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Research report

Somatosensory and sensorimotor consequences associated with the heterozygous disruption of the autism candidate gene, Gabrb3

Timothy M. DeLorey[a,*], Peyman Sahbaie[a], Ezzat Hashemi[a], Wen-Wu Li[c], Ahmad Salehi[b], David J. Clark[c]

[a] Molecular Research Institute, Palo Alto, CA 94303, United States
[b] Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Veterans Affairs Palo Alto Health Care System, Palo Alto, CA 94304, United States
[c] Department of Anesthesiology, Stanford University School of Medicine, Veterans Affairs Palo Alto Health Care System, Palo Alto, CA 94304, United States

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ABSTRACT

Autism spectrum disorder (ASD) is diagnosed based on three core features: impaired social interactions, deficits in communication and repetitive or restricted behavioral patterns. Against this backdrop, abnormal sensory processing receives little attention despite its prevalence and the impact it exerts on the core diagnostic features. Understanding the source of these sensory abnormalities is paramount to developing intervention strategies aimed at maximizing the coping ability of those with ASD. Consequently, we chose to examine whether sensory abnormalities were present in mice heterozygous for the Gabrb3 gene, a gene strongly associated with ASD. Mice were assessed for tactile and heat sensitivity, sensorimotor competence (accelerating rotarod task) and sensorimotor gating by prepulse inhibition of the acoustic startle reflex (PPI). All heterozygotes exhibited an increase in seizure susceptibility and similar reductions in Gabrb3 expression in the dorsal root ganglia, spinal cord, whole brain and amygdala. Interestingly, significant differences were noted between heterozygous variants in regards to tactile sensitivity, heat sensitivity, sensorimotor competence and PPI along with differences in Gabrb3 expression in the reticular thalamic nucleus and the bed nucleus of stria terminalis. These differences were influenced by the heterozygotes' gender and whether the Gabrb3 gene was of paternal or maternal origin. These results are not adequately explained by simple haploinsufficiency of Gabrb3, therefore, additional mechanisms are likely to be involved. In addition, this is the first report of the occurrence of tactile and heat hypersensitivity in an ASD mouse model, two features often associated with ASD.

1. Introduction

$GABA_A$ receptors are ligand-gated chloride channels that play a critical role in neurodevelopment and mediate most of the fast synaptic inhibition in adult brain. Functional $GABA_A$ receptors are constructed from a pool of heterogeneous subunits that assemble together to form multiple subtypes that exhibit differing GABA sensitivities and pharmacologies [4,50]. The β_3 subunit, encoded by the GABRB3 gene, is the major β isoform present during brain development and is strongly implicated in ASD [1,59,61]. Although no one gene will be universally responsible for all idiopathic cases of autism, two recent studies of postmortem ASD brain found that the majority of brains studied displayed significant reductions in the expression of the β_3 subunit protein [15,59]. Moreover, the first direct evidence of *GABRB3* involvement in ASD, due to a nucleotide coding variant of *GABRB3*, was recently reported by Delahanty et al. [9].

The prevalence of autism spectrum disorder (ASD) has been estimated to be as high as 1 out of 91 children [34] with a 4:1 male to female ratio [18]. The core diagnostic traits of ASD include impaired social interactions, stereotypical or restrictive behaviors, and communication deficits [21]. However, a number of other behavioral abnormalities are common to ASD, including hyperactivity, epilepsy, sensorimotor deficits, sleep disturbances and sensory processing abnormalities in auditory, visual, tactile and thermal modalities [6,33,57]. Although sensory processing abnormalities are common to ASD they manifest in diverse ways, in a study of 200 cases of ASD about 39% showed hypo-responsiveness, 19% hyper-responsiveness and 36% displayed mixed responsiveness [23].

Although mice lacking the *Gabrb3* gene (*Gabrb3*−/−) exhibit a number of abnormalities relevant to ASD [10,12,65], mice with a heterozygous deletion of the *Gabrb3* gene are likely to be a more accurate representation of the molecular constituency of ASD. However, to date *Gabrb3* heterozygous mice have not been typically reported as having significant behavioral abnormalities. These observations may be, in part, due to the unusually high behavioral variability displayed by heterozygous mice [11,38,65] that were of a mixed-strain background, C57BL/6J × 129Sv/SvJ, two substrains

* Corresponding author. Tel.: +1 650 269 9805; fax: +1 650 210 0318.
E-mail address: tdelorey@ayatanainstitute.com (T.M. DeLorey).

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commonly reported as being behaviorally different [30,44]. To avoid such confounds we backcrossed the *Gabrb3* gene disruption for seven generations against pure C57BL/6J, largely reducing the 129Sv/SvJ contribution. We hypothesized that haploinsufficiency of *Gabrb3* expression, on a stable background, would be sufficient to alter behaviors often associated with ASD such as abnormalities in somatosensory processing and sensorimotor function. In this report, behavioral assessments were done in conjunction with measurements of *Gabrb3* expression levels in several neural components associated with the assessed behaviors.

2. Materials and methods

Techniques used to disrupt the *Gabrb3* gene have been previously described [29]. Mice with a heterozygous disruption of the *Gabrb3* gene, were originally obtained from Dr. Gregg Homanics at University of Pittsburgh, PA. These mice are now available from the Jackson Laboratory (Bar Harbor, ME). The mouse line used in the current study was backcrossed for seven generations against the background C57BL/6J strain. The nomenclature used throughout this manuscript identifies heterozygous mice (p/m) as containing a disrupted (−) or intact (+) *Gabrb3* gene on the paternal allele (p) or maternal allele (m). Therefore, a p−/m+ designation indicates that the mouse has a disrupted *Gabrb3* gene on the paternal allele and an intact *Gabrb3* gene on the maternal allele. Mice were evaluated between 10 and 36 weeks of age, within a weight range of 19–32 g. Two cohorts were used in this study, the first completed the following series of behavioral experiments in the same order (tactile, heat, wire test, marble burying and lastly seizure susceptibility) with 1 week between each set of behavioral tasks. A second cohort underwent rotarod testing followed a week later by startle and PPI evaluation. Throughout the study heterozygous mice were gender matched to littermate controls. Mice were housed in groups of 3–5 on a 12/12-h light/dark cycle (lights on at 06:00 h) in a climate-controlled room with food and water provided *ad libitum*. Genotyping was conducted by PCR on tail tissue samples. All animal protocols conformed to the guidelines determined by the National Institute of Health (USA) Office for Protection from Research Risks and were approved by the Animal Care and Use Committee of the Veterans Affairs Palo Alto Health Care System. Assessments were made relative to gender and whether the disrupted gene was of paternal or maternal origin. In all behavioral assays the evaluators were blinded to the genotypes of the mice being tested.

2.1. Behavioral assays

2.1.1. Mechanical threshold

Static tactile allodynia (tactile hypersensitivity) is predominantly mediated by Aδ fibers and can be detected by a heightened response to the application of small, stiff probes (i.e. von Frey monofilaments) to the skin of humans or the hind-paws of rodents [17,48]. The assessment of mechanical (tactile) sensitivity was determined according to the "up-down" method described by Chaplan et al. [7]. Briefly, a series of Semmes–Weinstein calibrated von Frey monofilaments (Stoelting Co., Wood Dale, IL) were used, with nine filament strengths chosen at logarithmic intervals, ranging from 0.04 to 4 g. Mice were allowed to acclimate for 30 min within a nonrestrictive Plexiglas cylindrical enclosures (diameter 10 cm and height 30 cm) placed on top of a wire-mesh surface elevated 40 cm above the table's surface. After the adaptation period the smallest diameter filament was applied to the center of the plantar surface of the hind-paw. The stimulus was applied for 5 s with enough force to bend the fiber slightly with withdrawal of the hind-paw from the fiber scored as a response. When no response was observed, the next stiffest fiber in the series was applied to the same paw. If a response was obtained, a less stiff fiber was applied, if no response occurred the next stiffer fiber was once again applied. Testing proceeded in this manner until four fibers, after the one that elicited the first response, were applied. Mechanical withdrawal thresholds were calculated using a data fitting algorithm of the response data, which permitted the use of parametric statistics for comparison analysis [55].

2.1.2. Heat sensitivity

In order to determine whether heterozygous mice exhibited a change in sensitivity to heat, the method of Hargreaves, modified for use in mice, was employed to assess heat hyperalgesia [25]. Mice were allowed to acclimate for 30 min in nonrestrictive Plexiglas enclosures (10 cm diameter × 30 cm height) placed on a clear glass plate maintained at 29 °C. A radiant heat source was activated in conjunction with a timer and focused onto the planter surface of the hind-paw. When the paw was withdrawn, both heat and timer were halted. The radiant heat intensity was adjusted to provide approximately a 10 s paw withdrawal baseline for control mice (+/+), this setting was used in all subsequent experiments. A maximal cut-off of 20 s was used to prevent tissue damage. Four measurements were made per animal per test session.

2.1.3. Acoustic startle and prepulse inhibition

The methods used for assessing acoustic startle, a primitive brainstem reflex, and prepulse inhibition (PPI), a phenomenon in which a weak pre-stimulus reduces the startle response to subsequent startling stimulus, were adapted from Frankland et al. [19]. Startle testing was conducted using a SR-Lab startle response system (San Diego Instruments, San Diego, CA). Mice were placed in a nonrestrictive Plexiglas cylinder (3.2 cm internal diameter) resting on the sensor platform within the ventilated apparatus. A piezoelectric accelerometer, attached to the base of the sensor platform, detected and transduced all mouse movements, which were digitized and stored by computer. Acoustic startle stimuli, prepulse stimuli and the continuous background white noise of 65 decibels (dB) were delivered via a high-frequency speaker (frequency range of 0.4–16 kHz) located 28 cm above the containment cylinder. The speakers, containment cylinder and sensor platform were housed within a sound-attenuated chamber. The startle amplitude was taken to be the maximal response that occurred within 65 ms of the presentation of the startle stimulus (recorded every ms). The sound levels for background noise and startle/prepulse stimuli were verified with a digital sound level meter, and the SR-Lab calibration unit was used routinely to ensure uniform sensitivity between test chambers.

Following an acclimation period of 5 min within the containment cylinder, mice were exposed to an acoustic startle stimuli at each of five different startle stimulus intensities (80, 90, 100, 110, 120 dB) with the startle reflex amplitude being recorded and stored via computer interface. PPI was evaluated by the presentation of a non-startling acoustic prepulse just prior to a startling stimulus with measurements reflecting the percentage inhibition of the normal startle amplitude that occurred in the absence of the prepulse. Each PPI trial consisted of a startle stimulus (120 dB, 40 ms), which was preceded by a prepulse of 70, 80 or 90 dB with a fixed interval (100 ms) between onsets of the prepulse and startle stimuli. A prepulse was 20 ms in duration with a rise/fall time of less than 1 ms. Each trial was presented once in a pseudo-random sequence within each test session block with a variable inter-trial interval (ITI) of 12–30 s (average 15 s). A total of 10 trial blocks for each startle and PPI session were presented. The following formula was used to calculate % prepulse inhibition of a startle response: % PPI = 100 − [(startle response with prepulse presented before the startle stimulus/startle stimulus response alone) × 100].

2.1.4. Accelerating rotarod

In the accelerating rotarod task, a mouse is required to constantly make postural adjustments in order to maintain balance on the rotarod and avoid falling off. The rotarod (Smartrod Model SRTC, AccuScan Instruments, Columbus, OH) consists of a 10-cm-diameter rubber-coated cylinder that can revolve at varying speeds. Each trial was started by placing a mouse on the stationary rod for 30 s to acclimate, followed by the rod being accelerated from 3 to 19 rpm over a 180 s period. Each mouse received one trial per day for seven consecutive days. Latency time to fall off the accelerating rotarod was recorded automatically by the Smartrod.

2.1.5. Marble burying

The marble burying procedure has been reported to assess obsessive-compulsive tendencies as well as repetitive/perseverative responses in mice [63,67]. The marble burying procedure used was adapted, with minor modifications, from previous studies [67]. Briefly, mice were acclimated to the testing room for 1 h prior to test chamber adaptation (Day 1) and prior to testing (Day 2). During adaptation, each animal was individually acclimated for 30 min to the clear polypropylene testing chamber (13 cm × 20 cm × 30 cm) containing a 5 cm deep layer of sawdust bedding. On day 2, mice were individually placed back into the test chamber, which now contained 24 clear glass marbles (1 cm in diameter) placed in six rows of four, on top of a 5 cm deep layer of sawdust. The number of marbles fully buried in 30 min was recorded.

2.1.6. Wire-hanging test

The wire-hanging test is a simple approach used to measure neuromuscular ability (muscle tone and grip strength) of a rodent by assessing the animal's ability to hang suspended by its forepaws from a wire (2 mm diameter) 30 cm above a sawdust covered surface for a maximum time of 1 min [32,49]. Latency to fall was measured from the time a mouse was placed hanging by its forepaws on the wire until it fell. The test was performed twice for each mouse with results from each mouse being averaged and data analyzed.

2.1.7. Pentylenetetrazol (PTZ) induced seizures

PTZ-induced seizure sensitivity experiments were conducted using a minimal number of animals. A sub-convulsant dose of PTZ (35 mg/kg) in 0.9% sodium chloride solution was administered via intra-peritoneal (i.p.) injection. Mice were scored using the seizure scoring method of Schwaller et al. [60]. Specifically, seizures were scored as follows: (1) behavioral arrest and staring, (2) whole body twitching or mild tremor, (3) myoclonic jerking of the head and forelimbs numbering less than 20, (4) myoclonic jerking of the head and forelimbs number more than 20, (5) bilateral forelimb clonus (6) generalized tonic-clonic seizures, often involving wild running and jumping behavior with loss of postural control, (7) status epilepticus defined as 10 min or more of continuous or closely spaced seizures with no return to normal behavior, often resulting in death. All sessions were videotaped for 30 min and scored by two blinded observers.

2.1.8. Collection of dorsal root ganglia (DRG), spinal cord and whole brain

Mice were sacrificed by CO_2 inhalation. The posterior elements of the spine were removed and the segmental nerves were cut immediately distal to the ganglion