Behavioural Brain Research 229 (2012) 265–272

Contents lists available at SciVerse ScienceDirect

Behavioural Brain Research

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

Research report

GluA3-deficiency in mice is associated with increased social and aggressive behavior and elevated dopamine in striatum

Abby Adamczyk[a], Rebeca Mejias[a], Kogo Takamiya[b,1], Jennifer Yocum[c], Irina N. Krasnova[d], Juan Calderon[a], Jean Lud Cadet[d], Richard L. Huganir[b], Mikhail V. Pletnikov[c], Tao Wang[a,*]

[a] McKusick-Nathans Institute of Genetic Medicine, Department of Pediatrics, Johns Hopkins University School of Medicine, 733 North Broadway BRB 513, Baltimore, MD 21205, USA
[b] Department of Neuroscience and Howard Hughes Medical Institute, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA
[c] Department of Psychiatry and Behavioral Sciences, Johns Hopkins School of Medicine, Baltimore, MD 21205, USA
[d] Molecular Neuropsychiatry Research Branch, National Institute on Drug Abuse, NIH/DHHS, Baltimore, MD 21224, USA

ARTICLE INFO

Article history:
Received 9 May 2011
Received in revised form
30 November 2011
Accepted 4 January 2012
Available online 21 January 2012

Keywords:
Glutamate receptor
GluA3
Striatum
Dopamine
Serotonin
Olfactory bulb
Aggression
Sociability
Social interaction
Mice

ABSTRACT

Glutamate signaling has been implicated in the regulation of social behavior. AMPA-glutamate receptors are assembled from four subunits (GluA1–4) of mainly GluA1/2 and GluA2/3 tetramers that form ion channels of distinct functional properties. Mice lacking GluA1 showed a reduced anxiety and male aggression. To understand the role of GluA3 in modulating social behavior, we investigated GluA3-deficient mice (*Gria3* −/Y) on C57BL/6J background. Compared to wild type (WT) littermates ($n = 14$), *Gria3* −/Y mice ($n = 13$) showed an increase in isolation-induced male aggression ($p = 0.011$) in home cage resident–intruder test; an increase in sociability ($p = 0.01$), and increase in male–male social interactions in neutral arena ($p = 0.005$); an increase in peripheral activities in open field test ($p = 0.037$) with normal anxiety levels in elevated plus maze and light–dark box; and minor deficits in motor and balance function in accelerating rotarod test ($p = 0.016$) with normal grip strength. *Gria3* −/Y mice showed no significant deficit in spatial memory function in Morris-water maze and Y-maze tests, and normal levels of testosterone. Increased dopamine concentrations in stratum ($p = 0.034$) and reduced serotonin turnover in olfactory bulb ($p = 0.002$) were documented in *Gria3* −/Y mice. These results support a role of GluA3 in the modulation of social behavior through brain dopamine and/or serotonin signaling and different AMPA receptor subunits affect social behavior through distinct mechanisms.

1. Introduction

Glutamate mediates the majority of excitatory neurotransmission in the central nervous system via a family of receptors with distinct substrate specificity [1,2]. Glutamate receptors are widely expressed in brain structures that control emotion and behavior [3] suggesting a role of glutamate signaling in behavioral modulation. AMPA glutamate receptors are assembled from four subunits, GluA1–4, to tetrameric complexes [1–3] with a maximum of two different subunits present in each receptor complex [4,5]. The subunit composition of AMPA receptors influences their ion permeability, rectification, and kinetics [1,2,6].

The roles of AMPA receptors in modulating behavioral phenotype have been studied in animal models. Two competitive antagonists for AMPA receptors, CNZX and NBQX, reduced the biting component of aggressive behavior in Turku aggressive (TA) mice and GYKI, a non-competitive antagonist, suppressed all aggressive manifestations [7]. Mice lacking GluA1 [8] or carrying a mutation, R582Q, resulting in calcium-impermeable receptors [9] exhibited a reduced long-term potentiation, deficits in spatial working memory, reduced male aggression, and anxiety [10]. Mice-lacking GluA2 displayed an enhanced long-term potentiation, reduced exploratory activity, and impaired motor function [11]. Mice lacking GluA3 showed a significantly enhanced long term potentiation (LTP) and normal depotentiation after the establishment of LTP [12]. GluA3-deficient mice were also found to have a deficit in motor and balance, increased alcohol consumption after alcohol deprivation [13], a disturbance in non-rapid eye movement sleep and respiratory modulation, and an increased tendency of seizure [14]. A recent genome-wide scan of aggressive NZB/B1NJ and unaggressive A/J mice identified proximal

Abbreviations: AMPA, 2-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid; GluA3, AMPA glutamate receptor 3; NE, norepinephrine; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; 5-HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid.

* Corresponding author. Tel.: +1 443 287 3525; fax: +1 410 955 7397.
E-mail address: twang9@jhmi.edu (T. Wang).
[1] Present address: Department of Integrative Physiology, University of Miyazaki Faculty of Medicine, Miyazaki, 889-1692, Japan.

0166-4328/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbr.2012.01.007

chromosome X including *Gria3* as one of the two quantitative trait loci for aggression [15].

Despite these studies, little is known about the contribution of GluA3 to social behavior. In this study, we investigated behavioral phenotype and brain monoamine levels of *Gria3* −/Y mice on C57BL/6J background [16]. *Gria3* −/Y mice were found to have an increase in isolation-induced male aggression, sociability, male–male social interactions in neutral arena, elevated dopamine in striatum, and reduced serotonin turnover in olfactory bulb. The results support a role of GluA3 in the modulation of social behavior and implicate that different AMPA subunits influence social phenotype through distinct mechanisms.

2. Materials and methods

2.1. Animals

Gria3 −/Y mice were generated by standard gene targeting method on a mixed C57BL/6J and 129 (129X1/SvJ × 129S1) strain background [16]. These mice have been back-crossed with C57BL/6J inbred strain (Jackson Laboratory, ME) for over 20 generations in order to achieve a homogenous strain background. Male *Gria3* KO (*Gria3* −/Y) and wild type (WT) littermate control mice (*Gria3* +/Y) were generated by breeding WT C57BL/6J males with heterozygous (*Gria3* +/−) females. Mice were genotyped using PCR of tail DNA following a published protocol [16]. Adult *Gria3* −/Y (n = 14) and *Gria3* +/Y mice (n = 13) between 4 and 9 months of age were studied using standard behavioral tests for rodent. Mice were housed in temperature-controlled rooms with 12 h light/dark cycle (9:00 and 21:00) and had free access to water and standard mouse chow. Animal breeding and procedures were conducted in strict accordance with NIH Guide for Care and Use of Laboratory Animals. An animal study protocol for this project was approved by the Johns Hopkins University Animal Care and Use Committee.

2.2. Mouse behavioral testing

Mouse behavioral tests were conducted at Animal Behavioral Core of Johns Hopkins University School of Medicine following standard protocols from the Animal Behavior Core User Manual (http://www.brainscienceinstitute.org/index.php/cores) as described previously [17,18]. The test order and age of mice for individual test (in parenthesis) are provided as follow: open field (4 month), Y-maze (4 month), elevated plus maze (5 month), resident–intruder test (5 month), sociability and preference for social novelty (6 months), dyadic male–male interaction (6 month), rotarod (6 months), Morris water maze (7 month), light and dark box (9 month), grip strength (9 month), general olfaction (9 month), sample harvesting (9 month). For individual test, WT and knockout mice were always tested together to minimize potential variations. At least one week of rest was arranged between tests. Average ambient lighting (lux) for individual tests: open field (288–318), elevated plus maze (492), light–dark box (492 for light box and 0 for dark box, respectively), resident–intruder test, dyadic male–male interaction and sociability and social novelty (595).

2.2.1. Open-field test

Each individual test mouse was placed in a photo-beam (n = 16 at equal spacing of 2.5 cm) equipped clear plastic chamber (45 cm × 45 cm and was allowed to explore free from interference for 30 min. The peripheral area (425 cm^2) was defined by the two side-photo beams, #1–2 and #15–16 while the central area (1600 cm^2) was defined by photo beams #3–14 at each direction. Their movements were tracked using a SDI Photobeam Activity System (San Diego Instruments). Their patterns of ambulatory movement, fine movement, and rearing behavior at central and peripheral areas were recorded and analyzed.

2.2.2. Sociability and preference for social novelty

The test was carried out in a 45 cm × 45 cm × 37.5 cm (H) clear plastic chamber divided equally into four quadrants. Two small mesh cages (10 cm in diameter, 15 cm high) were placed at the opposite corners of two quadrants. The test mouse was allowed to explore the chamber freely for 5 min with the small empty mesh cages before starting test trials. For trial 1, a wild-type stranger male mouse was placed inside one of the mesh cages and the test mouse was allowed to explore the chamber freely for 10 min. For trial 2, a second wild-type stranger mouse was placed in the other mesh cage and the test mouse was allowed to explore freely for another 10 min. The time that the test mouse spent in each of the four quadrants was measured during each of the 10 min sessions and analyzed.

2.2.3. Resident–intruder test

The test was carried out in the individual home cage of the testing male after seven days of isolation. On the eighth day, a young and unfamiliar intruder male was placed in the home cage of the test mouse. The mice were then allowed to interact free from interference for 10 min. The intruders were 2-month-old male mice of C57BL/6J strain without significant fighting experience. They were housed in separate cages from resident mice. Each intruder mouse was used only once in this test on any given test day. The entire interactions were video-recorded. Aggressive behavior (attacks and tail rattles) and nonaggressive social behavior (sniffing and following) were scored and analyzed by two independent observers.

2.2.4. Dyadic male–male interaction in neutral field

The test was carried out in a 45 cm × 45 cm × 37.5 cm (H) clear plastic box that is unfamiliar to both test mouse and stranger mouse. A test mouse and a stranger male mouse of same age and strain background were placed in the box that was separated by a divider. The mice were allowed to explore half of the box freely for 5 min. The divider was then removed and the mice were allowed to interact for 10 min free from interference. Aggressive behavior (attacks and tail rattles) and nonaggressive social behavior (sniffing and following) of the test mouse were video-recorded and analyzed.

2.2.5. Elevated-plus maze

Elevated plus maze tests anxiety-related behavior in rodents. The maze, made of stainless steel, consists of two closed arms measuring 48 cm (L) × 10 cm (W) × 38 cm (H) and two open arms measuring 48 cm (L) × 10 cm (W) (San Diego Instruments). The four arms were connected by a middle 10 cm × 10 cm platform. The test mouse was placed on the middle platform and remained in the maze during the 5 min session. The total time spent and number of entries into the closed and open arms were recorded and analyzed.

2.2.6. Light–dark box test

This test is for anxiety-related behavior in rodents. A test mouse was placed in the dark side of a light–dark box [35 cm (W) × 17.5 cm (D) × 3 cm (H), Coulbourn Instruments] and allowed to explore free from interference for 5 min. The time elapsed before the mouse entered the light side as well as the total time spent in each side of the box was recorded and analyzed.

2.2.7. Y-maze

The Y-maze for spatial working memory in rodents consists of three identical arms [46 cm (L) × 6.25 cm (W) × 2.5 cm (H)] radiating at 120° angles from a central platform. The test was done in three trials. During the first trial, the test mouse was placed at the end of one arm, chosen at random prior to the test, and remained in the maze free from interference for 5 min. The total number of spontaneous alternations divided by the number of total possible alternations was recorded and analyzed. The second and third trials were run seven days after the first trial. During the second trial, one of the arms, chosen randomly for each mouse prior to the test, was blocked. The test mouse was allowed to explore the two unblocked arms for 5 min followed by a resting period of 10 min. During the third trial, the test mouse was returned to the maze with all three arms open and allowed to explore for another 5 min. The third trial was analyzed for time spent in the arm blocked during the second trial. The data was analyzed for the first 2 min and full 5 min of trial 3.

2.2.8. Morris water maze

Morris water maze for spatial reference memory was tested in WT (n = 9) and *Gria3* KO (n = 9) mice. A standard water maze (120 cm in diameter) containing deep, opaque water (25 °C) was set up with a rescuing platform (10 cm × 10 cm) just below the water surface and marked with a cue (flag) and four large spatial cues outside the maze. On test day 1, mice were subjected to four, 1-min swimming trials in the maze to locate the platform. In each of the four trials, the platform was placed at a different quadrant of the maze. On test days 8–10, the platform cue was removed and the mice were subjected to three, 1-min trials to locate the hidden rescuing platform placed at a fixed quadrant of the maze. On test day 11, mice were subjected to one trial of 3 min of free swimming without platform. Their time spent in the quadrant where the platform was located during the hidden platform trials was determined and compared between WT and KO groups.

2.2.9. Rotarod test

A test mouse was placed on a rotating rod that is accelerated from 5 to 30 rpm during a 5 min session in a standard testing apparatus (Rotamex-5 from Columbus Instruments with mouse spindle). The performance was graded by the time a mouse stays on the rotating rod. Each mouse was tested under the same parameters three times each day for three days and a total of 9 trials. Data from all nine sessions was obtained and analyzed for each mouse.

2.2.10. Grip strength

This is to test forepaw grip strength in rodent. A grip strength meter was placed horizontally and mice, held by the tail, were allowed to grasp the pull bar with their front paws only. Mice were pulled backward, horizontally, and the peak force was recorded in pounds. This was done three times in a row during each of two training trials with 1-h in between. One hour after the training trial, a test trial was conducted with each mouse tested five times in a row. The highest and lowest values are removed and means and SEM for three middle values were calculated.

2.2.11. Olfaction test

A test mouse was placed in a fresh, clean cage for this test. After 5 min of free exploration, three drops of vanilla extract and three drops of water were placed at