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Sarm1, a neuronal inflammatory regulator, controls social interaction, associative memory and cognitive flexibility in mice

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ABSTRACT

Impaired neurodevelopment leads to several psychiatric disorders, including autism, schizophrenia and attention deficiency hyperactivity disorder. Our prior study showed that sterile alpha and TIR motif-containing 1 protein (Sarm1) regulates neuronal morphogenesis through at least two pathways. Sarm1 controls neuronal morphogenesis, including dendritic arborization, axonal outgrowth and establishment of neuronal polarity, through the MKK–JNK pathway. Neuronally expressed Sarm1 also regulates the expression of inflammatory cytokines in the brain, which have also been shown to impact brain development and function. Because the reduction of Sarm1 expression negatively influences neuronal development, here we investigated whether Sarm1 controls mouse behaviors. We analyzed two independent Sarm1 transgenic mouse lines using a series of behavioral assays, and found that the reduction of Sarm1 protein levels had a limited effect on locomotion and anxiety. However, Sarm1 knockdown mice exhibited impairments in cued and contextual fear conditioning as well as cognitive flexibility. Moreover, the three-chambered social test, reciprocal social interaction and social transmission of food preference further illustrated deficiencies in Sarm1 knockdown mice in social interaction. These findings suggest that Sarm1, a molecule that regulates innate immunity and neuronal morphogenesis, regulates social behaviors and cognition. We conclude that Sarm1 is involved in immune response, neural development and psychiatric disorders.

1. Introduction

Neurodevelopmental disorders, including autism and schizophrenia, are complex neuropsychiatric diseases (Arnold and Trojanowski, 1996; Ehninger et al., 2008; Walsh et al., 2008). Both environmental factors and genetic deficits contribute to the pathogenesis of such disorders. The most well-known environmental factor is prenatal immune challenge caused by infection. Robust cytokine production induced by the innate immune response is believed to affect neural development and lead to several neurodevelopmental disorders (Baharnoori et al., 2009; Malkova et al., 2012; Patterson, 2009). Key components of innate immune responses, such as toll-like receptors (TLRs) and inflammatory cytokines, were recently found to be involved in regulating neural function and differentiation. For example, both interleukin (IL)-6 and IL-10 are involved in infection-induced schizophrenia in mice (Deverman and Patterson, 2009; Meyer et al., 2008; Smith et al., 2007), and IL-1ß, IL-6 and tumor necrosis factor (TNF)-α inhibit dendrite outgrowth in rat cortical neurons (Gilmore et al., 2004). The TLRs are expressed in neurons and modulate neural development. For example, TLR2, TLR3 and TLR4 regulate embryonic or adult hippocampal neurogenesis (Lathia et al., 2008; Rolls et al., 2007). TLR3, TLR7 and TLR8 negatively regulate neurite outgrowth (Cameron et al., 2007; Liu et al., 2013; Ma et al., 2006). TLR3 also affects memory retention (De Miranda et al., 2010; Okun et al., 2010). The prenatal administration of polyinosinic/polycytidylic (poly I:C), which is synthetic double strand RNA ligand of TLR3, also disturbs mouse behaviors later in adult life (Deverman and Patterson, 2009; Patterson, 2009).

Sterile alpha and TIR motif containing 1 (Sarm1), an evolutionarily conserved adaptor protein, interacts with different proteins that regulate signaling pathways that are involved in diverse cellular functions. Sarm1 has a Toll/Interleukin-1 receptor (TIR)-domain, and as such has been identified as the fifth member of the myeloid differentiation primary response gene 88 (MyD88) family and functions as a negative regulator of TLR3/TLR4 signaling in the TIR-domain-containing adapter-inducing interferon-β (TRIF)-dependent signaling pathway (Carty et al., 2006; Mink et al., 2001). Sarm1 is preferentially expressed by neurons in the brain (Chen et al., 2011; Kim et al., 2007), and its deficiency leads to a reduction in the level of TNF-α expression in the brainstem following infection with the West Nile virus (Szretter et al., 2009), demonstrating a role for Sarm1 in neuronal immune response.

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Even without viral infection, the knockdown of Sarm1 in mice alters the expression of inflammatory and antiviral cytokines in mouse brains (Lin et al., 2013). In cultured hippocampal neurons, Sarm1 regulates neuronal morphogenesis at multiple levels, including acting downstream of syndecan-2 in controlling dendritic arborization and regulating axonal outgrowth and neuronal polarity (Chen et al., 2011). In brains, knockdown of Sarm1 reduces the dendritic arbors of neurons and brain sizes (Chen et al., 2011), further supporting the role of Sarm1 in brain development.

Because Sarm1 regulates neuroinflammation and neuronal morphogenesis, both of which are critical in the pathogenesis of neurodevelopmental disorders, we hypothesized that Sarm1 may control cognitive behaviors. In a continuation of our prior research, in this study we analyzed the behaviors of Sarm1 knockdown transgenic mice by testing their performance in a series of standardized behavioral paradigms and demonstrated the roles of Sarm1 in associative learning, social behaviors and cognitive flexibility. Our findings demonstrate that a neuronal immune regulator can control cognition and social behaviors and therefore suggest that the innate immunity of neurons is involved in the etiology of psychiatric disorders.

2. Methods

2.1. Animals and behavioral analyses

All of the animal experiments were carried out with the approval of the Academia Sinica Institutional Animal Care and Utilization Committee. Sarm1 knockdown mice were generated in a C57BL/6J background (Chen et al., 2011). The animals used in the behavioral assays were the offspring of transgenic males and wild-type C57BL/6J females to rule out a maternal effect of Sarm1 knockdown on offspring behavior. Male mice were used for behavioral assays to avoid variations due to the estrus cycle. All of the procedures for the behavioral analyses have been described previously (Chung et al., 2011; Nadler et al., 2004; Wrenn et al., 2003) with minor modifications.

Behavioral testing was performed at eight weeks to 16 weeks of age as summarized in Table 1. The animals were moved from the housing room to the test room for accommodation for at least one week prior to the behavioral assays. Littermates were housed in mixed-genotype groups of three to five mice per cage based on the litter size. After each test, the mice were temporarily put into a new clean cage but not their home cage. Therefore, the tested mice did not affect the behaviors of the naïve mice. For fear conditioning, non-tested animals were temporally removed from the test room when the experiment was ongoing, minimizing the possible effect of auditory stimulation on non-tested animals.

Table 1
Sequence of administration of behavioral tests and age of animals for each task.

Test group	Order of test	Name of behavior test	Age of tested mice (weeks of age)
I	1st	Open field	8
I	2nd	Elevated plus maze	8
I	3rd	Light–dark box	8–9
I	4th	Three-chambered social test	9–10
II	5th	Reciprocal social interaction	11
II	6th	T maze	11–14
II	7th	Contextual or cued fear conditioning	15–16
–	–	Social transmission food preference	13
–	–	Olfaction test I and II	8–10

The test room was lit by fluorescent light with a light intensity of 280 lumen/m^2 (lux). A 12-h light/dark cycle (lights off at 20:00) was maintained in the test room. Food and water were available *ad libitum*, except during the T-maze and social transmission of food preference experiments. The first group of tests was conducted in the order: open field, elevated plus maze, light–dark box and finally the three-chambered social test. For the second-group tests, each subject mouse was housed individually for one week before the test of reciprocal social interaction and the T-maze. Littermates were housed as a group again for one week in advance of the fear conditioning experiments. To prevent fluctuations due to circadian rhythm, the first group of assays was carried out from 10:00 to 14:00 and the second group of assays was performed from ~14:00 to 19:00, before the onset of the dark phase. For social transmission of food preference, two mice of the same litter (WT or Sarm1 knockdown mice) were housed with one wild-type demonstrator for three days before the assay. The sample sizes for each experiment are indicated in the figures.

2.2. Open field

The apparatus for the open field test was an open transparent plastic box (40 × 40 × 32.5 cm). Four equal-sized squares were marked in the corners of the box on the bottom. The total area of the four corners was equal to the area of the central square region. For the open field test, mice were placed individually in a corner of the box, and their movements were recorded for 30 min by videotaping from the top. The total distance moved in the entire arena and the time spent in the four corner squares and center square were quantified using the Smart Video Tracking System (Panlab, Barcelona, Spain).

2.3. Elevated plus maze

The elevated plus maze was made in the shape of a horizontal cross consisting of two open arms (30 cm) and two closed arms (30 cm) enclosed by 14 cm-high walls. The arms were extended from a central platform, which was a 5-cm square. The plus maze was elevated to a height of 45.5 cm from the floor. An individual mouse was put on the central platform facing an open arm and allowed to explore the entire plus maze. The movement of each mouse was recorded for 10 min by videotaping from the top. The total distance travelled and time spent in the open and closed arms in the first 5 min and the entire 10 min were analyzed using the Smart Video Tracking System (Panlab, Barcelona, Spain).

2.4. Light–dark box

The apparatus for this test was modified from the open field box. An open black box (19 × 39 × 45 cm) was inverted and put into the open field box to divide it into two compartments of equal size. A small opening (5 cm in diameter) at the bottom of the black box allowed the access of mice between the two compartments. An individual mouse was placed into the lit compartment and then allowed to explore the apparatus for 10 min. The movement was recorded by videotaping and the percentage of time in the light box was analyzed using the Smart Video Tracking System (Panlab, Barcelona, Spain).

2.5. Contextual and cued fear conditioning

The training chamber (Box A) consisted of a transparent plastic box with side walls and a ceiling (14 × 14 × 19.5 cm). The floor of the chamber was composed of stainless steel grids, which were used to deliver a foot shock of 0.6 mA. The chamber was placed in a sound-attenuating box, which was illuminated by a 7.5 W