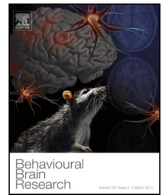
Behavioural Brain Research 292 (2015) 432–442

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr



Research report

Heterozygous L1-deficient mice express an autism-like phenotype

Bruno Sauce[a], Christopher Wass[a], Meera Netrakanti[a], Joshua Saylor[a], Melitta Schachner[b,**], Louis D. Matzel[a,*]

[a] Department of Psychology, Program in Behavioral and Systems Neuroscience, Rutgers University, Piscataway, NJ 08854, USA
[b] Keck Center for Collaborative Neuroscience and Department of Cell Biology and Neuroscience, Rutgers University, Piscataway, NJ 08854, USA

HIGHLIGHTS

- We investigate the behavior of mice with one copy of the L1 gene inactivated.
- Subjects are socially impaired, exhibit repetitive behaviors, and aversion to light.
- Subjects express normal levels of anxiety, motor abilities, and spatial learning.
- Reduced expression of L1 might contribute to the development of autism.

ARTICLE INFO

Article history:
Received 25 February 2015
Received in revised form 19 May 2015
Accepted 22 May 2015
Available online 14 June 2015

Keywords:
L1CAM
Social behavior
Mice
Autism
Spatial learning

ABSTRACT

The L1CAM (L1) gene encodes a cell adhesion molecule that contributes to several important processes in the developing and adult nervous system, including neuronal migration, survival, and plasticity. In humans and mice, mutations in the X chromosome-linked gene L1 cause severe neurological defects in males. L1 heterozygous female mice with one functional copy of the L1 gene show complex morphological features that are different from L1 fully-deficient and wild-type littermate mice. However, almost no information is available on the behavior of L1 heterozygous mice and humans. Here, we investigated the behavior of heterozygous female mice in which the L1 gene is constitutively inactivated. These mice were compared to wild-type littermate females. Animals were assessed in five categories of behavioral tests: five tests for anxiety/stress/exploration, four tests for motor abilities, two tests for spatial learning, three tests for social behavior, and three tests for repetitive behavior. We found that L1 heterozygous mice express an autism-like phenotype, comprised of reduced social behaviors and excessive self-grooming (a repetitive behavior also typical in animal models of autism). L1 heterozygous mice also exhibited an increase in sensitivity to light, assessed by a reluctance to enter the lighted areas of novel environments. However, levels of anxiety, stress, motor abilities, and spatial learning in L1 heterozygous mice were similar to those of wild-type mice. These observations raise the possibility that using molecules known to trigger L1 functions may become valuable in the treatment of autism in humans.

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1. Introduction

The glycoprotein L1 is a cell adhesion molecule that contributes to neuronal cell migration and survival, neuritogenesis, axon guidance, myelination, and synaptic activity and plasticityduring development and in adults [1–6]. The importance of L1 is evidenced by the severe neurological defects consequent to mutations in its gene. Mutations in the L1 gene in humans can produce the “L1 syndrome”, a severe and rare neurological disorder characterized by mental retardation, dilated cerebral ventricles, hypoplasia of the corticospinal tract, and agenesis of the corpus callosum [7], as well as Hirschsprung's disease [8]. Similarly, mice carrying null mutations in the L1 gene are hydrocephalic (to varying degrees depending on the genetic background) and have abnormal development in the projection of the corticospinal tract and in axons of the corpus callosum [9–11]. In addition, mice with developmentally delayed ablation of the L1 gene under the control of a neuron-specific promoter are impaired in learning and memory [12].

* Corresponding author at: Department of Psychology, Rutgers University, Piscataway, NJ 08854, USA. Fax: +1732 445 2263.
** Corresponding author at: Department of Cell Biology and Neuroscience, Rutgers University, Piscataway, NJ 08854, USA. Fax: +1 732 445 2063.
E-mail addresses: schachner@biology.rutgers.edu (M. Schachner), matzel@rci.rutgers.edu (L.D. Matzel).

http://dx.doi.org/10.1016/j.bbr.2015.05.040
0166-4328/© 2015 Published by Elsevier B.V.

The gene coding for L1 is located on the X chromosome. Owing to the random inactivation of genes on the X chromosome, female carriers of L1 mutations are heterozygous at the level of the L1-expressing cells, in that each cell expresses either the normal or the mutated gene. In humans, heterozygous carriers (L1+/−) of the L1 syndrome usually show a mild phenotype, being thus very different from the severe symptoms of L1-deficient (L1-/y) males [13]. And in mice, contrary to what one would expect, heterozygous females show a complex set of morphological features that are not intermediate between wild-type (L1+/+) and fully L1-deficient (L1-/y) mice [14].

Two features in L1 heterozygous female mice observed by Schmid et al. are of special relevance: (1) L1 heterozygous mice have a higher density of neurons, but not astrocytes, in the motor-sensory cortex relative to their wild-type littermates [14]. This suggests that L1 might modulate motor functions, a possibility that is supported by the observation that application of L1 to cultures of motor neurons prevented their death [15]. (2) Compared to wild-type mice, heterozygous females express a larger volume of the corpus callosum, a tendency to larger sizes of cortices and hippocampi, and, at the microscopic level, a higher density of neurons in the cortex and basal ganglia of both young and adult mice [14]. Furthermore, apoptosis was reduced in L1 heterozygous mice at early postnatal age, implying that not solely the density, but the overall number of neurons is increased in L1 heterozygous mice. Interestingly, increased neuronal density and growth during development has been described in autistic persons [16,17]. And in a case study, a boy with a mutation in the L1 gene presented autistic features with several stereotyped movements of hands and upper limbs [18].

To our knowledge, there are only a few studies measuring behavioral phenotypes in L1-deficient mice. Regarding L1 heterozygous mice, no systematic behavioral investigation has ever been carried out. Here, we designed a study to begin to fill this gap. Given the complexity and novelty in their neuroanatomical traits which are reminiscent of traits of autistic persons, we believe that an exploration of the behavior of constitutively L1-deficient heterozygous female mice is critical to gain more insights into the phenotypes of human female carriers with L1 null mutations.

Here, we investigated the behavior of L1-deficient heterozygous mice by performing a battery of tests in five different categories: anxiety and stress, motor abilities, spatial learning (and spatial learning under stress), social behavior, and repetitive behaviors. We chose/created these tests aiming for functional specificity, so they could be diagnostic of quantitative abnormalities and be easily replicated by other researchers. In particular, we hypothesized that the L1 heterozygote females would exhibit motor impairments as well as autism-like phenotypes of impaired social behavioral and increased repetitive behaviors.

2. Materials and methods

2.1. Animals

The constitutively L1-deficient heterozygous mouse line used in our study was created from animals with an insertion of a tetracycline-controlled transactivator into the second exon of the L1 gene, and then backcrossed for at least 10 generations onto a 129/SvJ background [9,10]. We tested nine female L1 heterozygous (L1−/+) mice and seven female wild-type (L1 +/+) littermate controls, weighing 23–37 g (weight did not differ significantly between the groups). At the start of the study, animals were four months old and were housed individually in a Plexiglas shoebox style cages in a controlled room under SPF conditions and a 12:12 h dark/light cycle with food and water ad libitum. During this time, we handled the mice daily (90 sec/day) for two weeks.

2.2. Behavioral tests

We assessed the animals' behavior with tests in five different categories: 5 tests of anxiety and stress, 4 tests in motor abilities, 2 tests in spatial learning, 3 tests in social behavior, and 3 tests in repetitive behaviors. This diversity of tests was designed to allow a broad spectrum screening. We applied the tests in the same order as reported below, with an interval of about 2 days between each test.

2.2.1. Anxiety, stress, and light responsivity

2.2.1.1. Open field. The open field test is a commonly used test of anxiety, where mice are allowed to explore a novel (typically stress-inducing) open space (for a review on the topic, see Prut and Belzung) [19]. Here, we used a 46 × 46 cm box with 20 cm high walls of white Plexiglas as the open field. The floor of the box was divided with tape into a 6 by 6 grid pattern (7.65 cm for each square), resulting in 20 squares next to the outer walls of the field (i.e., "walled squares"), and 16 squares in the interior of the field (i.e., "center squares"). The box was located in a brightly lit room (250 lx) in order to make the center squares even more anxiety-producing, as mice are averse to open spaces and bright lights. We placed the animals in the center of the box and allowed them to explore it for 5 min, while video recording for later scoring. As the measure of anxiety/stress reactivity, we used the relative time (in percentage) spent in the center squares. Lower numbers indicate more anxiety/stress reactivity.

2.2.1.2. Elevated plus-maze. The elevated plus-maze is a commonly used test of anxiety or stress reactivity where, similarly to the open field test, mice are allowed to explore stress-inducing open spaces (for a review, see Hogg) [20]. Here, we used a maze made of grey Plexiglas in the form of a "plus" shaped platform, 30 cm above the ground, and with four arms (each 4.4 cm wide, 28 cm long). Two opposing arms of the maze were enclosed by 8 cm high, grey Plexiglas walls, and the other two arms were open. The maze was located in a brightly-lit room (250 lx) in order to increase the difference in aversiveness between open and closed arms, as mice are averse to open spaces and bright lights. We placed the mice in the center of the maze facing an open arm and allowed them to explore for 4 min, while video recording for later scoring. As the measure of anxiety/stress reactivity, we used the relative time (in percentage) spent on the open arms. Lower numbers indicate more anxiety/stress reactivity.

2.2.1.3. Light/dark box. The light/dark box test is commonly used to measure anxiety, where mice are allowed to choose between staying in a dark compartment or in a brightly lit, aversive compartment (for a review, see Bourin and Hascoët) [21]. Here, we used a 56 × 15 × 10 (length × width × height) Plexiglas chamber divided into two equal sized compartments (28 cm in length). One compartment was the "dark side", black and lit at 10 lx, and the other compartment was the "light side", white and lit at 300 lx. The whole chamber had a covering lid and a center wall with a 3 cm opening connecting the dark and light sides. We started animals in the black side and observed their behavior for four minutes. As the measure of anxiety/stress reactivity, we used the time spent on the light side. Lower numbers indicate more anxiety/stress reactivity.

2.2.1.4. Light gradient. In order to assess if the results from the light/dark Box test were a reflection of differences in light sensitivity (as opposed to differences in anxiety and stress), we tested the mice in the light gradient test. The apparatus was a thin gray

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