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Research report

Gabrb3 gene deficient mice exhibit impaired social and exploratory behaviors, deficits in non-selective attention and hypoplasia of cerebellar vermal lobules: A potential model of autism spectrum disorder

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Abstract

Objective: $GABA_A$ receptors play an important regulatory role in the developmental events leading to the formation of complex neuronal networks and to the behaviors they govern. The primary aim of this study was to assess whether gabrb3 gene deficient ($gabrb3^{-/-}$) mice exhibit abnormal social behavior, a core deficit associated with autism spectrum disorder.
Methods: Social and exploratory behaviors along with non-selective attention were assessed in $gabrb3^{-/-}$, littermates ($gabrb3^{+/+}$) and progenitor strains, C57BL/6J and 129/SvJ. In addition, semi-quantitative assessments of the size of cerebellar vermal lobules were performed on $gabrb3^{+/+}$ and $gabrb3^{-/-}$ mice.
Results: Relative to controls, $gabrb3^{-/-}$ mice exhibited significant deficits in activities related to social behavior including sociability, social novelty and nesting. In addition, $gabrb3^{-/-}$ mice also exhibited differences in exploratory behavior compared to controls, as well as reductions in the frequency and duration of rearing episodes, suggested as being an index of non-selective attention. $Gabrb3^{-/-}$ mice also displayed significant hypoplasia of the cerebellar vermis compared to $gabrb3^{+/+}$ mice.
Conclusions: The observed behavioral deficits, especially regarding social behaviors, strengthens the face validity of the gabrb3 gene deficient mouse as being a model of autism spectrum disorder.

Keywords: Social behavior; Cerebellar vermis; Exploration; Non-selective attention; Nest building

1. Introduction

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder characterized by impaired social behaviors, stereotypical or restrictive behavioral patterns, and deficits in language and communication [31]. In addition to the three core deficits, a wide continuum of behavioral phenotypes have also been reported in association with ASD, including cognitive impairment, hyperactivity, epilepsy, motor deficits, attentional deficits, hypotonia and sleep disturbances [2,11,47,74]. To date, no single region of the brain or pathophysiological mechanism has yet been identified as being the cause of ASD. However, the cerebellum, frontal cortex, hippocampus, amygdala and the cerebello-thalamo-cortical circuit have all been implicated in ASD [68]. The neural underpinnings of ASD are also poorly understood, although, there is evidence for a strong genetic component [60], even though no candidate gene has been verified to date. Nevertheless, one part of the genome stands out, chromosomal region 15q11-q13, which has been strongly implicated in ASD via linkage and association studies [50,51,56,70]. Furthermore, maternal duplications of this region remain one of the most common cytogenetic abnormalities found in cases of idiopathic ASD, accounting for about 1–2% of the cases reported [22]. In addition, deletion of this region

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results in either Angelman syndrome or Prader-Willi syndrome depending from which parent the deletion was inherited. Both syndromes often meet the diagnostic criteria for ASD as defined by DSM-IV [61,65,69,73]. Within this chromosomal region there exists a gene cluster of $GABA_A$ receptor subunit genes: GABRB3, GABRA5, and GABRG3, encoding for the $GABA_A$ receptor subunits β_3, α_5, and γ_3, respectively.

$GABA_A$ receptors are heterooligomeric GABA-gated chloride channels constructed from eight classes of subunits exhibiting varying amino acid sequence homologies (α_{1-6}, β_{1-4}, γ_{1-3}, δ, ρ_{1-2}, π, ε and θ) that produce multiple $GABA_A$ receptor isoforms with various GABA sensitivities and associated pharmacologies [14,58]. These subunits exhibit unique regional and temporal distribution within the central nervous system. During development $GABA_A$ receptors play a role in proliferation, migration, and differentiation of precursor cells that orchestrate the development of the embryonic brain [9]. A developmental deficiency in any of these roles would adversely effect the temporal ordering of neurogenesis and synaptogenesis, thereby affecting maturation of circuits that are later involved in complex behaviors.

The high prevalence rate of ASD, 1 out of 150 births [20] has prompted an urgency to develop animal models as a fundamental step toward comprehending the complex molecular underpinnings associated with this disorder. A suitable animal model should meet three fundamental criteria (1) *face validity*: behavioral characteristics should mirror those present in the human disorder; (2) *construct validity*: similarities in the underlying etiology should exist between the human disorder and the animal model; and (3) *predictive validity*: the outcome of a treatment regime applied to the animal model should reflect the likely impact on humans with the disorder. While no model can be expected to replicate the full complexity of the human behavioral phenotype, an animal model that exhibits specific behavioral and morphological characteristics typically associated with ASD would be invaluable.

Numerous studies, employing a diverse set of approaches including autoradiographic, molecular biological, and genetics, provide overwhelming support for the role of GABAergic mechanisms in the etiology of ASD [13,17,38,70]. In light of these observations, mice that have a targeted disruption of the mouse equivalent of the human GABRB3 gene, which encodes the β_3 subunit of the $GABA_A$ receptor, can be argued to possess construct validity in reference to ASD. In addition, gabrb3 gene disrupted ($gabrb3^{-/-}$) mice exhibit numerous behavioral abnormalities, including many often reported in association with ASD [26,27,39]. In this present study we sought to further extend the face validity inherent to the $gabrb3^{-/-}$ mouse relative to behaviors associated with ASD. Given the primacy of its deficit in ASD, special emphasis was placed on the assessment of social behavior. In addition, we assessed other behaviors often impaired in ASD, including exploratory behavior and non-selective attention. Lastly, as the cerebellar vermis has been cited as being abnormal in ASD [64] we performed a simple morphological assessment of this region in $gabrb3^{-/-}$ and $gabrb3^{+/+}$ mice. In addition, this region has been reported to be crucial to the consolidation of aversely motivated contextual memory, an elementary form of spatial learning [66] and a feature previously found to be disrupted in $gabrb3^{-/-}$ mice [27]. The cerebellum has also been reported to be involved in exploratory behaviors [62], shifting attention [1] and spatial orientation [41], features likely to impact the behaviors being assessed within the current study. In light of the overwhelming evidence implicating the GABAergic system in ASD and the numerous parallels between the ASD phenotype and the phenotype observed in $gabrb3^{-/-}$ mice, this timely study provides a crucial connection between the disruption of the gabrb3 gene and the impairment of social behavior, a key diagnostic component of the ASD phenotype. Insights gained from the current efforts aid in bridging gaps in our understanding of the interconnectiveness between genetics, development and behavioral outcome.

2. Methods

2.1. Mice

All mice used in this study were male, consistent with the 4:1 male–female ratio prevalent in autism [29]. Gabrb3 gene knockout mice ($gabrb3^{-/-}$) and wildtype littermates $gabrb3^{+/+}$ were produced at the University of Pittsburgh and the Veterans Affairs Palo Alto Health Care System. Techniques used to disrupt the gabrb3 gene have been previously described [39]. C57BL/6J and 129/SvJ mice were obtained from Jackson Laboratory (Davis, CA) at 6–8 weeks of age. 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. All mice were housed in groups of 4–8 on a 12-h light/12-h dark cycle under controlled temperature with food and water provided *ad libitum*. A number of studies report that the phenotype of a given single gene mutation is modulated by the genetic background of the inbred strain(s) in which the mutation is maintained (for review see Ref. [24]). As the genetic background of the $gabrb3^{+/+}$ and $gabrb3^{-/-}$ mice is a hybrid between 129Sv/SvJ and C57BL/6J mice, similar progenitor strains were also assessed in this study alongside the wildtype and knockout mice to assure that observed differences were not merely the result of stain variations within the two progenitor strains. The age of mice used in this study ranged from 16 to 52 weeks. The precise age range used for each assessment is listed under each method subsection. Due to a 90% mortality rate at birth of mice lacking the gabrb3 gene [39], the number of $gabrb3^{-/-}$ mice available to pursue the goals of this study represent the minimal amount of mice required to perform adequate statistical analysis. This low survival rate was a contributing factor in the wide age range of mice available for the present study. In an effort to reduce the impact of age differences on the results, we age matched all controls to the available $gabrb3^{-/-}$ mice.

2.2. Social interaction and preference for social novelty

The social interaction apparatus used in this study was a modified conditioned place preference apparatus (Med Associates Inc., St. Albans, VT), consisting of a polycarbonate box with two partitions dividing the apparatus into three separate chambers (two interaction chambers and one neutral chamber). Attached to each interaction chamber was a stimulus cage separated from the interaction chamber by a wire mesh wall with a 1 cm^2 grid mesh (Fig. 1). With the partitions open, a test mouse is able to move freely between chambers. Partitions are automatically opened and closed by a computer controlled interface (Med PC for Windows) and the output interface ENV-313/BD (Med Associates Inc.). The three chambers were equipped with infrared photo-sensors spaced 2 cm apart and 1 cm above the chamber floor with data collected via a Med Associates input module ENV-2561 and interface SG-6080/C. Both interaction chambers are identical in size, shape, color, floor texture and lighting with removable flooring for cleaning between experiments. The neutral chamber is similar in color and floor texture to the interaction chambers so as not to be aversive. During testing, "stranger" mice were placed in one or both stimulus

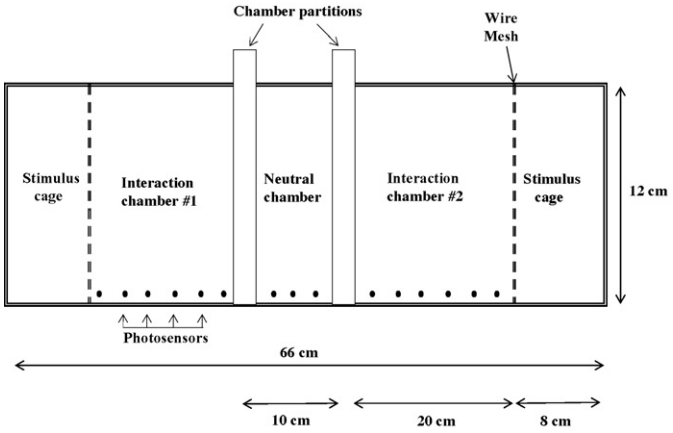

Fig. 1. Diagram of the automated apparatus used in assessing social behavior in mice. Side view showing location of infrared photo-sensors and chamber partitions. Partitions can be automatically opened to create a 5 cm wide opening, allowing the test mouse to pass freely from one chamber to the next. A wire mesh wall separates each of the interaction chambers from an adjacent stimulus cage, which will either be empty or contain a "stranger mouse".

cages. The wire mesh separating the interaction chamber from the stimulus cage allows nose contact between test mouse and stranger mouse, while preventing aggressive interactions and also prevents the "stranger" mouse from initiating the social contact.

2.2.1. Sociability

All test mice were experimentally naïve to the social interaction test and were habituated to the test room for at least 30 min prior to assessment. Testing was done on all four mouse genotypes: C57BL/6J, 129/SvJ, gabrb3$^{-/-}$ and gabrb3$^{+/+}$. The age range of the mice tested in these social assessments were between 16 and 52 weeks of age. Stranger mice were male C57BL/6J housed in groups of four per cage. The experimental design of both the social interaction and social novelty tests were adapted from Nadler and co-workers [53,54]. Briefly, baseline adaptation was collected by placing the test mouse in the central neutral chamber for a 2 min habituation period, followed by the partitions being raised and allowing the mouse to explore freely all three chambers (two interaction chambers and the central neutral chamber) for a 10 min period with data being collected in 5 min bins. During baseline assessments there were no "stranger" mice in either of the stimulus cages. Time spent in each chamber was used as baseline data to assess general activity, willingness to circumnavigate the testing apparatus and to assure that no mouse exhibited a side preference. Both ambulatory locomotor activity and the total number of entrances (transitions between chambers of the apparatus) during the 10 min baseline period were assessed using the photo-sensors in the chamber walls. An exclusion criterion required each mouse to explore each of the three chambers for a minimum of 90 s (15% of the total test time) each during the initial 10 min exploratory period. Mice that failed to meet this criterion were eliminated from further consideration. Following baseline data collection, each mouse was returned to the neutral central chamber for 2 min with partitions closed. During this time, an unfamiliar adult C57BL/6J mouse (stranger #1) was placed within one of the stimulus cages that were attached to each interaction chamber. Again partitions were raised, allowing the test mouse to move freely throughout the three chambers for a 10 min test session. Location of the 'stranger mouse' was alternated between the left and right stimulus cages on consecutive sessions. Again, measurements were collected in 5 min time bins and broken down into time spent in each the three chambers (the interaction chamber adjacent to a stimulus cage containing 'stranger mouse #1', interaction chamber adjacent to an empty stimulus cage, and the neutral central chamber). Also measured was the number of entries made into each interaction chamber.

2.2.2. Preference for social novelty

To quantify preference for social novelty, a second 10 min test session was performed immediately following the 10 min sociability test. The original unfamiliar mouse (stranger mouse #1) remained in its stimulus cage while a second unfamiliar mouse (stranger mouse #2) was added to the second stimulus cage, which was previously empty. Stranger #1 and stranger #2 mice originated from different home cages and had never come into physical contact with each other or with the test subject. After the partitions were again raised, automated measurements were collected in an identical fashion to the social interaction test.

To assure that the time spent in the interaction chamber adjacent to the stimulus cage containing an unfamiliar mouse was social in nature, a blinded observer scored, from videotape, each test session for explorations or sniffing directed towards a "stranger mouse" (nose within 2 cm of the wire mesh wall and directed toward the stranger mouse). This data was then compared to exploration and sniffing behavior exhibited by the test mouse towards the wire mesh wall separating the second interaction chamber from a stimulus cage that contained no mouse. The observer scores were compared to the automated scoring. The cumulative scores for the 10 min observer scored test sessions were expressed as total duration of direct social interactions in seconds.

2.2.3. Data analysis

Baseline locomotor activity, number of entries to and the time spent in side chambers was examined by an overall repeated measure ANOVA for the four genotypes during the adaptation period followed by Bonferroni *post hoc* multiple comparison tests. The social data was initially analyzed by an overall repeated measures ANOVA with the factors of genotype and side preference (e.g. "stranger mouse #1" side versus the empty side in the sociability test and "stranger mouse #1" side versus "stranger mouse #2" side in the preference for social novelty test). Subsequently, statistical significance was determined by within group repeated measures ANOVA with Bonferroni *post hoc* multiple comparison tests to determine side preferences following a significant overall ANOVA. All statistical analyses were performed using GraphPad Prism 4.0 (GraphPad, San Diego, CA).

2.3. Nest building and utilization

Prior to evaluation of nest building, each mouse was housed individually for 1 h in a clean cage with fresh sawdust bedding. The age of the mice used in these assessments ranged between 16 and 52 weeks of age. The observation period took place during the light cycle and was initiated by the introduction of a nestlet, a 2.5 g, 5.5 cm^2 wafer of compressed cotton (Ancare, Bellemore, NY) into the cage. Assessment of the total amount of time spent building a nest during the first hour was performed during three separate 10 min epochs each separated by 10 min. Time building the nest included the amount of time a mouse was involved in shredding the nestlet using either its mouth or forepaws as well as transporting the material around the cage. After the first hour a second nestlet was provided by hanging the nestlet from a clip attached to the food hopper and after 24 h a third nestlet was introduced in the same manner. The amount of nest material utilized after 1, 24 and 48 h of exposure was assessed by subtracting the weight of unutilized nestlet material from the total weight of material made available to the mouse. After 48 h, the nest quality was scored and the nest location recorded [16,46,52,72]. Nest quality was scored according to the following scale modified from Moretti et al. [52]: 1, nestlets largely unmodified; 2, nestlets modified (shredded) but no distinct nest shape; 3, flat nest with partially shredded nestlet and large segments of the nestlet wafer remaining intact; 4, shallow nest with partially shredded nestlets; 5, shallow nest with well shredded material; 6, nest with well developed walls; 7, nest in a shape of a cocoon with small opening of a diameter of <3 cm or complete roof. Nest building time and nestlet usage were analyzed for statistical significance by a one-way ANOVA with Bonferroni *post hoc* multiple comparison test. Nest quality scores were analyzed using Kruskal–Wallis nonparametric one-way ANOVA with Dunn's *post hoc* multiple comparison test. Lastly, during the light period on 3 separate days, the resting location of each mouse, relative to the location of the nest was noted on three separate occasions throughout the day.

2.4. Exploratory behavior

Methods used for assessing exploratory behavior were adapted from 'open field' protocols [30]. Briefly, mice in the age range of 24–52 weeks were placed individually into a brightly lit circular arena (diameter 54 cm, height

25 cm) for a period of 1 h in order to acclimate to the novel environment. Twenty-four hours later each mouse was returned to the open field arena that now contained a novel object (circular mesh cage 3 in. in diameter and 2 in. in height) placed in the center of the open field chamber. During the 10 min test period several assessments were manually assessed including the time it took to first make contact with the object, the total time spent investigating the object, the number of contacts with the object and the total number of times each mouse reared. A rearing episode was defined as either rearing on hindlimbs or leaning with one or both forepaws against the wall or the novel object placed within the open field chamber. Instances in which the mouse sat back on its hindlimbs in order to groom itself were not considered a rearing event. The frequency and duration of rearing episodes, suggested as being a measure of non-selective attention, were also assessed according to methods adapted from Aspide et al. [5]. Briefly, recordings taken during the other exploratory assessments were visually monitored in 1-min time blocks in order to determine the rearing frequency of each mouse when placed in a novel environment. These values were averaged within genotype for each 1-min time block. Subsequently, the mean duration of each rearing episode was also assessed for each mouse and averaged within genotype. The open field chamber was cleaned with a wet sponge in between trials. Each session was videotaped in order to verify all assessments and to produce diagrams of the exploratory path that each mouse pursued. Data was analyzed for statistical significance by a repeated measures ANOVA with Bonferroni *post hoc* multiple comparison test following a significant ANOVA. Differences in rearing frequency were analyzed by a two-way ANOVA genotype × time block, as the dependent variable.

2.5. Brain preparation

Mice from both the gabrb3$^{-/-}$ and gabrb3$^{+/+}$ groups were sacrificed between the ages of 51 and 52 weeks by CO_2 inhalation followed by rapid transcardial perfusion with 20 ml of 0.1 M phosphate buffered saline (PBS) and then 20 ml of 4% paraformaldehyde (PFA) in 0.1 M phospate buffer (PB). Perfusion was performed using a 22-guage 1 in. needle inserted into the left ventricle with a small cut made in the right atrium for fluid to exit the circulatory system. Following perfusion, brains were carefully removed from the skull and incubated for 5 h in 4% PFA and then cryoprotected by incubation in 30% sucrose in 0.1 M PB overnight at 4 °C. Brains were placed in a 1 mm mouse brain matrix (Ted Pella, Redding CA) and separated along the midline using a razor blade. Both the right and left hemispheres were frozen separately in M1 imbedding agent (Shandon Lipshaw Inc., Pittsburgh, PA) at −20 °C and then stored at−80 °C until used. Prior to sectioning, brains were moved to a freezer and kept at −20 °C for 15 h. Each hemisphere was then mounted on a specimen holder of a freezing stage microtome (Leica, CM1850) that was kept at −15 °C. Tissue was cut in serial sagittal sections from the mediosagittal line at a thickness of 40 μm then thaw-mounted on room temperature Superfrost plus slides (Fisher Scientific, Pittsburgh, PA) and dried for 4 h at room temp. Slide mounted tissue sections were stained for 20 min with toluidine blue (0.5%, in 1.0% sodium tetraborate (w/v) in deionized H_2O) then destained by 15 dips in 70%, 90% and 100% ethanol followed by four xylene washes lasting 3 min each. Slides were then cover-slipped using Permount (Fisher Scientific, Pittsburgh, PA).

Images of the cerebellar vermis from each section were taken using a SPOT RT slider camera (software v4.0.9, Diagnostic Instruments Inc, Sterling Heights, MI) mounted on an Olympus BHTU-BH2 microscope (Scientific Instrument Company, Sunnyvale, CA). Images were superimposed onto a corresponding diagram of a sagittal section of mouse cerebellar vermis taken from Paxinos and Franklin [59] at the level of the vermis between 0.36 and 0.60 mm lateral from the midline. Three separate sections were examined per animal (right or left vermal lobules) with surface areas being averaged per animal then pooled within genotype (gabrb3$^{-/-}$ and gabrb3$^{+/+}$). Semi-quantitative measurements were done at a magnification of 4× by two independent observers that were blind to the mouse genotype. The entire lobule (molecular layer, granule layer and white matter) area (see Fig. 9 for example) was included in the measurement with statistical significance determined by a two-tailed Student's *t*-test (Prism 4, GraphPad, San Diego CA).

3. Results

3.1. Social behavior

A repeated measures ANOVA of the baseline locomotor activity of the four genotypes, gabrb3$^{-/-}$ ($n=6$), gabrb3$^{+/+}$ ($n=6$), C57Bl/6J ($n=6$) and 129/SvJ ($n=6$) mice indicated a significant effect of genotype ($F_{3,20}=9.55$, $p<0.001$), with gabrb3$^{-/-}$ mice displaying a significantly higher degree of ambulatory activity than control mice (Table 1), in agreement with previous reports of hyperactivity in gabrb3$^{-/-}$ mice [39]. In addition, following a significant ANOVA ($F_{11,60}=11.95$, $p<0.001$), gabrb3$^{-/-}$ mice were found to exhibit a significantly higher number of entries relative to control mice in regards to the total transitions between all chambers of the social interaction apparatus during the adaptation period (Table 1). All mice, regardless of genotype, were found not to exhibit a significant difference between the amount of time spent in either interaction chamber ($F_{1,40}=0.52$, $p=0.48$) or the number of entries into either chamber ($F_{1,40}=0.04$, $p=0.84$), thereby demonstrating a lack of side preference (Table 1). Additionally, the number of entries made to either side during the 10 min sociability or social novelty assessments were likewise found not differ significantly within each mouse genotype (data not shown), similar to what has been previously reported [54]. The initial 5 min test epoch tended to be more informative than the second 5 min epoch, in regards to the duration of the interaction in both the sociability and social novelty assessments, as has been previously reported [54]. In order to verify both the robustness of the automated data collection procedure and to verify that the data reflected interactions that were social in nature, a blind observer also eval-

Table 1
Baseline locomotor activity

Mouse genotype	Ambulatory activity[a]	Total transitions between chambers	Chamber entrances		Time spent in each chamber (s)	
			Left	Right	Left	Right
gabrb3$^{-/-}$	1936 ± 129	186 ± 40	49 ± 8	57 ± 19	208 ± 30	213 ± 33
gabrb3$^{+/+}$	1278 ± 92***	132 ± 14*	38 ± 3	31 ± 7	245 ± 29	173 ± 26
C57BL/6J	1459 ± 45**	106 ± 15**	29 ± 4	26 ± 6	229 ± 33	159 ± 28
129/SvJ	1342 ± 100***	84 ± 14***	23 ± 4	20 ± 4	237 ± 39	309 ± 37

Baseline locomotor data was collected during the 10 min phase of the social behavior assessment, prior to introducing a "stranger mouse" to the social interaction apparatus. Data is presented as the mean ± S.E.M. Gabrb3$^{-/-}$ $n=6$, gabrb3$^{+/+}$ $n=6$, C57Bl/6J $n=6$, 129/SvJ $n=6$.

[a] Arbitrary units. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

uated the interactions from videotape. Due to the fragility of the gabrb3$^{-/-}$ mouse genotype (see methods) there was a wider than normal age distribution, three mice in the 16–22 weeks age range and the remaining three in the 50–52 week range. Therefore, within genotype analysis was also performed, in regard to each assessment, in order to determine whether the age difference was likely to influence the outcome of the assessment. Differences between the two age groups were not significant.

3.1.1. Automated measurements of the sociability test

In agreement with Nadler et al. [54], we found that the number of chamber entries was independent of the time spent in the chambers, regardless of whether the chamber was adjacent to a cage containing an unfamiliar mouse or was empty. An overall repeated measures ANOVA of the initial 5 min period revealed a significant difference in side duration between the four strains; $F_{1,40} = 62.66$, $p < 0.001$. On further analysis it was found that there was no significant difference between the three control strains on measures of side duration ($F_{2,30} = 0.65$, $p > 0.05$). Control strains were found to exhibit a significant preference for spending time in the interaction chamber adjacent to the stimulus cage containing an unfamiliar mouse "stranger mouse #1" as opposed to spending time in the interaction chamber adjacent to an empty stimulus cage, C57Bl/6J: $F_{5,25} = 6.55$, $p < 0.01$; 129/SvJ: $F_{5,25} = 5.21$, $p < 0.05$ and gabrb3$^{+/+}$: $F_{5,25} = 2.82$, $p < 0.01$ (Fig. 2). Similar results have been previously reported for C57BL/6J mice in this task [54]. In contrast, the time gabrb3$^{-/-}$ mice spent, during the first 5 min epoch, in either the chamber adjacent to the cage containing "stranger mouse #1" or the chamber adjacent to an empty stimulus cage was found not to differ significantly ($F_{5,25} = 1.92$, $p > 0.05$; Fig. 2).

3.1.2. Automated measurements of the preference for social novelty test

Ten minutes after exposure to stranger mouse #1 a second unfamiliar mouse "stranger mouse #2" was placed into the previously empty stimulus cage adjacent to interaction chamber #2 (see Fig. 1). An overall repeated measures ANOVA of the first 5 min epoch revealed a significant difference for side between the four strains; $F_{1,40} = 52.5$, $p < 0.001$. There was no significant difference between the three control strains on measures of side duration during the initial 5 min test epoch; $F_{2,30} = 0.27$, $p > 0.05$. *Post hoc* tests revealed the control strains to exhibit a preference for interacting with the new mouse (stranger mouse #2), as opposed to the now familiar mouse (stranger mouse #1), C57Bl/6J: $F_{5,25} = 13.58$, $p < 0.001$; 129/SvJ: $F_{5,25} = 14.11$, $p < 0.01$ and gabrb3$^{+/+}$: $F_{5,25} = 7.19$, $p < 0.001$ (Fig. 3). This preference was also detectable in the second 5 min test epoch in both the C57BL/6J and 129/SvJ mouse strains, but not in gabrb3$^{+/+}$ mice (Fig. 3). Gabrb3$^{-/-}$ mice failed to display a significant preference for the unfamiliar mouse over the now familiar mouse in either test epoch ($F_{5,25} = 0.70$, $p > 0.05$; Fig. 3). Furthermore, gabrb3$^{-/-}$ mice exhibited an increased preference for the middle/neutral chamber (175.3 ± 55.4, mean $\pm$ S.E.M.) compared to the control strains gabrb3$^{+/+}$ (115.3 ± 25.5), C57BL/6J (97.3 ± 32.5) and 129/SvJ (60.7 ± 11.4) during the 10 min social novelty test, though the difference between genotypes was not found to be statistically significant (compare grey bars in Fig. 3).

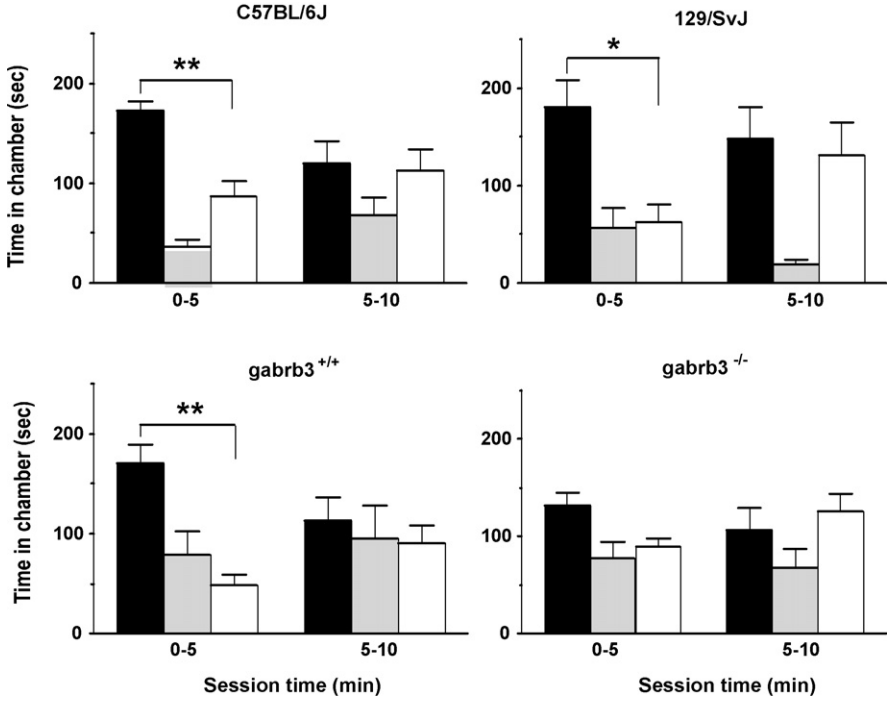

Fig. 2. Sociability histograms of the time that mice spent in each of the three chambers of the social interaction apparatus. The black bars represent the time spent in interaction chamber #1, adjacent to the stimulus cage containing an unfamiliar mouse (stranger mouse #1), grey bars represent time in the neutral central chamber and white is time in interaction chamber #2, adjacent to an empty stimulus cage. Data presented as the mean $\pm$ S.E.M. C57BL/6J $n = 6$, 129/SvJ $n = 6$, gabrb3$^{+/+}$ $n = 6$, gabrb3$^{-/-}$ $n = 6$. $^{*}p < 0.05$, $^{**}p < 0.01$.

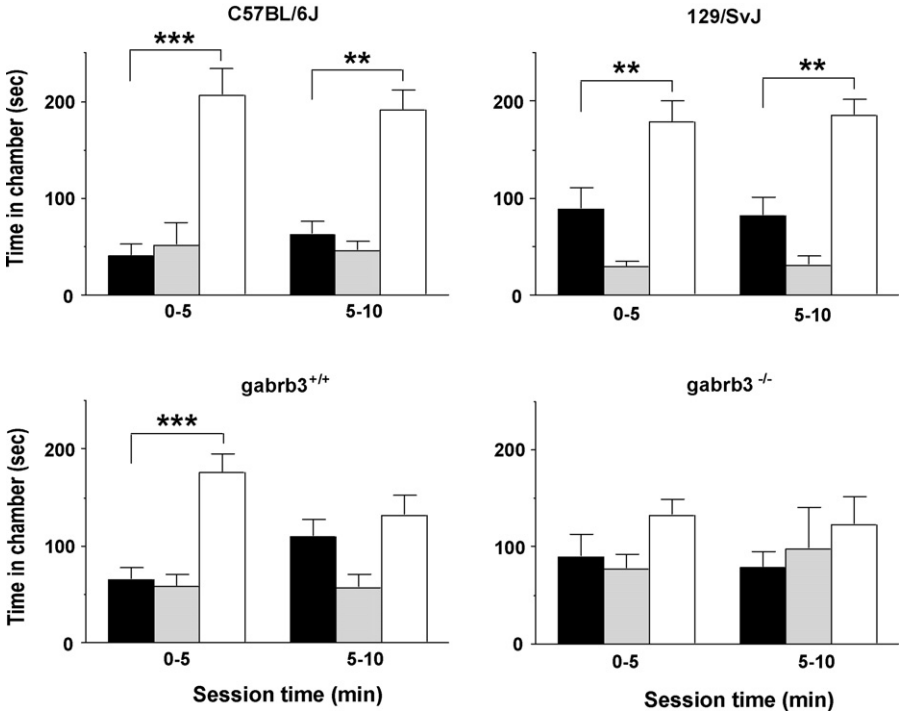

Fig. 3. Social novelty histograms of the mean time (±S.E.M.) mice spent in the interaction chamber adjacent to the now familiar stranger mouse #1 (black bar), the unfamiliar stranger mouse #2 (white bar) or in the neutral central chamber (grey bar). As expected all three control genotypes exhibited a significant preference for the unfamiliar mouse (stranger mouse #2) over the now familiar mouse (stranger mouse #1). The preference for stranger mouse #2 remained significant into the second 5 min epoch for both C57BL/6J and 129/SvJ mice. C57BL/6J $n=6$, 129/SvJ $n=6$, gabrb3$^{+/+}$ $n=6$, gabrb3$^{-/-}$ $n=6$. $^{**}p<0.01$, $^{***}p<0.001$.

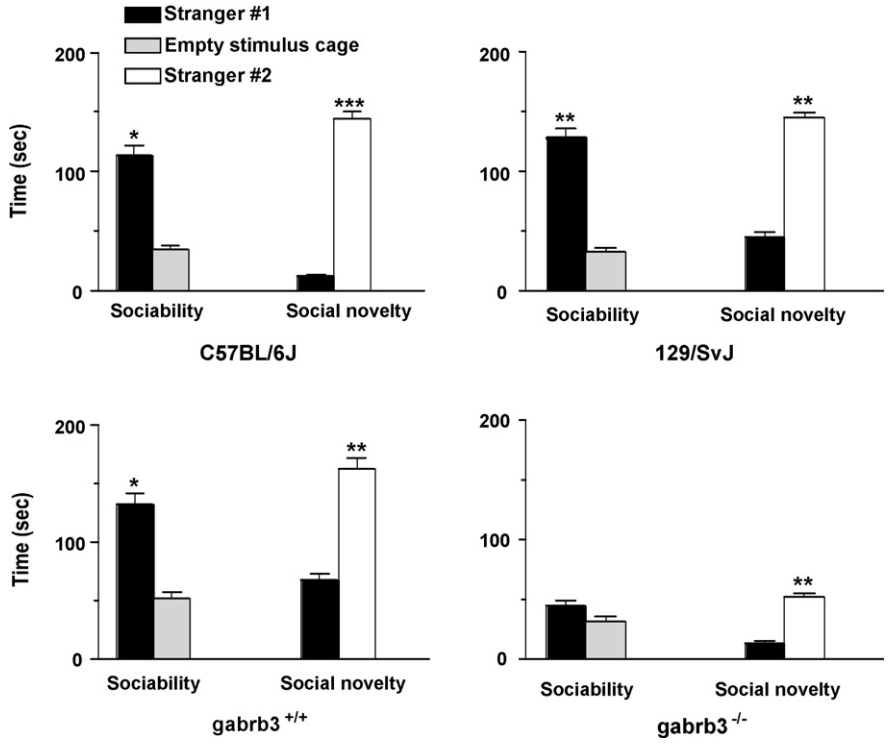

Fig. 4. Histograms of the sociability and the preference for social novelty assessed from videotape, by an observer, blind to the genotype of the test mice. Observer scores in the sociability test are presented as the total time, during a 10 min test period, that test mice spent with their noses within 2 cm of the wire mesh of the stimulus cage containing an unfamiliar mouse (stranger mouse #1) versus the total time the test mice spent with their noses within 2 cm of the wire mesh of the stimulus cage that was empty. In the preference for social novelty test the data is presented as the amount of time mice were observed to have their noses within 2 cm of the stimulus cage containing stranger mouse #1 (the now familiar mouse) versus the stimulus cage containing stranger mouse #2 (the new unfamiliar mouse). $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

3.1.3. Observer scoring of the sociability and preference for social novelty tests

Analysis of the observer scored data during the 10 min test period of the sociability tests was found to agree favorably with the automated data for both control and gabrb3$^{-/-}$ mice (compare Figs. 3 and 4). Similar agreement between the automated and observer scored sociability data has been previously reported in C57BL/6J mice [54]. *Post hoc* tests revealed significant differences in the amount of time mice spent sniffing at the cage containing stranger mouse #1 versus the empty stimulus cage for C57BL/6J: $F_{1,5} = 11.07, p < 0.05$; 129/SvJ: $F_{1,5} = 16.78, p < 0.01$ and gabrb3$^{+/+}$: $F_{1,5} = 15.62$, $p < 0.05$, but not for the gabrb3$^{-/-}$ mice: $F_{1,5} = 1.10$, $p > 0.05$ (Fig. 4).

Comparison between the automated and observer scoring of the social novelty test again found the control genotypes to behave in a similar manner under both assessments. However, subtle differences in the performance of gabrb3$^{-/-}$ mice were detected by observer scoring that were not detected by the automated scoring method. Whereas, the amount of time gabrb3$^{-/-}$ mice spent in the proximity of either stranger mouse #1 or stranger mouse #2 was not found to be significantly different when assessed by the automated method (Fig. 3), the observer scoring, which only takes note of direct interactions (nose within 2 cm of the wire mesh of the stimulus cage containing stranger mouse #1 or #2), revealed a significant difference in the amount of time involved in direct interactions with stranger mouse #2 versus stranger mouse #1 (Fig. 4). *Post hoc* tests indicated a significant preference for sniffing at stranger mouse #2 over stranger mouse #1, C57BL/6J: $F_{1,5} = 64.48$, $p < 0.001$; 129/SvJ: $F_{1,5} = 29.29$, $p < 0.01$; gabrb3$^{+/+}$: $F_{1,5} = 20.68$, $p < 0.01$ and gabrb3$^{-/-}$ mice: $F_{1,5} = 20.16$, $p < 0.01$. Upon closer examination of the observer data, in regards to the percentage of the total time spent sniffing the "stranger mice", the nature of the contrasting results for the gabrb3$^{-/-}$ mice between the automated versus the observer scored methods was revealed. By examining the total time that each mouse genotype spends interacting with stranger mouse #1 in the sociability test, you will note a markedly reduced level of sniffing of stranger mouse #1 by gabrb3$^{-/-}$ mice in comparison to the three control strains ($F_{3,15} = 5.38$, $p < 0.01$, Fig. 5A). Observer assessment of the social novelty test reveals a similar trend in respect to the total amount of time gabrb3$^{-/-}$ mice spend sniffing at stranger mouse #2 compared to the time the control strains spend sniffing at stranger mouse #2 ($F_{3,15} = 13.65$, $p < 0.001$, Fig. 5B). Moreover, when considering total sniffing directed at both stranger mouse #1 and stranger mouse #2 in the social novelty test, again gabrb3$^{-/-}$ mice clearly displayed a significant reduction in the overall amount of time spent in sniffing directed at either stranger mouse #1 or #2, compared to control genotypes ($F_{3,15} = 20.04$, $p < 0.001$, Fig. 5C). Thereby revealing that gabrb3$^{-/-}$ mice exhibit low levels of social engagement towards other mice.

Nesting behavior was likewise assessed as it represents a form of homecage-activity often linked to social behavior [52]. The amount of time mice spent interacting with nesting material during the first hour of exposure to nestlets, was analyzed by a repeated measures ANOVA, which indicated strain differences: $F_{3,26} = 3.73$, $p < 0.05$ with *post hoc* tests revealing significant

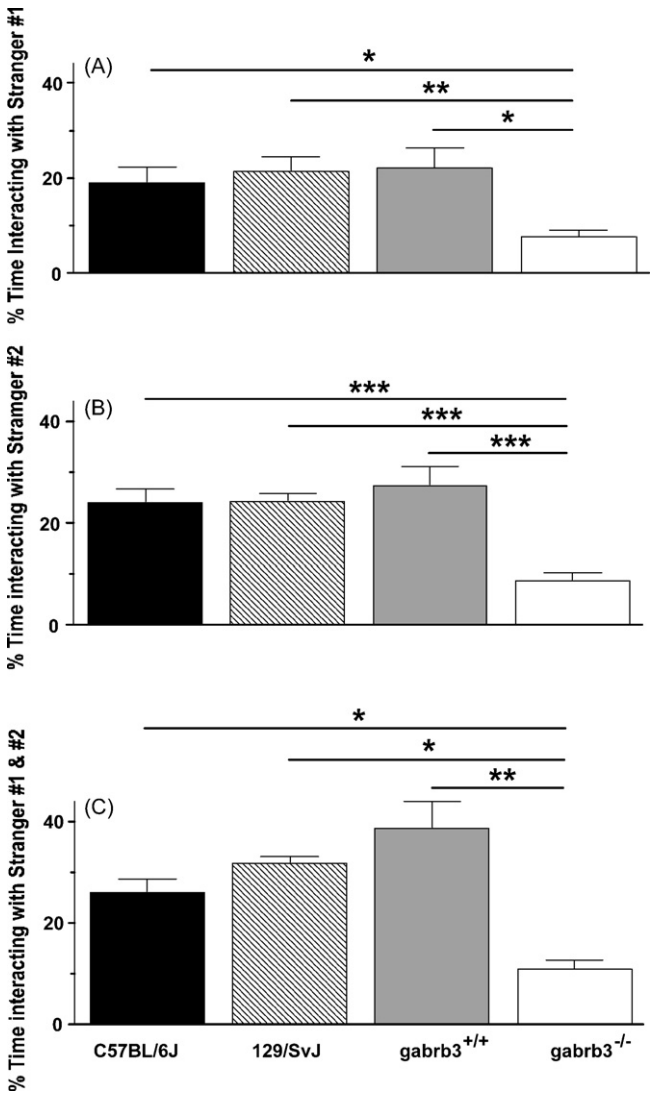

Fig. 5. Histogram of the percentage of the total 10 min test period that each mouse genotype spent sniffing at the stimulus cage containing (A) stranger mouse #1 in the sociability test. (B) Stranger mouse #2 in the social novelty test and (C) percentage of total time spent sniffing at both stranger mouse #1 and stranger mouse #2 in the social novelty test. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

differences between gabrb3$^{-/-}$ mice and both C57BL/6J and gabrb3$^{+/+}$ mice (Fig. 6A). Although all mouse genotypes demonstrated an increase in material usage over the 48 h observation period, a repeated measures ANOVA again revealed a significant strain difference in nestlet material usage at the 1, 24 and 48 h test intervals: $F_{3,26} = 8.32$, $p < 0.001$; $F_{3,26} = 44.53$, $p < 0.001$; $F_{3,26} = 13.95$, $p < 0.001$, respectively with *post hoc* tests revealing gabrb3$^{-/-}$ mice to differ significantly from controls at all three time-intervals (Table 2). The nest quality scores (see Section 2.3 for scoring) were assessed 48 h after nestlets were first introduced to the cages. A Kruskal–Wallis analysis revealed a significant variation between mouse genotypes ($\chi^2 = 18.47$, $p < 0.001$) with *post hoc* tests indicating gabrb3$^{-/-}$ mice to be significantly impaired in their ability to achieve the nest complexity exhibited by the control genotypes (Fig. 6B). Whereas, control mice typically built well-assembled walls

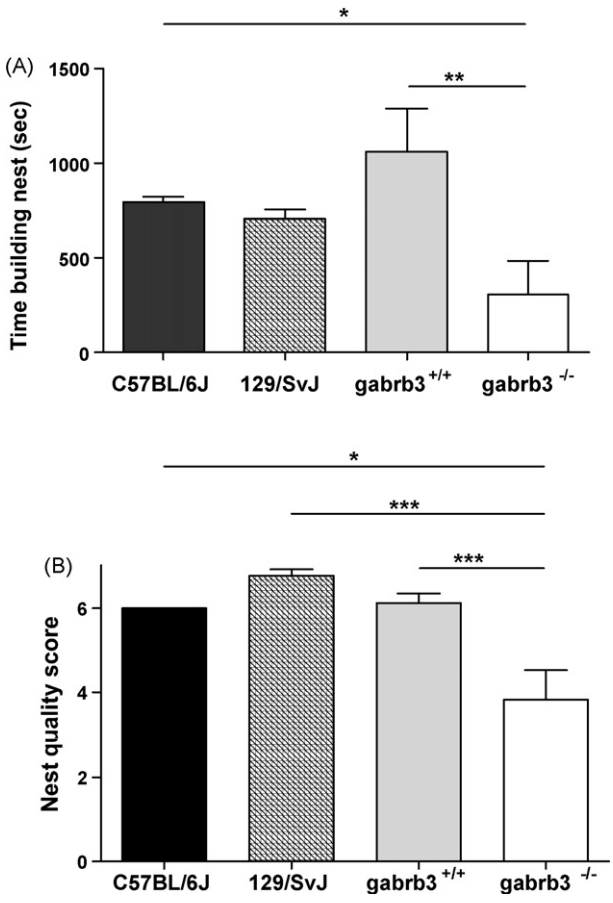

Fig. 6. (A) Histogram of the mean time (±S.E.M.) that each mouse genotypes spent interacting with freshly introduced nesting material (nestlet) during three separate 10 min observations taken over a 1 h period immediately after the introduction of the nestlet. (B) Histogram of average nest scores (mean ± S.E.M.), taken 48 h after nestlet introduction. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$. Gabrb3$^{-/-}$ $n=6$, gabrb3$^{+/+}$ $n=8$, C57BL/6J $n=8$, 129/SvJ $n=8$.

or a cocoon-like nest from fully shredded nestlets, gabrb3$^{-/-}$ mice generally built flat nests consisting of mostly unshredded nesting material. It is noteworthy to mention that all control mice built their nests in either a corner or along the cage wall, while gabrb3$^{-/-}$ mice often built their nests away from the corners and walls, if they constructed one at all. Utilization of the nests during resting periods also differed between control and gabrb3$^{-/-}$ mice: the controls were found resting in their nest in 100% of the observations taken over a 3 day period, but the gabrb3$^{-/-}$ mice were observed sleeping away from the nesting material in 50% of the observations.

Table 2
Material utilization in nest construction

Genotype	1 h	24 h	48 h
gabrb3$^{-/-}$	0.3 ± 0.3	1.6 ± 0.4	4.1 ± 0.9
gabrb3$^{+/+}$	1.9 ± 0.4***	4.6 ± 0.2***	7.0 ± 0.3***
C57BL/6J	2.0 ± 0.2***	4.9 ± 0.0***	7.4 ± 0.1***
129/SvJ	2.0 ± 0.2***	5.0 ± 0.0***	7.5 ± 0.0***

Data is presented as the mean ± S.E.M. of material usage, in grams, measured at three time, intervals following initial introduction of nestlet material. Bonferroni *post hoc* comparison, of material usage between each control mouse genotype and gabrb3$^{-/-}$ mice at each time, interval. Gabrb3$^{-/-}$ $n=6$, gabrb3$^{+/+}$ $n=8$, C57Bl/6J $n=8$, 129/SvJ $n=8$. $^{***}p<0.001$.

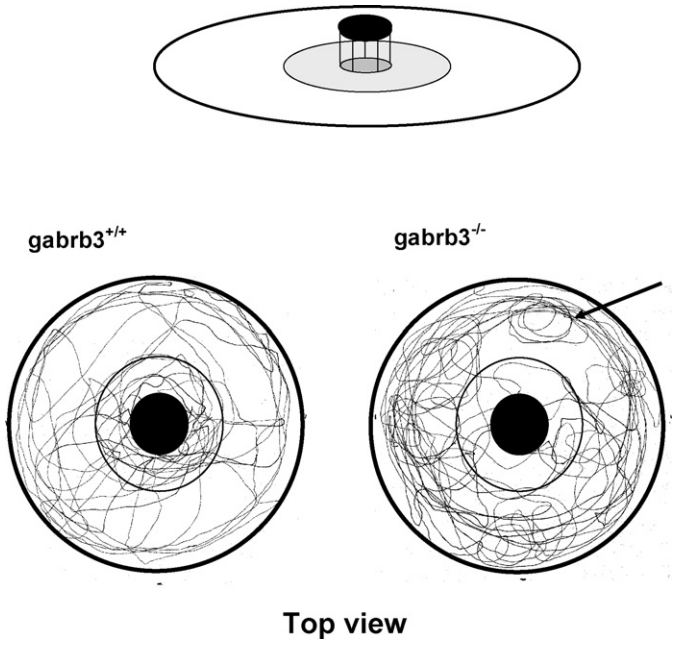

Fig. 7. Representative examples of the exploratory paths taken by a gabrb3$^{+/+}$ and gabrb3$^{-/-}$ mouse in an open field chamber that contained a novel object (an empty round cage, depicted as a solid black disc) placed in the center, that was not present during the acclimation phase of the test. The arrow indicates an example of the stereotypical circling often exhibited by gabrb3$^{-/-}$ mice.

3.2. Exploratory behavior

Fig. 7 depicts representative examples of the exploratory paths followed by a gabrb3$^{+/+}$ and a gabrb3$^{-/-}$ mouse. The higher overall line density/length of the gabrb3$^{-/-}$ mouse, compared to the gabrb3$^{+/+}$ mouse, is reflective of the hyperactive nature of gabrb3$^{-/-}$ mice. The relative proportion of the path density enclosed within the inner open circle, containing the novel object, is qualitatively higher in the gabrb3$^{+/+}$ mouse compared to the gabrb3$^{-/-}$ mouse and is associated with the significantly longer time gabrb3$^{+/+}$ mice spent investigating the novel object compared to gabrb3$^{-/-}$ mice (Table 3). These exploratory path diagrams also reveal the stereotypical circling pattern common to gabrb3$^{-/-}$ mice [39] (see arrow, Fig. 7) that was not observed in the more random locomotor pathways exhibited by gabrb3$^{+/+}$ mice.

Significant genotype differences were detected in both the time that elapsed before mice first made contact with a novel object placed in the open field chamber ($F_{3,26}=8.55$, $p<0.001$) and the amount of time mice spent investigating the novel object ($F_{3,26}=36.9$, $p<0.001$). *Post hoc* analysis revealed significance difference between gabrb3$^{-/-}$ mice and control genotypes in both assessments (Table 3). In addition, the number of contacts mice made with the novel object also revealed an overall significant effect of strain; $F_{3,26}=19.4$, $p<0.001$ with *post hoc* tests indicating statistically that only the 129/SvJ mice differed significantly from gabrb3$^{-/-}$ mice (Table 3).

Table 3
Exploratory behavior

Mouse genotype	Time to first contact with novel object (s)	Time spent investigating novel object (s)	Number of contacts with novel object	Total number of rearings
gabrb3$^{-/-}$	54.9 ± 12.0	63.6 ± 10.5	15.9 ± 4.5	14.2 ± 6.6
gabrb3$^{+/+}$	12.1 ± 1.7***	236.1 ± 24.6***	20.4 ± 1.4	51.6 ± 7.9**
C57BL/6J	18.9 ± 3.9**	180.8 ± 8.8**	21.8 ± 0.7	53.0 ± 6.4***
129/SvJ	15.0 ± 5.2***	426.4 ± 39.7***	46.2 ± 4.2***	30.8 ± 3.1

Data was quantified from 10 min videotape sessions of mice placed in an open-field chamber containing a novel object, scored by an observer blind to the mouse genotype. Data represents the mean ± S.E.M. Gabrb3$^{+/+}$ $n = 7$, gabrb3$^{-/-}$ $n = 7$, C57Bl/6J $n = 8$, 129/SvJ $n = 8$. $^{**}p < 0.01$, $^{***}p < 0.001$.

The total number of rearings, another indicator of exploratory behavior [12], was also found to exhibit a significant strain difference; $F_{3,25} = 8.57$, $p < 0.001$, with *post hoc* tests demonstrating a significance difference between gabrb3$^{-/-}$ mice and both gabrb3$^{+/+}$ and C57Bl/6J mice, but not 129/SvJ mice (Table 3). As shown in Fig. 8A, gabrb3$^{-/-}$ displayed a lower frequency of rearing activity, as compared with the corresponding control mice. A two-way ANOVA revealed a significant effect of genotype in the frequency of rearing ($F_{3.225} = 6.84$, $p < 0.01$) with no genotype × time block interaction. A statistically significant reduction in the mean duration of a rearing event was also noted in the gabrb3$^{-/-}$ mice compared to the control genotypes (Fig. 8B) following a significant repeated measures ANOVA in regards to duration; $F_{3,25} = 16.64$, $p < 0.001$.

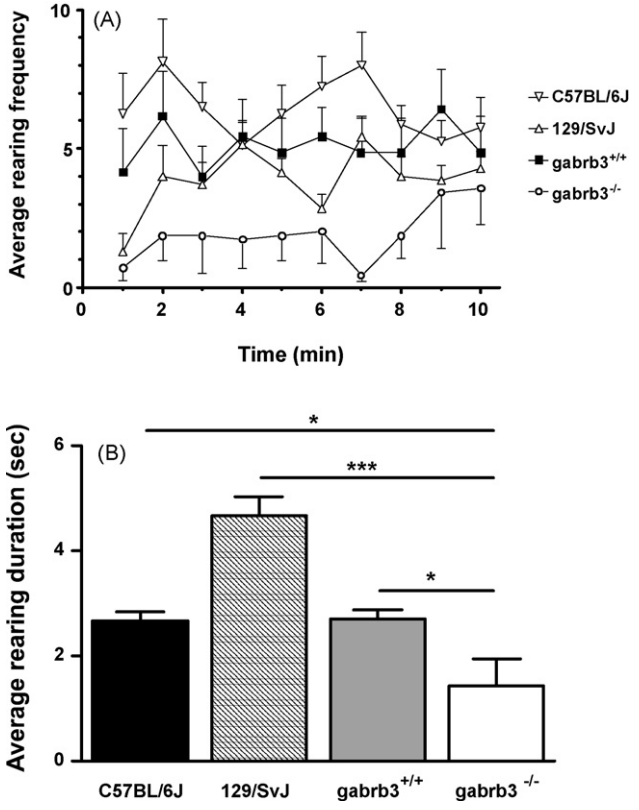

Fig. 8. (A) Average rearing frequency (mean ± S.E.M.) demonstrated by each genotype, when placed in an open field chamber containing a novel object. *Post hoc* analysis revealed a significant difference ($p < 0.001$) between gabrb3$^{-/-}$ mice and each control genotype in rearing frequency over the entire 10 min test period. (B) Mean duration of a rearing event for each mouse genotype, averaged over the entire 10 min testing period in a novel environment. Gabrb3$^{-/-}$ $n = 7$, gabrb3$^{+/+}$ $n = 7$, C57BL/6J $n = 8$, 129/SvJ $n = 8$. $^{*}p < 0.05$, $^{***}p < 0.001$.

3.3. Cerebellar vermis

Semi-quantitative measurements of the sagittal surface area of cerebellar vermal lobules (Fig. 9) of gabrb3$^{+/+}$ and gabrb3$^{-/-}$ mice revealed significant decreases in the surface areas of vermal lobules II, III, IV & V, and VI & VII in gabrb3$^{-/-}$ mice compared to gabrb3$^{+/+}$ (Fig. 10). A non-significant reduction in the surface areas of vermal lobules VIII, IX and X in gabrb3$^{-/-}$ mice compared to gabrb3$^{+/+}$ mice, were also noted (Fig. 10).

4. Discussion

The GABRB3 gene is vital to proper brain development and to mature brain function. The consequences of disrupting this gene have been demonstrated in mice, which exhibit numerous abnormalities, many of which have been observed in association with the neurodevelopmental disorders Angelman syndrome and ASD [26,27,39]. The present study provides a crucial addition to this body of work by demonstrating that gabrb3$^{-/-}$ mice exhibit deficits in social behavior, a core feature of ASD. In addition, other inclinatory traits often associated with ASD were also detected in these mice, including hypoplasia of cerebellar vermal lobules and deficits in both exploratory behaviors and non-selective attentional processes related to the orientation of attention [5,35,64].

Gabrb3$^{-/-}$ mice clearly exhibited social deficits, as indicated by a significant reduction in overall social engagement in both sociability and social novelty testing, however, some aspects of social curiosity appear to remain intact. While gabrb3$^{-/-}$ mice spent less time in the proximity of unfamiliar mice than did their control counterparts (see Fig. 4), we were able to discern from closely examining the direct interactive contact that gabrb3$^{-/-}$ mice exhibited towards either of the two stranger mice in the social novelty test, that there was a preference for the novel mouse over the now familiar mouse (compare Fig. 3 with Fig. 4). The discrepancy between the two measurements can be partially explained by noting that the automated scoring method collects data on total time spent in all three chambers including the central/neutral chamber, whereas the observer scoring method only collects data pertaining to direct social engagement

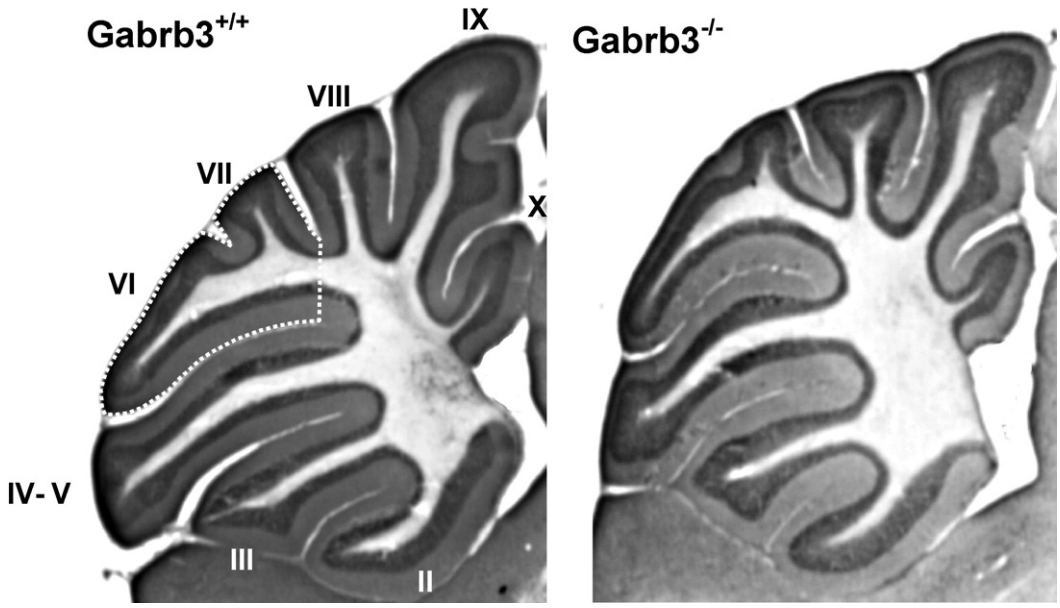

Fig. 9. Representative examples of toluidine blue stained midsagittal sections through the cerebellar vermis (0.36–0.60 mm lateral from the midsagittal line) from gabrb3$^{+/+}$ and gabrb3$^{-/-}$ mice. A roman numeral identifies each cerebellar vermal lobule. Lobules VI–VII on the gabrb3$^{+/+}$ brain section have been outlined using a white dashed line to indicate the area being measured and compared.

(nose touching or sniffing within 2 cm of the unfamiliar mouse) as opposed to just being in the social proximity of the unfamiliar mouse. If one examines the total amount of direct social engagement that each mouse genotype exhibits, one easily discerns that gabrb3$^{-/-}$ mice displayed an attenuated level of interaction towards unfamiliar mice in general, compared to controls (Fig. 5). We also observed differences in the nesting behavior of gabrb3$^{-/-}$ mice, a behavioral feature associated with abnormalities in social behavior [52] and that has likewise been observed in other mouse models linked to ASD, including Mecp2 gene mutation mice, a model of Rett syndrome and in mice deficient in the Dvl1 gene [46,48,52].

As a rodent's social behavior is highly dependent on olfactory cues, one must consider whether olfaction differences between gabrb3$^{-/-}$ and gabrb3$^{+/+}$ may have contributed to the observed differences in social behavior. Indeed, gabrb3$^{-/-}$ mice have been reported to exhibit subtle differences in olfactory discrimination, however, gabrb3$^{-/-}$ mice were found to exhibit better odor discrimination than gabrb3$^{+/+}$ mice in certain tasks [57]. We also noted that gabrb3$^{-/-}$ mice were as adept as gabrb3$^{+/+}$ mice in locating a buried piece of cookie (data not shown). Although these observations do not eliminate the possibility of an olfaction confound, they do shed doubt on olfactory irregularities as being the primary explanation for the observed social impairment exhibited by gabrb3$^{-/-}$ mice. Taken together, the deficits that gabrb3$^{-/-}$ mice exhibit in regards to sociability, social novelty, nest building and previous observations that gabrb3$^{-/-}$ mouse mothers fail to care for their young [39], add up to strongly indicate that normal social behavior is significantly impaired in gabrb3$^{-/-}$ mice.

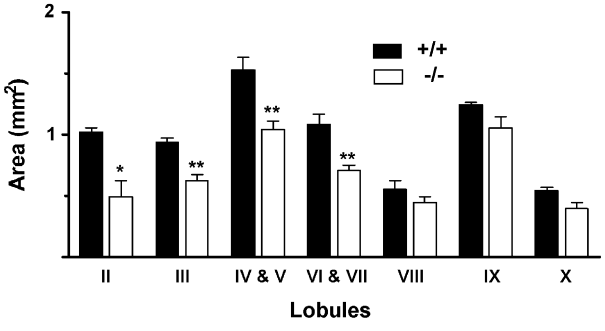

Fig. 10. Histogram of the measured surface area of cerebellar vermal lobules in gabrb3$^{+/+}$ ($n=4$) and gabrb3$^{-/-}$ ($n=5$) mice. $^{*}p<0.05$, $^{**}p<0.01$.

Gabrb3$^{-/-}$ mice were also found to exhibit significant deficits in a variety of exploratory parameters. The diagrams depicted in Fig. 7 represent a typical path that either a gabrb3$^{+/+}$ or gabrb3$^{-/-}$ might pursue. Differences between gabrb3$^{+/+}$ and gabrb3$^{-/-}$ mice can be gleaned from such diagrams, including the hyperactivity typically exhibited by gabrb3$^{-/-}$ mice (high path density in outer region), low exploration of the novel object (low path density in the inner ring) as well as the stereotypical circling pattern typical of these mice (see arrow Fig. 7). Whereas, gabrb3$^{+/+}$ mice exhibit the opposite pattern, lower path density in the outer region and a higher path density in the inner ring containing the novel object, reflecting the higher amount of time spent investigating the object. Compared to control mice, gabrb3$^{-/-}$ mice took longer to initiate the investigation of a novel object placed in the center of the testing chamber and spent less time devoted to exploring the novel object. Whereas, the total number of contacts a mouse made with the novel object did not appear to be as informative as the total amount of time the mouse actually spent investigating the object (Table 3).

Another component of exploratory behavior is rearing, which is associated with a rodent's motivational state and arousal level when exposed to a novel environment [67]. Consequently, novel stimuli, in all sensory modalities, are highly effective in attracting and focusing attention. Significantly less rearing occurred

in gabrb3$^{-/-}$ compared to gabrb3$^{+/+}$ and C57BL/6J, but not 129/SvJ mice (Table 3). However, the duration of an average rearing event in 129/SvJ mice was observed to be much longer than in the other genotypes (Fig. 8B), therefore, potentially accounting for the lower rearing number. On the whole, 129/SvJ mice appeared to be more inquisitive, in regards to the exploratory parameters assessed, than the other mouse genotypes. Gabrb3$^{-/-}$ mice also exhibited a lower rearing frequency and lower mean rearing duration compared to control mice (Fig. 8 A and B). Studies conducted by Aspide et al. [5,6] have demonstrated that the frequency and duration of rearing episodes exhibited by a rodent placed in a novel environment also indexes the scanning and orienting phase of attention (non-selective attention). The significant reduction in both the frequency and duration of rearing episodes exhibited by the gabrb3$^{-/-}$ mice, relative to controls (Fig. 8A and B) suggests gabrb3$^{-/-}$ mice exhibit a deficit in non-selective attention. This is of interest as individuals with ASD often display deficits across many attentional domains, including selective and sustained attention with the inability to shift and orient attention rapidly and accurately among spatial targets and between sensory modalities [2,35,75]. This attentional deficit likely contributes to an autistic individuals reduced tendency and motivation to thoroughly explore novel environments [64] and has been suggested to be a contributing factor in the limited social interactions that autistic individuals display toward others [31].

In addition to the above behavioral deficits, gabrb3$^{-/-}$ mice have been previously found to be poor swimmers, exhibit difficulty walking on grid floors, often run in circles and do not perform as well as controls on the accelerating rotarod [27,39]. Therefore, one possible explanation for the previously observed deficits and reduction in rearing, may involve changes to the vestibular system of the inner ear, which controls balance and orientation in space. Subsequently, two studies have reported changes in components of the vestibular system of the gabrb3$^{-/-}$ mouse [42,49]. While mindful of these observations, the current assessments made were not dependent on high levels of motor coordination or postural control. Furthermore, upon close observation of gabrb3$^{-/-}$ mice, they were found to be quite capable of rearing and able to groom themselves for extended periods while sitting back on their hindlimbs. Therefore, the moderate motor coordination and potential postural control issues associated with gabrb3$^{-/-}$ mice may contribute, but are unlikely to be the primary cause of reduced rearing behavior. Another potential confound to the behavioral assessments performed on gabrb3$^{-/-}$ mice is that these mice are prone to seizures [27,39]. Therefore, an accumulated seizure load, especially in the older gabrb3$^{-/-}$ mice could potentially contribute to the observed behavioral differences as well as differences in brain morphology. We are not able to comment on whether there would be differences in the cerebellar hypoplasia between younger and older gabrb3$^{-/-}$ mice as the cerebellar assessments were all done on mice between 51 and 52 weeks of age. However, younger gabrb3$^{-/-}$ mice were found to perform in a similar fashion to older gabrb3$^{-/-}$ mice in regards to the behaviors assessed in this study (data not shown). Furthermore, as demonstrated in the gabrb3$^{-/-}$ mouse, GABAergic deficits leads to numerous phenotypic traits including epilepsy, therefore, one would anticipate that ASD cases that exhibit GABAergic deficits may likewise present with epilepsy. Subsequently, about 25% of the ASD population exhibit epilepsy [34] and would also be subject to an accumulated seizure load as they age. This increasing seizure load could also potentially influence the manifestation of the ASD phenotype over the lifetime of this population.

A variety of neuroimaging studies suggest that the cerebellum is involved in more than just motor coordination, but also in a diverse set of higher cognitive functions [45], many of which have been observed to be affected in ASD. These include the processing of spatial information [44,63], spatial orientation [41], exploratory behavior [62] and shifting attention [1]. The cerebellar vermis, especially lobules VI and VII, have been reported as being abnormal in ASD [64]. Semi-quantitative assessments of the cerebellar vermal regions of gabrb3$^{-/-}$ mice relative to gabrb3$^{+/+}$ mice found significant reductions in the surface areas of lobules II–VII (Fig. 10) with non-significant reductions occurring in lobules VIII–X. As large sections of lobules IX and X mature earlier than lobules VI and VII, the current observation suggests that the developmental aberrations leading to reductions in the surface areas of lobules VI and VII in gabrb3$^{-/-}$ mice may have, in part, occurred later in vermal development [4,40]. Similarly, the L1 cell adhesion molecule knockout mouse, which likewise exhibits hypoplasia of the cerebellar vermis, especially in lobule VI, also displays a reduced tendency to explore unfamiliar environments, novel objects placed within their environments, are in continual motion and display stereotypical circling [30]. It is not clear what the circling pattern in these mice or gabrb3$^{-/-}$ mice represents, though it is reminiscent of the peripheral circling exhibited by rodents with induced cerebellar lesions. Moreover, it has been noted that there is a proclivity towards spinning (circling) in the ASD population [15]. The observed hypoplasia of the cerebellar vermis of the gabrb3$^{-/-}$ mice could conceivably contribute to a variety of behavioral deficits observed in gabrb3$^{-/-}$ mice including poor exploratory performance, reduced rearing, impaired contextual memory, poor motor coordination, hyperactivity, reduced digging, stereotypical circling and hypotonia [27,37,39]. It is noteworthy that the reduced exploratory and presence of stereotypical patterns observed in ASD have both been significantly correlated with the magnitude of cerebellar hypoplasia in vermal lobules VI and VII [64]. Furthermore, lobules VI and VII are part of the oculomotor vermis, which controls saccadic eye and head movements [32] and are therefore essential for adequate exploration of the external world. Although, it is compelling to consider that the behavioral deficits exhibited by gabrb3$^{-/-}$ mice are linked with its cerebellar hypoplasia, disrupting other brain regions can also lead to similar behavioral deficits. For example lesioning of the hippocampus in normal mice likewise results in poor nest building and reduced rearing and exploration [25]. Subsequently, gabrb3$^{-/-}$ mice also exhibit abnormal $GABA_A$ receptor binding and function in the hippocampus [39,43]. In addition, we recently discovered that the locus coeruleus (LC) in gabrb3$^{-/-}$ mice have an expanded plexus of LC dendrites relative to gabrb3$^{+/+}$, C57Bl/6J and 129/SvJ mice [37]. This is of

Table 4
Overlap between ASD characteristics and phenotypic traits of gabrb3$^{-/-}$ mice

Characteristic	ASD	gabrb3$^{-/-}$ mice	Reference
Core deficits			
Impaired social behavior	√	√[a]	[31]
Repetitive, stereotypical behavior	√	√	[10,39]
Deficits in communication	√	?	[55]
Inclinatory deficits			
Hyperactivity	√	√	[10,27]
Poor motor coordination	√	√	[10,27]
Tactile hypersensitivity	√	√	[8,76]
Temperature hypersensitivity	√	√	[36,76]
Cognitive impairment	√	√	[27,78]
Sleep disturbances	√	√	[10,79]
Epilepsy	√	√	[19,27]
Abnormal exploratory behavior	√	√[a]	[64]
Deficits in orientation of attention	√	√[a]	[35]
Reduced cerebellar vermis size	√	√[a]	[23]
Reduced benzodiazepine binding in hippocampus	√	√	[13,71]

√: Feature is present.
[a] Observation from the current study.

potential interest as the LC has also been implicated in processes involved in focusing, orienting, scanning and shifting attention [7,28,77]. However, while the cerebellum [3] and hippocampus [33] have both been implicated in ASD, to date, the LC has not been highly investigated in regards to ASD.

In summary, the current results clearly indicate that gabrb3$^{-/-}$ mice exhibit abnormalities in social behavior. This coupled with an earlier observation that these mice exhibit stereotypical behavior [39], demonstrates these mice to possess two of the triad of core behavioral deficits required in humans in order to receive a diagnosis of ASD. We also report that gabrb3$^{-/-}$ mice exhibit deficits in exploratory behavior and in non-selective attention along with hypoplasia of the cerebellar vermis, all features associated with ASD. These current findings are added to a lengthy list of abnormalities previously reported in gabrb3$^{-/-}$ mice, which bear obvious parallels to symptoms often reported in association with ASD (Table 4) [26]. Taken together, these observations provide overwhelming support for the face validity of the gabrb3$^{-/-}$ mouse as a model of ASD. In addition, strong construct validity of this mouse model is supported by an abundant body of evidence implicating the GABRB3 gene in ASD [18,21,70]. Therefore, a compelling argument can be made for the strength of the gabrb3$^{-/-}$ mouse as a model of ASD and that further investigations into the predictive validity of this model are highly warranted.

Acknowledgments

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