as also shown in the case of Neureilgin-2/3 KO mice [50–53]. Interestingly, both SynI−/− and SynII−/− mice showed a decreased marble burying behavior and SynII−/− mice displayed a decreased total time spent exploring both the cup containing the unfamiliar mouse and the empty cup in the modified three chambers test. These results are reminiscent of the reduced interest for environment observed in autistic patients [54,55]. It has been shown that autistic children during an environmental exploration task, used to assess restricted interests, displayed significantly less object exploration than the normal subjects [55].

Several functional differences have been observed among members of the Syn family. While Synl and Synll start to be expressed during synaptogenesis and their levels are high in mature neurons, Synll expression is high during early neuronal development and becomes downregulated in adult animals [56,57]. Accordingly, in Synll−/− mice the impairments in social novelty were present in young animals and disappeared in adult mice. However, some behavioral deficits such as altered approach-avoidance behavior, social interaction or impaired social transmission of food preference persisted in adult Synll−/− mice. Social communication in mice has been less studied because of the difficulty of relating the social communication in rodents to that in humans. Nevertheless, several procedures have been established, such as recording ultrasound vocalizations [58,59], the scent marking paradigm [60] and the social transmission of food preference task [61–63]. Although the latter task has been mainly developed to study social learning in rats [64], there is a growing interest to use it for studying olfactory communication in mice [59–61]. Interestingly, an olfactory subsystem that includes olfactory sensory neurons expressing the receptor guanylyl cyclase GC-D, the cyclic nucleotide–gated channel subunit CNGA3 and the carbonic anhydrase isoform CAII was found to be implicated in the acquisition of social transmission of food preference in mice [65].

We have found that, both young and adult Synll−/− and Synll−/− mice exhibit deficits in the social transmission of food preference. Such deficits could not be ascribed to motivational or olfactory defects, because mutant and control mice ate the same amount of food and displayed similar olfactory abilities. In line with our results, Porton et al. [32] showed that Synll−/− mice were impaired in social transmission of food preference assessed after 20 min and 24 h, indicating the possible existence of both short- and long-term
memory deficits. In order to minimize the involvement of long-term memory processes, our tasks were performed with a short delay (1 h) between social interaction with demonstrator and the test. Moreover, no detectable short-term object recognition deficits have been observed in the various strains of Syn KO mice [26,32]. However, it is still possible that the social transmission of food preference impairments observed in Syn KO mice are also contributed, at least in part, by cognitive impairments. Studies measuring ultrasound vocalizations in mice could help to further clarify this issue. Moreover, SynII−/− mice, but not Syn−/− or SynIII−/− mice, display an excessive self-grooming behavior, which may reproduce the increased repetitive behavior typical of the core symptoms of ASD.

The behavioral abnormalities associated with ASD occur prior to the onset of seizures in children with ASD and epilepsy [66,67]. A similar developmental relationship between autism-like behaviors and epilepsy was also observed in SynIII−/− and SynII−/− mice, [22–24,26]. Interestingly, we found that autism-related behaviors in SynI−/− and SynII−/− mice precede the onset of seizures and that epilepsy does not significantly affect the expression of the behavioral alterations in adult mice. Moreover, SynIII−/− mice, that are not epileptic, also display several traits of social deficits. Thus, the behavioral abnormalities found in mutant mice likely reflect an autistic phenotype and not secondary changes due to epilepsy.

Mutations of ASD-related genes encoding for various synaptotrophic proteins mimic specific autism-related behavioral impairments. Indeed, it has been reported that Neuroligin-3 or Shank-3 KO mice show impairments in social interactions [53,68]; Neuroligin-3/4 and Shank-1/3 KO mice display deficits in social communication [52,69,70]; Shank-2 KO mice exhibit reduced social interaction, social communication and repetitive jumping [71]. Moreover, Neurexin-1α and CNTNAP2 KO mice display decreased nest-building behavior [72,73]. CNTNAP2 KO mice were also characterized by altered social behavior, stereotypic motor movements and behavioral inflexibility [73], while Neuroligin-1, Neurexin-1α and Shank-3 KO mice displayed excessive grooming behavior [68,72,74]. Although these genes account only for a limited number of ASD cases, they map into a “synaptic autism pathway” in which dysfunctions of any of the genes essential for the regulation of synapse formation, excitation/inhibition balance and activity-dependent synaptic rearrangements can result in a similar autistic behavioral phenotype [6–8].

Synapsins are not essential for synaptic transmission, but play a key role in synaptic homeostasis and plasticity [15]. They are differentially expressed in inhibitory and excitatory neurons and their deletion causes excitatory/inhibitory imbalance [16,19,45,48]. Previous studies indicated a role for GABAergic dysfunction in isolated behaviors, such as altered learning and memory and impaired social behavior [44,75]. The prominent alterations in inhibitory transmission that characterize Syn KO mice could play a causal role not only in seizure development, but also in the expression of the autistic traits. Among single Syn KO mice, SynII−/− mice display the strongest phenotype, although the mechanisms underlying these behavioral differences are still unclear. SynII−/− mice are more severely epileptic than SynI−/− mice [26], are more impaired in social behavior than SynI−/− and SynII−/− mice (our results) and display more prominent deficits in spatial memory which are absent in SynI−/− and SynII−/− mice [26,32]. The available data on the functional role of Syn isoforms in neuronal physiology indicate that they play, to a large extent, non-redundant functions. The behavioral differences observed among single Syn KO mice could depend on the developmental expression of the various isoforms. In particular, the lack of epilepsy and of consistent social deficits in SynII−/− mice could be ascribed to the marked downregulation of Syn I expression that occurs in the adult, while epilepsy and the other behavioral traits of SynI−/− and SynII−/− mice can be explained by their high expression levels in the postnatal brain, where they play key functions in the regulation of neurotransmitter release and short-term plasticity [15]. Moreover, in the postnatal brain, Syn and SynII are differentially expressed in excitatory and inhibitory synapses and in specific regions [76]. The more severe cognitive deficits of SynII−/− mice also correlate with more intense impairments in the hippocampal short-term-plasticity than SynI−/− KO mice [24] and are in line with the idea that Syn II plays a more structural role than Syn I in nerve terminal physiology [15].

In conclusion, the data indicate that Syns could represent a common genetic basis for epilepsy and ASD and that Syn KO mice represent an interesting animal model for autism-like phenotypes.

### Conflict of interest statement

The authors declare no conflict of interest.

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### References

1. Diagnostic and statistical manual of mental disorders. Washington, DC: American Psychiatric Association; 1994.
2. Gillberg C, Billstedt E. Autism and Asperger syndrome: coexistence with other clinical disorders. Acta Psychiatrica Scandinavica 2000;102:321–30.
3. Rapin I, Dunn M. Language disorders in children with autism. Seminars in Pediatric Neurology 1997;4:86–92.
[4] Tuchman R, Cuccaro M, Alessandri M. Autism and epilepsy: historical perspective. Brain Dev 2010;32:79–8.
[5] Dravet C. The behavioral disorders in epilepsy. Revue Neurologique 2002;158:453–85.
[6] Bourgeron T. A synaptic trek to autism. Current Opinion in Neurobiology 2000;10:19–24.
[7] Ramocki MB, Zoghbi HY. Failure of neuronal homeostasis results in common neurodevelopmental syndromes. Nature 2008;455:912–8.
[8] Sudhof TC, Malenka RC. Understanding synapses: past, present, and future. Neuron 2008;60:459–76.
[9] Deonna T, Routel E. Autistic spectrum disorder: evaluating a possible contributing or causal role of epilepsy. Epilepsia 2006;47(Suppl. 2):79–82.
[10] Nobile E, Tramontini 3V, Ryan A. The genetics of autism. Pediatrics 2004;113:472–86.
[11] Garcia CC, Blair HJ, Seager M, Couthard A, Tannant S, Buddles M, et al. Identification of a mutation in synapsin I, a synaptic vesicle protein, in a family with epilepsy. Journal of Medical Genetics 2004;41:183–6.
[12] Fassio A, Patry Le, Congia S, Onofri F, Piton A, Gauthier J, et al. SYN1 loss-of-function mutations in autism and partial epilepsy cause impaired synaptic function. Human Molecular Genetics 2011;20:2397–307.
[13] Cavalleri GL, Weale ME, Shinnava KV, Singh R, Lynch JM, Grintor B, et al. Multicentre search for genetic susceptibility loci in sporadic epilepsy and seizure types: a case–control study. Lancet Neurol 2007;6:582–90.
[14] Lakhan K, Kalita J, Misra UK, Kumar R, Mittal B. Association of intrinsic polymorphism rs37733634A>G in synapsin-2 gene with idiopathic epilepsy. Synapse 2010;64:403–8.
[15] Cesa F, Baldelli P, Valtorta F, Benfenati F. The synapsins: key actors of synapse function and plasticity. Progress in Neurobiology 2010;91:313–48.
[16] Baldelli P, Fassio A, Valtorta F, Benfenati F. Lack of synapsin I reduces the readily releasable pool of synaptic vesicles at central inhibitory synapses. Journal of Neuroscience 2007;27:1520–31.
[17] Bragina L, Cadoracchi C, Barbarelli P, Giovedi S, Benfenati F, Conti F. Heterogeneity of glutamatergic and GABAergic release machinery in cerebral cortex. Neuroscience 2007;146:1829–40.
[18] Chiappalone M, Casagrande S, Tedesco M, Valtorta F, Baldelli P, Martinouva S, et al. Opposite changes in glutamatergic and GABAergic transmission underlie the diffuse hyperexcitability of synapsin I-deficient cortical networks. Cerebral Cortex 2009;19:1422–39.
[19] Feng J, Chi P, Blanpied TA, Xu Y, Magarinos AM, Ferreira A, et al. Regulation of neurotransmitter release by synapsin III. Journal of Neuroscience 2002;22:4372–80.
[20] Bojko O, Farinato P, Cesca F, Fererra E, Valtorta F, Benfenati F, et al. Cortico-hypocampal hyperexcitability in synapsin I/III knockout mice: age-dependency and response to the antiepileptic drug levetiracetam. Neuroscience 2010;171:268–83.
[21] Ethisom L, Heggeland P. Seizure elements and seizure element transitions during tonic–clonic seizure activity in the synapsin I/II double knockout mouse: a neuroethological description. Epilepsy and Behavior 2009;14:582–90.
[22] Ketzel M, Kahn J, Weisberg I, Becker AJ, Friedman A, Gitler D. Compensatory network alterations upon onset of epilepsy in synapsin triple knock-out mice. Neuroscience 2011;189:108–22.
[23] Li J, Chen LS, Shugov O, Brodin L,狮莎 TS, Hvalby O, et al. Impairment of synaptic vesicle clustering and of synaptic transmission, and increased seizure propensity, in synapsin I-deficient mice. Proceedings of the National Academy of Sciences of the United States of America 2009;106:9235–9.
[24] Rosahl T, Geppert M, Spillane D, Herz J, Hammer RE, Malenka RC, et al. Short-term synaptic plasticity is altered in mice lacking synapsin I. Cell 1993;75:661–70.
[25] Cambiaghi M, Cursi M, Monzani E, Benfenati F, Comi G, Valtorta F, et al. Temporal evolution of neurophysiological and behavioral features of synapsin I/III triple knockout mice. Epilepsy Research, 2012, PMID 22846639.
[26] Conradi A, Zanardi A, Giacomini C, Onofri F, Valtorta F, Zoli M, et al. Synapsin I- and synapsin II-null mouse display an increased age-dependent cognitive impairment. Journal of Cell Science 2008;121:3042–51.
[27] Silva AJ, Rosahl TW, Chapman PF, Marowitz Z, Friedman E, Frankland PW, et al. Impaired learning in mice with abnormal short-lived plasticity. Current Biology 1996;6:1509–18.
[28] Dyck BA, Bayert MG, Ferro MA, Mishra RK. Medial prefrontal cortical synapsin II knock-down induces behavioral abnormalities in the rat: examining synapsin II in the pathophysiology of schizophrenia. Schizophrenia Research 2011;130:250–9.
[29] Dyck BA, Skolienick KJ, Castellano JM, Ki K, Thomas N, Mishra RK. Behavioral abnormalities in synapsin II knockout mice impact a causal factor in schizophrenia. Synapse 2009;63:662–72.
[30] Dyck BA, Skolienick KJ, Castellano JM, Ki K, Thomas N, Mishra RK. Knock-out mice show sensorimotor gating and behavioural abnormalities similar to those in the pheno-imide-induced preclinical animal model of schizophrenia. Schizophrenia Research 2007;97:292–3.
[31] Dyck BA, Tan ML, Daya RP, Basu D, Sookram CD, Thomas N, et al. Behavioral effects of non-viral mediated RNA interference of synapsin II in the medial prefrontal cortex of the rat. Schizophrenia Research 2012;137:32–8.
[32] Porton B, Rodrigues RM, Phillips LE, Gilbert JW, Feng J, Greengard P, et al. Mice lacking synapsin III show abnormalities in explicit memory and conditioned fear. Genes, Brain and Behavior 2010;9:257–68.
[64] Galef BC. A case study in behavioral analysis, synthesis and attention to detail: social learning of food preferences. Behavioural Brain Research 2012;231:266–71.

[65] Munger SD, Leinders-Zufall T, McDougall IM, Cockerham RE, Schmid A, Wandernoth P, et al. An olfactory subsystem that detects carbon disulfide and mediates food-related social learning. Current Biology 2010;20:1438–44.

[66] Tuchman R, Rapin I. Epilepsy in autism. The Lancet Neurology 2002;1:352–8.

[67] Volkmar FR, Nelson DS. Seizure disorders in autism. Journal of the American Academy of Child and Adolescent Psychiatry 1990;29:127–9.

[68] Peca J, Feliciano C, Ting JT, Wang W, Wells MF, Venkatraman TN, et al. Shank3 mutant mice display autistic-like behaviours and striatal dysfunction. Nature 2011;472:437–42.

[69] Wang X, McCoy PA, Rodriguez RM, Pan Y, Je HS, Roberts AC, et al. Synaptic dysfunction and abnormal behaviors in mice lacking major isoforms of Shank3. Human Molecular Genetics 2011;20:3093–108.

[70] Wohr M, Roulet FI, Hung AY, Sheng M, Crawley JN. Communication impairments in mice lacking Shank1: reduced levels of ultrasonic vocalizations and scent marking behavior. PLoS ONE 2011;6:e20631.

[71] Won H, Lee HR, Gee HY, Mah W, Kim JJ, Lee J, et al. Autistic-like social behaviour in Shank2-mutant mice improved by restoring NMDA receptor function. Nature 2012;486:261–5.

[72] Etherton MR, Blais CA, Powell CM, Sudhof TC. Mouse neurexin-1alpha deletion causes correlated electrophysiological and behavioral changes consistent with cognitive impairments. Proceedings of the National Academy of Sciences of the United States of America 2009;106:17998–8003.

[73] Penagarikano O, Abrahams BS, Herman EI, Winden KD, Gdalyahu A, Dong H, et al. Absence of CNTNAP2 leads to epilepsy, neuronal migration abnormalities, and core autism-related deficits. Cell 2011;147:235–46.

[74] Blundell J, Blais CA, Etherton MR, Espinosa F, Tabuchi K, Walz C, et al. Neuroligin-1 deletion results in impaired spatial memory and increased repetitive behavior. Journal of Neuroscience 2010;30:2115–29.

[75] Chattopadhyaya B, Cristo CD. GABAergic circuit dysfunctions in neurodevelopmental disorders. Frontiers in Psychiatry 2012;3:51.

[76] Kielland A, Ersir A, Wallas SI, Heggelund P. Synapsin utilization differs among functional classes of synapses on thalamocortical cells. Journal of Neuroscience 2006;26:5786–93.