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Genetic Background Effects in Neuroligin-3 Mutant Mice: Minimal Behavioral Abnormalities on C57 Background

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Abstract

Neuroligin-3 (NLGN3) is a postsynaptic cell adhesion protein that interacts with presynaptic ligands including neurexin-1 (NRXN1) (Ichtchenko et al., 1996). Mice harboring a mutation in the *NLGN3* gene (NL3R451C) mimicking a mutation found in two brothers with autism spectrum disorder (ASD) were previously generated and behaviorally phenotyped for autism-related behaviors. In these NL3R451C mice generated and tested on a hybrid C57BL6J/129S2/SvPasCrl background, we observed enhanced spatial memory and reduced social interaction (Tabuchi et al., 2007). Curiously, an independently generated second line of mice harboring the same mutation on a C57BL6J background exhibited minimal aberrant behavior, thereby providing apparently discrepant results. To investigate the origin of the discrepancy, we previously replicated the original findings of Tabuchi et al by studying the same NL3R451C mutation on a pure 129S2/SvPasCrl genetic background. Here we complete the behavioral characterization of the NL3R451C mutation on a pure C57BL6J genetic background to determine if background genetics play a role in the discrepant behavioral outcomes involving NL3R451C mice. NL3R451C mutant mice on a pure C57BL6J background did not display spatial memory enhancements or social interaction deficits. We only observed a decrease startle response and mildly increased locomotor activity in these mice suggesting that background genetics influences behavioral outcomes involving the NL3R451C mutation.

Keywords

autism spectrum disorder; autism; Neuroligin-3; genetic background; mouse behavior

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INTRODUCTION

Neuroligins are postsynaptic cell adhesion proteins that play a functional role in regulation of excitatory and inhibitory synapses (Levinson and El-Husseini, 2005a, b; Hu et al., 2015). Alterations in *NLGN3* and *NLGN4* genes have been implicated in autism and intellectual disability (Jamain et al., 2003; Cao and Tabuchi, 2016). Early autism genetic studies identified a missense mutation in *NLGN3* resulting in an arginine to cysteine substitution at conserved amino acid position 451 in two brothers, one with severe autism and one with Asperger's syndrome, a milder form of autism (Jamain et al., 2003). Heterogeneity involving the same genetic mutation raises an interesting question as to what may influence behavioral differences in two brothers with the same NL3R451C mutation. One explanation for phenotypic variability involving the same mutation is genetic heterogeneity (Hummel et al., 1972; Threadgill et al., 1995; Crawley, 1996; Gerlai, 1996). While the mutation may be the same, the differences in a number of other genes can play a role in phenotypic variation.

Phenotypic variation has also been observed in mouse models involving the *NLGN3* gene mutation NL3R451C. Initial behavioral studies of mice harboring a NL3R451C mutation on a hybrid C57BL6J/129S2/SvPasCrl genetic background identified decreased social behavior and enhanced spatial learning (Tabuchi et al., 2007). Social behavioral deficits were subsequently reproduced independently by the Südhof laboratory (Ehret et al., 2011a), followed by our own independent replication of both social and spatial learning differences on a pure 129S2/SvPasCrl genetic background (Jaramillo et al., 2014). A single study by the Crawley laboratory failed to replicate these findings in a similar NL3R451C mutation model on a C57BL6J background (Chadman et al., 2008). This suggested differences due to either genetic background or due to differences in behavioral protocols or expertise across laboratories. To address the question of whether genetic background modulates the behavioral phenotype of NL3R451C mutant mice, we have now examined the same mice characterized by the Crawley laboratory with the NL3R451C mutation on a pure C57BL6J background. Our findings replicate essentially the same behavioral results observed by Chadman et al. On a C57BL6J genetic background, the NL3R451C mouse model does not display social deficits or cognitive enhancement seen on either hybrid or pure 129S2/SvPasCrl genetic backgrounds. Our findings suggest that genetic background can modify behavioral features of the NL3R451C mutation. We conclude that genetic background rather than differences in behavioral testing or expertise across laboratories accounts for the discrepant findings in the literature.

METHODS

Generation of NL3R451C mice

NL3R451C on a pure C57BL6J background (NL3R451C^{C57}) were initially generated in the laboratory of Dr. Nathaniel Heintz on a pure C57BL6J genetic background (Chadman et al., 2008). Frozen spermatozoa were obtained as a gift from Dr. Heintz, and mice were re-derived by The Jackson Laboratory through *in vitro* fertilization. Mice were subsequently maintained on a pure C57BL6J genetic background.

Behavioral Overview

Behavioral tests were performed on a cohort of 42 male mice that ranged in age from 2.3 to 6.6 months of age. The cohort was generated by breeding wild-type males with heterozygous females (*Nlgn3* is an X-linked gene). Mice from the same litter were housed as pairs to ensure similar environmental experiences. Littermate pairs from multiple litters were used to decrease litter-specific effects.

Mice were kept on a 12-h light:12-h dark cycle, and behavioral testing was conducted during the light cycle. Behavioral testing started when the mice were between 2–6 months of age by an experimenter blind to genotype and blind to the purpose of the experiment and discrepancies in previous behavioral results.

Behavioral tests were conducted by testing less stressful behaviors before more stressful ones with attention to previous reports in the literature of order effects in behavioral testing (McIlwain et al., 2001). The order of behavioral testing was as follows: day 1: elevated plus maze (EPM), days 2-3 off, day 4: dark/light test (D/L), day 5: open field test (OF), days 6-7 off, day 8: locomotor test, days 9-10: rotarod, days 11-12: olfactory preference test, day 13: nesting, day 14-18 off, day 19: Startle test, day 20: prepulse Inhibition (PPI), days 21-27 off, days 29-32: social interaction with a juvenile mouse, day 33: 3-chamber sociability, day 34 off, day 35: Caged conspecific social approach, day 36: Olfactory test, days 37-38: Fear conditioning, days 39-67 off, days 67-85: Morris water maze. Days “off” were a result of non-availability of behavioral equipment, facility, or technician performing the experiment and were not predetermined. All behavioral testing of NL3R451C mice was performed by a behavioral core technician (LP) managed by Dr. Shari Birnbaum outside of the auspices of the Powell laboratory. The technician had no prior knowledge of any differences in behavioral outcomes in the NL3R451C mice (Tabuchi et al., 2007; Chadman et al., 2008; Etherton et al., 2011a; Jaramillo et al., 2014), and was blind to both the genotype and purpose of the study.

Prior to testing, mice were transported from their housing room to the site of behavioral testing for approximately one hour to habituate. Results are not presented in the order of testing to ease in the presentation and interpretation of the findings. All experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Texas of Southwestern Medical Center.

Behavioral tests

Elevated Plus Maze (EPM)—The EPM was conducted as previously described (Etherton et al., 2009; Jaramillo et al., 2014). Mice were placed in the center of a white Plexiglas elevated plus maze (each arm is 33 cm long and 5 cm wide, with 15-cm-high black Plexiglas walls on closed arms) and allowed to explore for 5 min. The test was conducted in dim white light (~7 lux). Data were recorded and analyzed using CleverSys® TopScan Software (Reston, VA).

Dark/Light Test (D/L)—The D/L test was conducted as previously described (Powell et al., 2004; Blundell et al., 2009; Jaramillo et al., 2014). Mice were placed in the dark

chamber of D/L box and allowed to habituate for 2 min. Following habituation, the technician removed a divider separating the dark side from the light side and mice were allowed to freely explore both chambers for 10 min while photobeams along the inside of the box tracked the mouse's time and frequency in each chamber. Boxes were custom made with each chamber 25 × 26 cm with 1,700 lux on the light side and ~0.1 lux on the dark side. Software and photobeam hardware are from Med Associates (St Albans, VT). Due to errors by the experimenter, 1 mouse pair (WT and corresponding littermate mutant) was removed from analysis.

Open Field Test (OF)—The OF was conducted as previously described (Etherton et al., 2009; Jaramillo et al., 2014). Mice were placed in the center of a white open field arena (44 × 44 × 44 cm) and allowed to explore freely for 10 min. The test was done under dim white light ~7 lux. CleverSys® TopScan software (Reston, VA) was used to measure and analyze the mouse's duration and frequency in a center zone (15 × 15 cm) and a thigmotaxis zone (4 cm from the wall of the open field).

Locomotor Activity—Locomotor activity was measured as described previously (Powell et al., 2004; Tabuchi et al., 2007; Etherton et al., 2009). Mice were placed in novel clean cages with minimal bedding (cage dimensions: 27.3 × 16.5 × 12.7 cm) and allowed to freely explore for 2 h. Horizontal locomotor activity (i.e. the number of photobeam breaks) was measured by computer software (San Diego Instruments; San Diego, CA), and data were analyzed in 5-min bins. This test was conducted under red light (~0.1 lux).

Accelerating Rotarod—The Accelerating rotarod was conducted as previously described (Powell et al., 2004; Kouser et al., 2013). Mice were placed on a stationary rotarod (IITC Life Sciences, Woodland Hills, CA) in a well-lit room that, when started, accelerated from 0 to 45 revolutions/min over 5 min. Mice were placed on the rod and the latency for mice to fall off was recorded. If a mouse clings onto the rod for one full revolution, that mouse's time was stopped and their latency was recorded. The test was conducted for 2 consecutive days with 4 trials per day for a total of 8 trials. Inter-trial intervals were 30 min.

Olfactory Discrimination Test—Initially test cages were prepared by inserting a dry cotton applicator that was 6" in length on a clean cage lid, so that the applicator hangs from the center of the lid, inside the cage. Mice were individually placed inside the test cage and allowed to habituate to the dry applicator for 15 minutes. After a 15-min habituation to a dry, cotton-tipped applicator, mice were exposed consecutively to 3, 2-min trials of both a swab dipped in water and a swab scented with dirty cage of an unfamiliar mouse. Order of presentation of water versus mouse scent was counterbalanced across trials and subjects.

Olfactory Cookie Test—One day prior to testing, food was removed from each mouse's home cage. The following day, a small portion of peanut butter cookie (Nutter Butter, Nabisco ®) was placed randomly ~1 cm under bedding in a novel clean mouse cage (Wersinger et al., 2004; Blundell et al., 2010b; Etherton et al., 2011b). A test mouse was taken from home cage and placed into the cage with the hidden cookie. Latency to find the cookie was measured. All mice found the hidden cookie in less than 10 min. One mouse pair

(WT and corresponding littermate) was removed from the study due to experimenter error (cookie was not buried sufficiently).

Nesting—Nesting behavior was performed in a well-lit (~80 lux) room. Mice were placed in a clean home cage that contained a cotton nestlet ($5.5 \times 5.5 \times 0.5$ cm). Height and width of the nests were measured at 30, 60, and 90 min.

Prepulse Inhibition and Startle Test—Both tests were conducted as previously described (Blundell et al., 2010a). For startle testing, mice were placed into startle chamber (San Diego Instruments, San Diego, CA) mounted atop a piezoelectric accelerometer that detected and transduced animal movements. Acoustic stimuli were delivered by high-frequency speakers mounted 33 cm above the cylinders. Animal movements were digitized and stored using computer software supplied by San Diego Instruments. For the startle reactivity test, eight presentations of six trial types were given in pseudorandom order: no stimulus, 80, 90, 100, 110, or 120 dB pulses. At the onset of startle stimuli, 65, 1-ms readings were recorded, and the amplitude of the startle responses was obtained in arbitrary units. Chambers were calibrated before each set of mice, and sound levels were monitored using a sound meter.

For prepulse inhibition (PPI), mice were subjected to five trial types within a 22 min session: pulse alone (40 ms, 120 dB, white noise pulse), three different prepulse/pulse trials (20 ms prepulse of 4, 8, or 12 dB above background noise level of 70 dB preceded the 120 dB pulse by 100 ms onset–onset interval), and no stimulus. All trials were presented pseudo-randomly with an average of 15 s (7–23 s) between the 62 trials. Testing began with a 5-min acclimation to the cylinders, followed by four blocks of test trials. The first and last blocks consisted of six pulse-alone trials. Blocks 2 and 3 each contained six pulse-alone trials, five of each level of prepulse/pulse trials, and five no-stimulus trials.

Social Interaction and Social Learning Behaviors—The 3-chamber sociability/preference test and caged conspecific social approach test were conducted as previously described (Moy et al., 2004; Nadler et al., 2004; Blundell et al., 2009; Kouser et al., 2013). In the 3-chamber sociability test mice were placed in the middle chamber and allowed to explore the apparatus for 10 min. Mice were removed from the box upon which a novel stimulus mouse was placed in the empty cage of one of the side chambers. Test mice were placed back into the box and were allowed to interact with an empty cage in one chamber or a caged social target (i.e. novel mouse) in the other for another 10 min. The locations of empty cage and target mouse were counterbalanced. Stimulus mice used for the 3-chamber sociability/preference test were 4–6 month old C57BL/6 male mice purchased from Jackson Laboratory (Bar Harbor, ME).

In the caged conspecific test, mice were placed into the same arena as the open field test and allowed to initially explore an empty cage for 10 min, then a cage housing a novel mouse for an additional 10 min. Time between 10-min trials was ~1 min. All social tests were done under dim white light ~7 lux. Data were recorded and analyzed using CleverSys® TopScan software (Reston, VA).

Fear Conditioning—Fear conditioning was performed as previously described (Blundell et al., 2008) with a few exceptions noted here. Mice were placed in a Plexiglas shock box (Med Associates, St Albans, VT) for 2 min, and then a 30 sec, 90 dB tone co-terminating in a 2 s, 0.5 mA foot shock was delivered twice with a 1-min interstimulus interval. Mice remained in the context for 2 min before being returned to their home cage. While the mice were in the box, freezing behavior in the training context was monitored every 5 s for 5 min by an observer blind to the experimental manipulation to measure contextual fear conditioning. The following day mice were placed back into the box and freezing behavior was observed for another 5 min. For cue fear conditioning, the inside of the fear conditioning box was altered with blue Styrofoam, and a paper towel sprayed with vanilla extract was placed under the chamber to change the look and smell of the chamber. Mice were re-exposed to the tone given initially, but no foot shock was given. Freezing behavior was monitored every 5 s for 5 min and percent freezing was measured before and during the 90 dB tone.

Morris water maze—The Morris water maze and visible platform tests were performed as described (Powell et al., 2004; Jaramillo et al., 2014) except that the probe trial was performed on day 12. Mice were trained to find a submerged platform in a pool (144 cm in diameter) of opaque water, with a thigmotaxis zone 9 cm from the wall of the pool. The water temperature was 22°C and was made opaque using gothic white, non-toxic tempera paint. Mice received 4 training trials per day. In all trials, mice were allowed to swim until they reached the platform or 60 s had elapsed. On day 12, 24 h following the conclusion of training, a probe test was conducted with the platform removed from the pool. Following the probe test, the platform was submerged in the opposite quadrant for reversal training. Following five days of reversal training, a probe trial was conducted. Reversal training was conducted similar to the initial training.

Statistical Analyses—Statistical analyses were conducted using StatPlus and Excel. All data were initially analyzed using two-way, unpaired Student's t-test or 2-way repeated measures ANOVA, as appropriate. Genotype was always used as the between-subject factors, and for the repeated measures ANOVAs, interaction target (for social interaction with a caged conspecific and 3-chamber sociability test), trial (for social interaction with a juvenile), day (for Morris water maze), or bin (for locomotor activity) were used as a within-subjects factor. Further planned comparisons were conducted using two-way, paired or unpaired t-tests, as appropriate. A thorough description of statistical analysis results can be found in Supplementary Table 1.

RESULTS

NL3R451C^{C57} mutant mice do not display enhanced spatial memory

Initially, we reported enhanced spatial memory in the NL3R451C mutant mice on a hybrid genetic background (Tabuchi et al., 2007), and replicated this finding on a 129S2/SvPasCrl genetic background (Jaramillo et al., 2014). To determine genetic background impacted enhanced spatial memory, we tested NL3R451C mutant mice on a pure C57BL6J background (NL3R451C^{C57}) in the Morris water maze. NL3R451C^{C57} mutant mice did not

display enhanced spatial memory as previously shown in hybrid or pure 129S2/SvPasCrl genetic background. NL3R451C^{C57} mice did not display a significant difference from WT mice in their latency to reach a hidden platform over an 11-d training session (Fig. 1A). Their distance traveled (Fig. 1B) and percentage of time spent in the thigmotaxis zone (Fig. 1C) were not significantly different from WT mice. Swim speed varied across days and genotypes with no significant interaction between genotype and day (Fig. 1D). On the 12th day we conducted a probe test with the hidden platform removed and observed WT mice showing a significant preference for target quadrant versus the other three quadrants. NL3R451C^{C57} mice also showed a preference for the target quadrant, though it was only significant versus one of the other three quadrants (Fig. 1E). Following the probe trial, we replaced the hidden platform in the opposite quadrant from previous training and conducted a reversal test. We did not observe a difference between WT and NL3R451C^{C57} in their latencies to find the new hidden platform location in the opposite quadrant (Fig. 1A, reversal). A probe test following 5 days of reversal training showed that NL3R451C^{C57} mice displayed a significant preference for the target quadrant versus the other three quadrants, while WT showed a significant preference for the target quadrant versus two of the other three quadrants (Fig. 1F).

NL3R451C^{C57} mutant mice do not display altered social interaction

Previously we observed social deficits in the NL3R451C mutant mice on hybrid (Tabuchi et al., 2007) and pure 129S2/SvPasCrl genetic backgrounds (Jaramillo et al., 2014). To determine whether background genetics influence social deficits, we tested NL3R451C mutant mice on a pure C57BL6J background using the 3-chamber sociability and caged conspecific social interaction tests. Initially in the 3-chamber sociability test, we did not observe a chamber preference for either group when the mice explored the empty chambers (Fig. 2A). However, when one of the chambers contained a social target (novel mouse under a cage) and the other contained just an empty cage (upside down wire pencil cup holder), both the mutant and WT mice showed a preference for the chamber that contained the social target (Fig. 2B). Time spent interacting with each target was equivalent across genotypes. In the caged conspecific test, both WT and NL3R451C^{C57} mice displayed similar levels of interaction with the social target (Fig. 2C). However, NL3R451C^{C57} mice showed a significant increase in total distance moved in the caged conspecific test compared to WT mice (Fig. 2D).

Anxiety and locomotor test in NL3R451C^{C57} mutant mice

Previously, NL3R451C mutant mice on a 129S2/SvPasCrl genetic background (Jaramillo et al., 2014) displayed increased locomotor activity and decreased anxiety-like behavior. NL3R451C^{C57} mice also displayed an increase in locomotor activity over a 2-h time period in the locomotor boxes compared to WT mice expressed by 5-min bins (WT vs. NL3R451C P= 0.003; Fig. 3A) and by total beam breaks during the 2-h session (WT vs. NL3R451C P= 0.0132, Fig. 3B).

We next conducted a series of tests to investigate potential anxiety-like behavior. In the open field test both mouse groups displayed similar total distance traveled (Fig. 3C) and spent similar time in the central anxiogenic region (Fig. 3D) and the thigmotaxis region (Fig. 3E).

We next tested motor ability and learning with the Rotarod test and found that both groups displayed similar latencies to fall over a 2-d period with 4 trials per day (Fig. 3F). In the elevated plus maze test, both groups displayed similar distances traveled (Fig. 3G), along with similar time in both the open and closed arms (Fig. 3H). The number of entries into the open and closed arm was also similar between groups (Fig. 3I). In the dark/light test, time spent in the dark or light chambers was equivalent between the groups (Fig. 3J). The latency to explore the light side was also similar between groups (Fig. 3K).

Associative learning, sensory response, and innate behaviors in NL3R451C^{C57} mutant mice

Because genetic background might reveal novel atypical behaviors in the NL3R451C^{C57} mutant mice, we also tested whether learning was altered in this model. In both context- (Fig. 4A) and cue-dependent (Fig. 4B) fear conditioning, we did not observe a difference between mouse groups. To further assess sensory responses in these mice we tested their response to tones of increasing volume and found NL3R451C^{C57} mutant mice displayed less acoustic startle to high decibel tones (Fig. 4C). (100dB: $P=.034$, 110dB: $P=.00022$, 120dB: $P=.00003$) Prepulse inhibition of acoustic startle was unchanged among the genotypes (Fig. 4D). Finally, we also tested innate behaviors such as nest building (Fig. 4E–F) and olfaction (Fig. 4G–H). We found that NL3R451C^{C57} mice formed slightly wider nests (Fig. 4E) of similar height (Fig. 4F) compared to WT littermates. No difference in latency to find an appealing food treat was observed (Fig. 4G). In an olfactory discrimination test, both WT and mutant mice showed significant preference for the mouse scent compared to the water scent (Figure 4H, **Trial 1**). In subsequent trials, repeat exposures to the same mouse scent show significantly decreased interaction time in both WT and mutants (Figure 4H, **Trials 2 & 3**).

DISCUSSION

Phenotypes associated with a given genetic mutation can vary among closely related individuals, such as the NL3R451C substitution identified in two Swedish brothers diagnosed with ASD. One brother displayed severe intellectual disability and epilepsy, while the other displayed a non-verbal learning disorder with no apparent epilepsy (Jamain et al., 2003). This phenotypic variation in the two brothers can be reasonably explained because of variation in genetic background due to either incomplete penetrance or to variability in the expression of the disease-associated genotype.

We observed similar phenotypic variation in several studies involving the NL3R451C mutation in mice of different genetic backgrounds. The focus of our previous study (Jaramillo et al., 2014) and this study is to determine if genetic background plays a role in phenotypic variability involving the NL3R451C mutation. The initial NL3R451C mutant mouse model published by Tabuchi et al was generated on a mixed C57BL6J $\times$ 129SvJ background and displayed deficits in social interaction along with enhanced spatial learning abilities. However, a subsequent study by Chadman et al involving the same mutation on a pure C57BL6J background did not observe social interaction deficits nor enhanced spatial learning (Chadman et al., 2008), but instead displayed minimal aberrant behavior. One

possible explanation for this discrepancy could involve genetic heterogeneity due to the 129SvJ strain of the C57BL6J $\times$ 129SvJ mixed background. The 129SvJ genetic background may provide a susceptibility locus to allow for more phenotypic penetrance or to allow for enhanced expressivity of the phenotype. Another possibility is that susceptibility to a wider range of phenotypes can be a result of a cumulative effect from modifiers originating from both strains on a mixed genetic background. The former remains a strong possibility considering we previously reported deficits in social interaction along with enhanced spatial learning abilities in NL3R451C mutant mice on a pure 129SvJ genetic background (Jaramillo et al., 2014). We provide a table comparing the behavioral results among the various NL3R451C mutant models in Supplementary Table 2.

In this study, we sought to test the behaviors previously tested in the NL3R451C mutant mice on a pure 129SvJ background. We also included additional behavioral tests to determine if the NL3R451C mutation on a pure C57BL6J genetic background provided any additional phenotypes. While it would have been ideal to perform exactly the same behavioral tests in exactly the same order in this and all previous publications (Tabuchi et al., 2007; Jaramillo et al., 2014), in all cases WT and mutant mice were tested as age- and sex-matched littermates together in the same facility within each study.

In the Morris water maze, we did not observe a difference in the rate of learning between WT and mutant mice, and both groups showed some preference for the target quadrant over other quadrants in the probe test. Curiously, while the WT mice appropriately showed increased latencies to reach the target platform on the first day of reversal training, the WT mice did not. This might be explained by a relative decrease in spatial strategy learned by the mutants compared to the WTs. Indeed the WT mice showed significant preference for the target quadrant compared to all three other quadrants while the mutants only showed significant preference for the target quadrant compared to a single other quadrant. Still, our conclusion is that NL3R451C mice on a C57 background behave differently on this task compared to the same mutants on other backgrounds.

We also did not observe differences in social interaction in NL3R451C mutant mice on a C57BL6J background compared to WT littermates. Our findings are similar to the negative findings in test of both social interaction and Morris water maze observed by Chadman et al. The similarity of these two studies in the C57 background suggests that the 129SvJ genetic background plays a role in the enhanced spatial learning and social interaction deficits associated with the NL3R451C mutation.

Additionally behavioral testing involving associative learning, innate behaviors, anxiety-related traits, and motor function was also unchanged in mutants compared to WT mice in our study. In the auditory startle response test, however, NL3R451C mutant mice displayed a significant decrease in startle response compared to WT mice. Although startle response was not tested in mutant mice on the mixed or pure 129SvJ background, it may be of interest to determine if this phenotype is exclusive to the pure C57BL6J background considering other studies have observed differences in startle responses between mice of 129/SvJ and C57BL6 genetic backgrounds.

Several previous publications have highlighted behavioral differences among various genetic background strains and even within substrains (Kiselycznyk and Holmes, 2011). 129/SvJ mice have been shown to have a lower maximal startle response to both tactile and acoustic stimuli than C57BL6J mice (Crawley et al., 1997). However, substrains of 129 and C57 background have shown the opposite in startle response with 129 having the higher response compared to C57 (Bullock et al., 1997). Behavioral differences among the 129/SvJ and C57BL6 backgrounds have also been reported in prepulse inhibition (Crawley et al., 1997; Paylor and Crawley, 1997) with 129/SvJ having a larger response than C57BL6, Morris water maze (Upchurch and Wehner, 1989; Owen et al., 1997) with 129/SvJ being poorer learners than C57BL6, anxiety (Dockstader and van der Kooy, 2001) with 129/SvJ displaying higher anxiety, and differences in behavioral responses to several pharmacological agents (Homanics et al., 1999; Varnado-Rhodes et al., 2000). Additionally, behavioral studies involving substrains of 129 and C57 genetic background have been shown to display differences, such as in startle response (Bullock et al., 1997), water maze (Owen et al., 1997; Nguyen et al., 2000; Voikar et al., 2001; Wolff et al., 2002), and locomotor activity (Gerlai, 1996; Baron and Meltzer, 2001; Holmes et al., 2002).

In summary, this study supports the importance of background strain on behavior in mutant mice. Our findings are consistent with the conclusion that genetic background accounts for differences in behavioral findings in the NL3R451C mutant models published to date (Tabuchi et al., 2007; Chadman et al., 2008; Etherton et al., 2011a; Jaramillo et al., 2014). In general, if studies involving mutant mice are to be reproducible and reliable, the importance of conducting these studies on models whose backgrounds are well defined, stable, and clearly communicated cannot be over-emphasized.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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LAY SUMMARY

Behavioral symptoms of autism can be highly variable, even in cases that involve identical genetic mutations. Previous studies in mice with a mutation of the Neuroligin-3 gene showed enhanced learning and social deficits. We replicated these findings on the same and different genetic backgrounds. In this study, however the same mutation in mice on a different genetic background did not reproduce our previous findings. Our results suggest that genetic background influences behavioral symptoms of this autism-associated mutation.

Figure 1. NL3R451C Mutants Exhibit Similar Levels of Spatial Learning in the Morris Water Maze Task as WT Littermates

(A–C) NL3R451C mice exhibit normal spatial learning in the Morris water maze during training when compared to WT littermates using both latency (A) and distance traveled (B) prior to reaching the hidden platform. NL3R451C mice spend similar time to WT littermates swimming near the wall of the water maze (thigmotaxis) compared to WT littermates during training trails (C). Swim speed over the 11 days of training revealed no interaction between genotype and day, a main effect of day, and a main effect of genotype overall. (D). Both WT and NL3R451C mice showed a statistically significant preference for the Target quadrant at least one other quadrant during a probe trial (E). When the platform location was reversed and mice were re-trained, WT and NL3R451C mice showed a statistically significant

preference for the Target quadrant over at least two of the three other quadrants (**F**). (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$; n=20 littermate pairs)

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Figure 2. NL3R451C Mutants Exhibit Similar Levels of Social Interaction as WT Littermates

(A) NL3R451C mutants display equal preference for both end-chambers in the 3-chamber apparatus. (B) NL3R451C and WT littermates both display a preference for the social target (caged novel mouse) over the empty cage. (C) In the caged conspecific test, both groups of mice displayed similar interaction time with the caged novel mouse. Additionally, both groups of mice traveled similar distances in the caged conspecific test (D). (* $P < 0.05$, **** $P < 0.0001$; $n = 20$ littermate pairs)

Figure 4. Fear conditioning, Startle and PPI, Nesting, and Olfactory test in NL3R451C mice
 NL3R451C mutant mice displayed similar freezing levels as WT littermates in the both contextual (A) and cued (B) fear conditioning. NL3R451C mutant mice exhibited decreased startle amplitude compared to WT mice (C). Prepulse inhibition of startle was similar between groups (D). Also, NL3R451C mutant mice displayed a significant increase in nest width at 60 and 90 min when compared to WT mice (E) but did not display a difference in nest height (F). NL3R451C mutant mice displayed similar results in two olfactory tests, latency to find a hidden treat (G) and olfactory discrimination (H). In panel H, both WT and mutants show significantly increased preference for the novel mouse smell compared to the water smell (Trial 1, asterisks over horizontal comparison lines). On subsequent trials 2 & 3, the time interacting with the now familiar mouse scent is significantly decreased compared to Trial 1 for both WT and mutants (Trials 2 & 3, vertically arrayed asterisks denote significant difference for interaction with familiar mouse scent compared to Trial 1).

interaction with the initially novel mouse scent for the same genotype). (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$; n=20 littermate pairs)

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