Exploring Parent-of-Origin Effects on Contextual and Cued Conditioned Threat Learning in Type III Neuregulin 1 Transmembrane Domain Mutant Mice

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1. INTRODUCTION

Schizophrenia is a complex psychiatric disorder caused by both genetic and environmental factors (Sullivan et al., 2003). Genetic disruptions and prenatal complications suggest that schizophrenia is a neurodevelopmental disorder (Murray et al., 1987; Walsh et al., 2008). Interactions between multiple genes and environmental factors alter brain development in ways that increase the risk of developing the disorder (Tsuange, 2000).

Genome wide association studies (GWAS) of populations in Iceland and Scotland identified Neuregulin 1 (Nrg1) as a schizophrenia susceptibility gene (Stefansson et al., 2002). Nrg1 encodes for a family of signaling molecules that play fundamental roles in the development of the nervous system (Harrison et al., 2005). When Nrg1 signaling is disrupted *in vivo*, there are morphological and psychological defects, as evidenced by a variety of mouse models. Mice heterozygous for a Nrg1 nonsense mutation that truncates the protein in the transmembrane domain display a hyperactive phenotype (Karl et al., 2007). Additionally, synapse maintenance is disrupted in mice with a mutation that eliminates expression of Type III Nrg1 (Wolpowitz et al., 2000). Nrg1 mice with partial deletion of epidermal growth factor like domains display decreased sociability and reduced freezing during contextual fear conditioning (Ehrlichman et al., 2007). These distinct phenotypes indicate that a variety of brain functions are affected by mutations within Nrg1. There are a number of phenotypes unique to Type III Nrg1 mutant animals. In Type III Nrg1 mutant mice, axon pathfinding is disrupted, and there are deficits in Schwann cell development (Hancock et al., 2011; Birchmeier et al., 2008). Type III Nrg1 mutant mice also exhibit impaired memory and sensorimotor gating (Chen et al., 2008).

The Type III Nrg1 cell receptor spans the cell membrane in a hairpin-like fashion. There are two transmembrane domains, one which is a cysteine-rich domain and one which is located at the c-terminus (Fleck at al., 2013). Type III Nrg1 goes through a series of complex proteolytic cleavages (Fleck at al., 2013). After juxtacrine signaling, the intracellular domain (ICD) at the c-terminus is liberated by gamma secretase and enters the nucleus, where it interacts with other proteins to alter gene expression in a process known as back-signaling (Bao, 2003). A valine to leucine hypomorphic mutation, or a mutation causing partial loss of gene function, in the transmembrane domain disrupts cleavage by gamma secretase and is associated with morphological defects in the development of cortical neurons (Chen et al., 2010).

This valine to leucine substitution in the human Nrg1 gene was originally identified in an isolated population in Costa Rica and was shown to be associated with an increased risk of psychosis, a key symptom of schizophrenia (Walss-Bass et al., 2006). Among the symptoms of schizophrenia are delusions, hallucinations, disorganized speech and behavior, and negative emotional response (American Psychiatric Publishing, 2013). The areas of the brain most connected to these behaviors are the

hippocampus and the amygdala, the former of which has structural abnormalities in chronic schizophrenics (Velakoulis et al., 2006). Associative, or conditioned threat, learning, analyzes functions that require both the amygdala and hippocampus (Clark et al.,1998; Goosens et al., 2001). The paradigm teaches animals to associate neutral sensory inputs (e.g. a general context or a discrete tone) with an aversive stimulus and monitors their subsequent response to these sensory cues (Curzon et al., 2009). In mice heterozygous for a mutation that eliminates expression of Type III Nrg1, abnormalities in contextual threat conditioning were found to be influenced by both sex (males showed a phenotype, females did not) and the parent-of-origin of the mutation (Shang et al., 2017). The hypothesis that schizophrenia is influenced by parent-of-origin is supported by a study analyzing a locus on chromosome 2p12-q11 that is associated with schizophrenia (Francks et al., 2003) and supporting evidence that the locus contains an imprinted gene, LRRTM1 (Ludwig et al., 2009).

This study used both the contextual and cue conditioned threat learning paradigm to ask if there is a link between the valine to leucine mutation in the transmembrane domain and a behavioral phenotype across wild type, heterozygous and mutant mice, and if so, whether the phenotype was influenced by the parent-of-origin effect of the mutation.

2. METHODOLOGY

2.1 Animals

The use of animals was approved by the Institutional Animal Care and Use Committee of State University of New York, Stony Brook (Stony Brook, NY, USA). Procedures are in accordance with NIH guidelines.

The Talmage lab had previously used homologous recombination mediated gene targeting to introduce the valine to leucine substitution into the mouse Nrg1 gene. Mice homozygous for the valine/leucine substitution are viable and fertile. Two breeding schemes were used to generate the cohorts used in this study. Breeding scheme one used crosses of males and females that were heterozygous for the mutant allele and resulted in wild type (valine/valine), heterozygous (valine/leucine). and homozygous (leucine/leucine) mutant offspring. The parent-of-origin of the leucine allele was unknown in this cohort.

Breeding scheme two used crosses of wild type males/females and heterozygous males/females that generated wild type and heterozygous offspring where the parent-of-origin of the mutant allele was known. For example, a heterozygous female crossed with a wild type male produced a mutant mother offspring.

Sample sizes of all test groups are shown in Table 1. All animals were on a pure C57Bl6/J background. Animals were weaned on postnatal day 21.

Genotypes were determined via PCR performed with genomic DNA isolated from tail biopsies. DNA was isolated using the DNeasy Blood & Tissue Kit following the manufacturer's protocol (Qiagen, 2006). PCR was then performed with the genotyping primers, NDEL1 and NDEL2. The NDEL1 primers sequence is 5'- GGT GAT CCC ATA CCC AAG ACT CAG -3'. The NDEL2 primer sequence is 5'- CTG CAC ATT TAT AGA GCA TTT ATT TTG G -3'. PCR conditions: one minute at 95°C followed by 35 cycles between 15 seconds at 95°C, 15 seconds at 65°C, and 15 seconds at 72°C. PCR products were separated using electrophoresis on a 2% agarose gel (Figure 1).

Animals were maintained in a vivarium at Stony Brook University (Stony Brook, NY, USA). Housing facilities were temperature- and humidity-controlled. Food and water were available *ad libitum*. Animals were kept on a 12 hour light/dark cycle with the light cycle running from 7AM to 7PM. Cages had a maximum of four animals; no animals were single housed prior to behavioral testing. Animals were tested at 10-14 weeks of age. The experimenter was blind to genotype throughout.

Genotype	Sex	Sample Size
Wild Type		
	Female	2
	Male	7
Mutant		
	Female	4
	Male	5
Heterozygous, Parent-of-Origin Unknown		
	Female	4
	Male	4
Heterozygous, Mutant Mother		
	Female	4
	Male	5
Heterozygous, Mutant Father		
	Female	4
	Male	3

Table 1. Sample Sizes by Genotype and Sex.

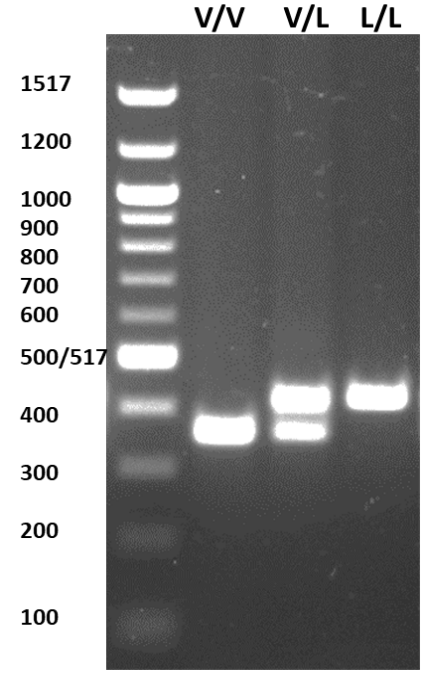

Figure 1. PCR gel depicting the differences in the bands corresponding to each of the three genotypes. V/V signifies valine/valine, the wild type genotype. V/L signifies valine/leucine, the heterozygous genotype. L/L signifies leucine/leucine, the homozygous genotype. (Image kindly provided by Dr. David Talmage)

2.2 Conditioned Threat Learning

A schematic of the conditioned threat learning protocol is provided below (Figure 2). This study utilized both contextual and cued conditioned threat learning in order to examine both contextual associative learning and cued associative learning. The conditioned threat learning protocol was performed for six consecutive days, each test 24 hours after the previous. Throughout the procedure, a white noise generator was used to eliminate background noise. The behavioral paradigm had 4 distinct phases: handling, training, contextual recall, and cue recall:

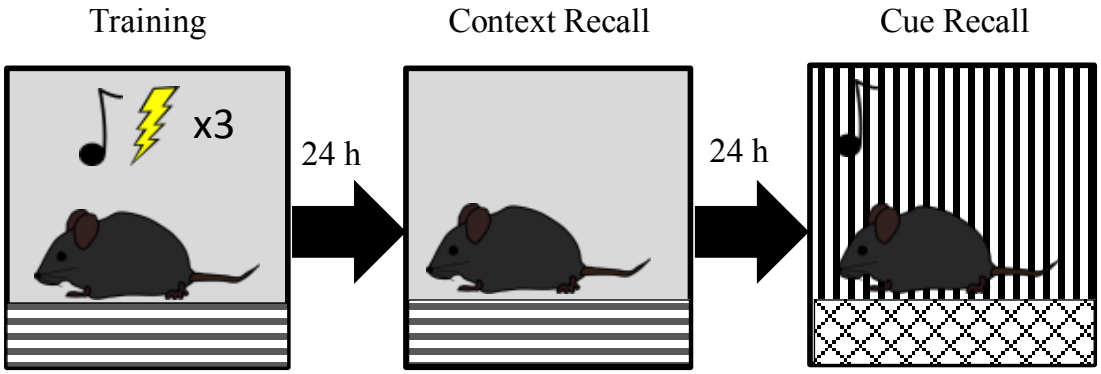

Figure 2. Conditioned Threat Learning Protocol. Schematic of days four, five, and six of the protocol. The music note signifies the 30 second tone and the lightning bolt signifies the 2 second shock. Image created by the author of this paper.

2.2.1 Handling

The first three days of the procedure consisted of handling sessions to reduce extraneous anxiety. On each day, individual animals were handled (cupped in researcher's hands with minimal or no restraint) for a total of five minutes.

2.2.2 Training

On the fourth day of the procedure, mice were trained. Individual mice were placed in fear conditioning cubicles with silver walls and metal grid floors, both scented with ethanol. The protocol consisted of 120 seconds of free exploration, a 30 second long 80 decibel tone, and a 2 second 0.8 mAmp shock presented via the metal floor. Note that the shock was presented during the last 2 seconds of the tone. At 240 and 360 seconds into the session, the tone and foot shock pairing was repeated. After the final pairing, mice remained in the chamber for an additional 90 seconds. A schematic of the training procedure is provided below (Figure 3).

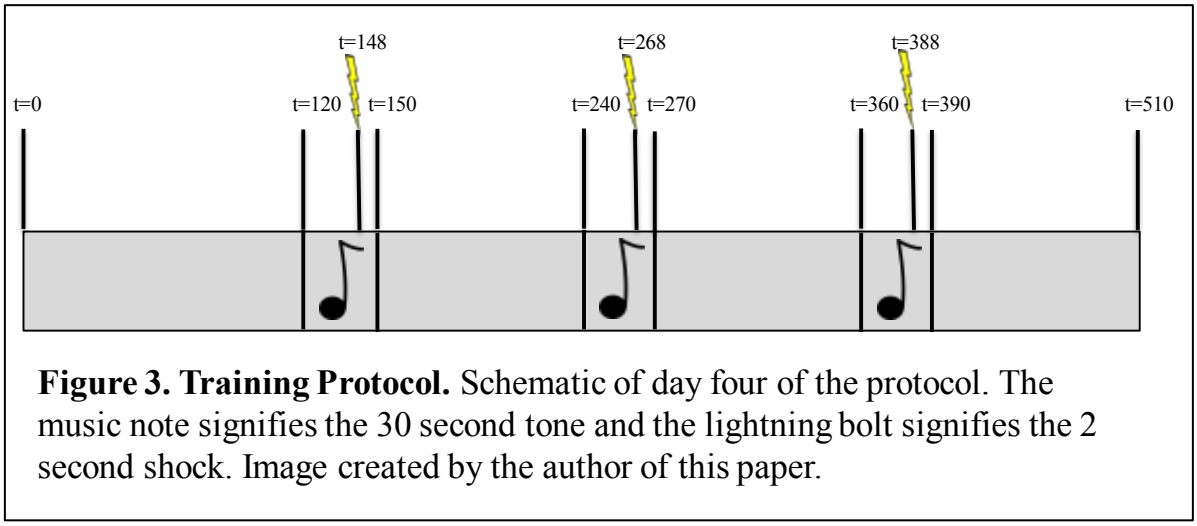

Figure 3. Training Protocol. Schematic of day four of the protocol. The music note signifies the 30 second tone and the lightning bolt signifies the 2 second shock. Image created by the author of this paper.

2.2.3 Context Recall

Twenty-four hours after the training session, animals were returned to the same conditioning chamber and their freezing was recorded during a 480 second session. Freezing was defined as lack of movement aside from breathing. This session provides a measure of the animals' freezing in the same context in which they were trained.

2.2.4 Cue Recall

Forty-eight hours after training, mice were placed in a cubicle with black and white striped walls and a mesh floor that was scented with lemongrass citrus. This altered visual, tactile, and olfactory cues, relative to the training environment. After 120 seconds of free exploration, mice were subjected to the same tone used during the training session for 30 seconds. As opposed to the training protocol, mice were not shocked, and the tone was only administered once. Following the single tone presentation, mice remained in the chamber for an additional 150 seconds. Freezing was also recorded during cue recall.

2.3 Video Analysis

During the training and both recall sessions, mice were filmed with cameras connected to Freeze Frame 4 trial recorder software. The software analyzes motion with a motion detection algorithm (ActiMectrics Software, 2018). After the videos were recorded, Freeze Frame 4 trial viewer was used to identify and record the times at which each mouse froze. The protocols were broken up into 30 second time bins and analyzed for the percentage of time spent freezing during that time bin.

2.4 Statistics

The percentage of time spent freezing in 30 second time bins was exported from Freeze Frame 4 first into a Microsoft Excel workbook and then into an IBM SPSS file. All graphs were created in Microsoft Excel, and statistical analyses were performed in IBM SPSS. Differences between groups of wild type, heterozygous (parent-of-origin unknown), and mutant mice during training were analyzed with a repeated measures (RM) analysis of variance (ANOVA) for each 30 second time bin. The same statistical analyses were used when comparing heterozygous mice with mutant mothers (MM) and mutant fathers (MF). Differences between groups of male and female mice within genotypic groups during training were analyzed with RMANOVA. Genotypic effect on percent freezing across the full 480 seconds of context recall was averaged and then analyzed using a one way ANOVA. The same statistical analyses were performed when comparing heterozygous mice MM and MF. Differences between male and female mice within genotypic groups during context recall were also analyzed with a one way ANOVA. The percent freezing data for cue recall was averaged into three time bins: pre-tone, tone, and tone. Differences between groups of heterozygous (parent-of-origin unknown), mutant, and wild type mice during cue recall were analyzed with a RMANOVA for each of the three time bins. The same statistical analyses were performed when comparing heterozygous mice with MM and MF. Differences between groups of male and female mice within genotypic groups during cue recall were analyzed with RMANOVA as well. Response to context was isolated by comparing the naïve portion of training (i.e. the first 120 seconds in the novel context), the first two minutes of context recall, and the naïve portion of cue recall (i.e. the first 120 seconds in the novel context). Differences between groups of heterozygous (parent-of-origin unknown), mutant, and wild type mice were analyzed with a RMANOVA each time bin. The same statistical analysis was performed when comparing heterozygous mice with MM and MF. Differences between groups of male and female mice within genotypic groups during training were analyzed with RMANOVA as well. Significance was defined as $p < .05$.

3. RESULTS

A previous behavioral study found that parent-of-origin impacted male but not female mice within mice heterozygous for a mutation that eliminates expression of Type III Nrg1 (Shang et al., 2017). Therefore, this study analyzed female and male mice separately in order to insure that a reduced phenotype within females or males did not mask a genotypic effect. This is also necessary due to the small sample sizes of experimental groups, in particular, the female wild type mice.

3.1 Genotypic Effect within Females

During training, a RM ANOVA revealed that there were no significant differences between the percent freezing of wild type, heterozygous, and homozygous female mice [$F(6.23, 21.91) = 1.31$, $p = 0.293$; Figure 4A]. Freezing increased throughout the procedure, indicating that female animals learned to associate the context and/or the tone with the aversive stimulus. The one way ANOVA for context recall revealed that there was no significant difference between wild type, heterozygous, and homozygous female mice [$F(2, 7) = 3.16$, $p = 0.105$; Figure 4B]. Within the isolated context recall analysis, there was not a significant difference between genotypes, but there was a strong trend [$F(2.23, 7.80) = 4.16$, $p = 0.056$; Figure 4C]. Additionally, statistical analyses of freezing during cue recall that analyzed female mice grouped by genotype yielded no significance [$F(4, 14) = 0.31$, $p = 0.856$; Figure 4D].

3.2 Genotypic Effect within Males

During training, a RM ANOVA revealed that there were no significant differences between the percent freezing of wild type, heterozygous, and homozygous male mice [$F(10.18, 66.15) = 1.48$, $p = 0.165$; Figure 5A]. The one way ANOVA for context recall revealed that there was a significant difference between wild type, heterozygous, and homozygous male mice [$F(2, 13) = 7.98$, $p = .005$; Figure 5B]. Post hoc analyses indicated significant differences between wild type and mutant male mice [$p = 0.031$] as well as between heterozygous and mutant male mice [$p = 0.007$]. However, there was no significant difference between wild type and heterozygous male mice [$p = 0.686$]. Within the isolated contextual recall analysis, there was a significant difference between genotypes [$F(4, 24) = 3.05$, $p = 0.036$; Figure 5C]. Post hoc analyses revealed significant differences between heterozygous and mutant male mice [$p = 0.043$] but not between wild type and mutant male mice [$p = 0.136$] or between wild type and heterozygous male mice [$p = 0.832$]. Statistical analyses of freezing during cue recall that analyzed male mice divided by genotype yielded no significance [$F(4, 24) = 1.52$, $p = 0.228$; Figure 5D].

3.3 Parent-of-Origin Effect within Females

Throughout the training procedure, there was not a significant difference for percent freezing between MF and MM female mice [$F(3.59, 21.52) = 0.86$, $p = 0.496$; Figure 6A]. Additionally, during context recall, a one way ANOVA revealed that there was no significant difference between MM and MF female mice [$F(1, 6) = 2.70$, $p = 0.151$; Figure 6B]. The parent-of-origin data within the isolated contextual recall analysis also yielded no significance [$F(1.11, 6.64) = 3.61$, $p = 0.100$; Figure 6C]. However, there was a significant difference between MF and MM female mice during cue recall [$F(2, 12) = 5.92$, $p = 0.016$; Figure 6D].

3.4 Parent-of-Origin Effect within Males

Throughout the training procedure, there was no significant difference for percent freezing between MF and MM male mice [$F(3.73, 22.36) = 2.27$, $p = 0.098$; Figure 7A]. Additionally, during context recall, a one way ANOVA revealed that there was no significant difference between MM and MF male mice [$F(1, 6) = 0.15$, $p = 0.713$; Figure 7B]. The parent-of-origin data within the isolated contextual recall analysis also yielded no significance [$F(2, 12) = 0.28$, $p = 0.758$; Figure 7C]. There was also no significant difference between MF and MM male mice during cue recall [$F(2, 12) = 0.44$, $p = 0.657$; Figure 7D].

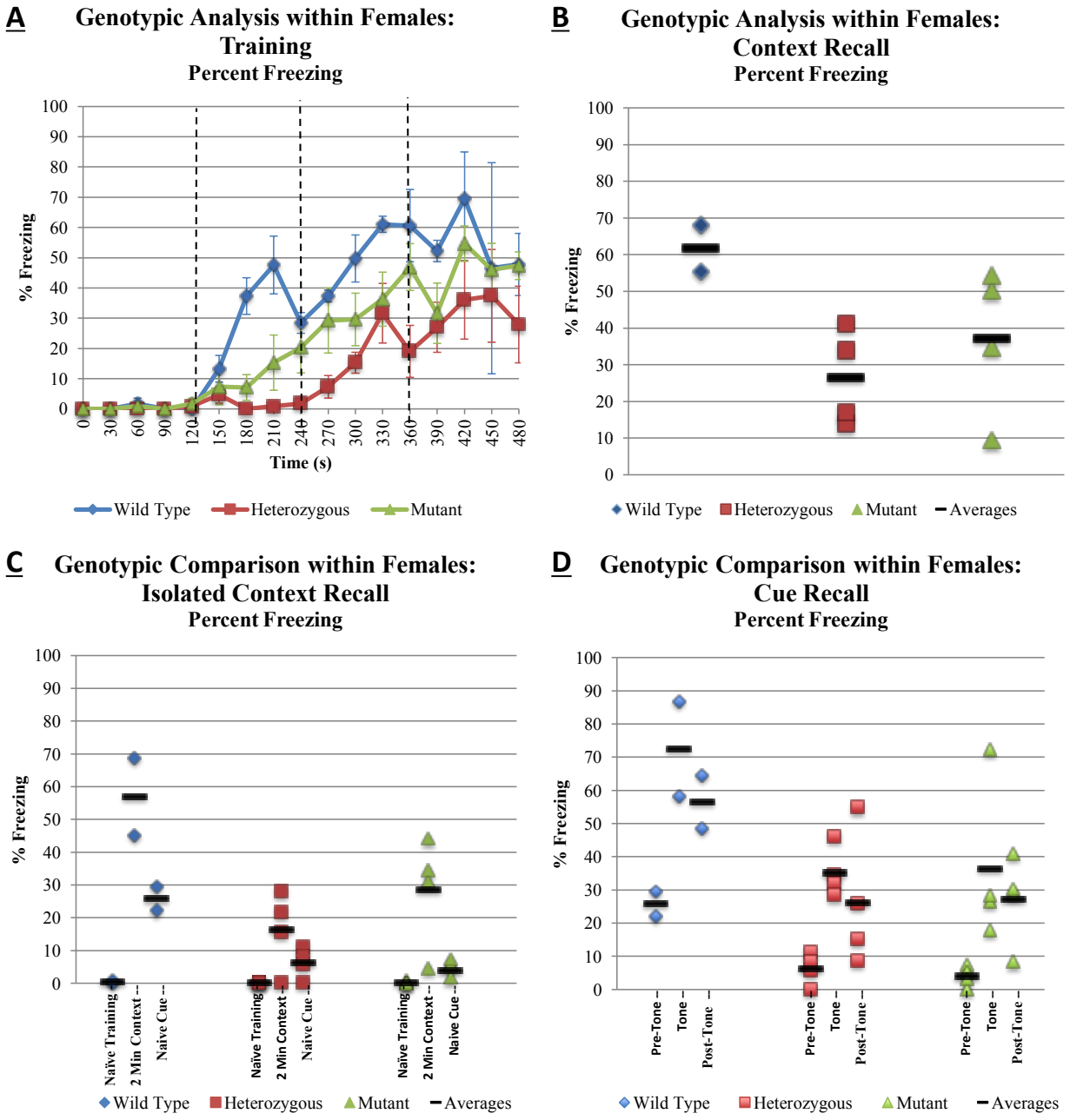

Figure 4. Female Percent Freezing by Genotype. Percent freezing in female wild type, heterozygous with parent-of-origin unknown, and mutant mice **(A)** during training protocol, **(B)** during context recall, **(C)** with context recall isolated, **(D)** during cue recall. Data for Figure 4A is presented as mean ± SEM. Data for Figures 4B-D are presented as percent freezing per individual animal with a mean per experimental group. In Figure 4A, dashed lines indicate tone onset.

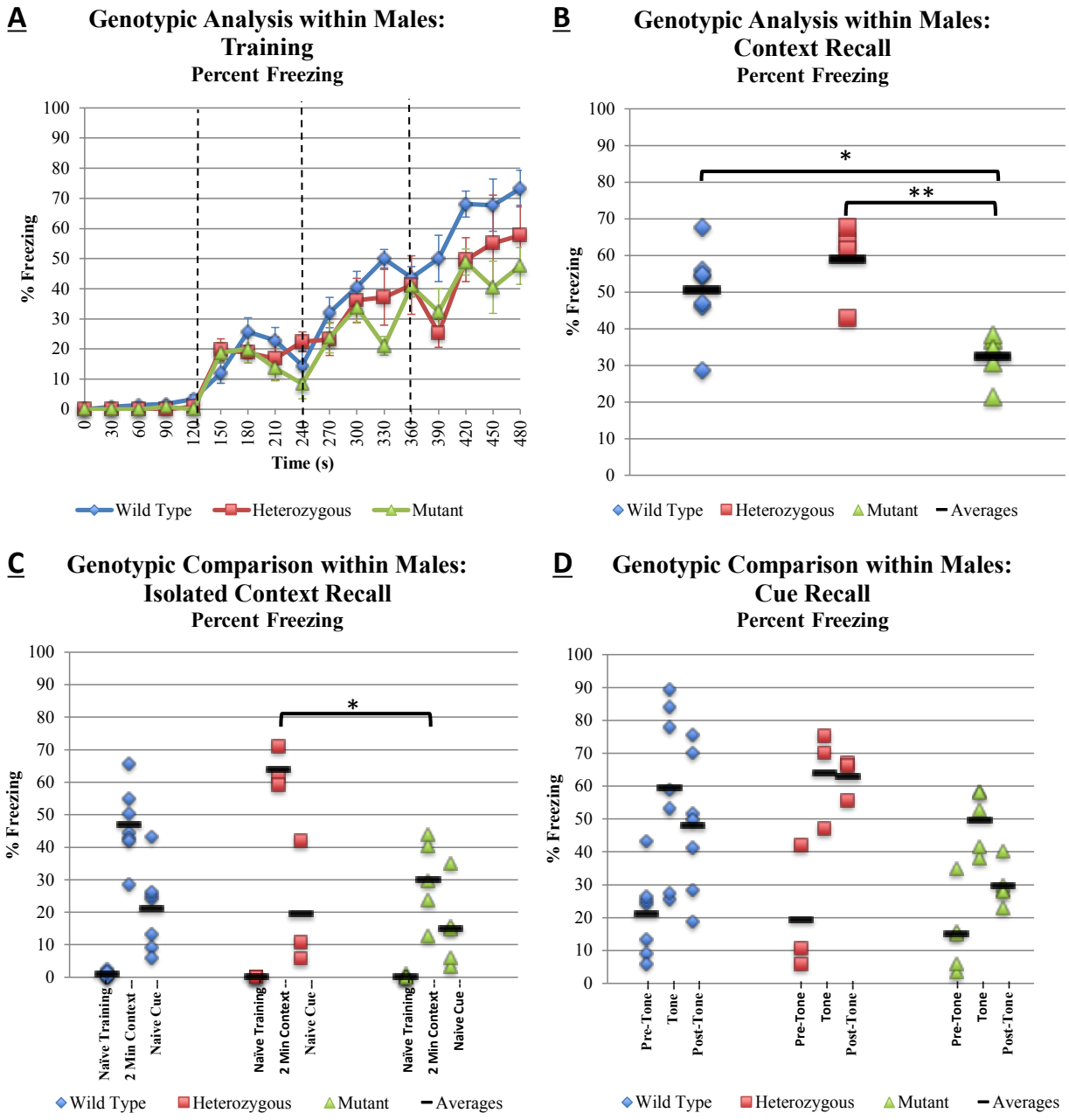

Figure 5. Male Percent Freezing by Genotype. Percent freezing in male wild type, heterozygous with parent-of-origin unknown, and mutant mice **(A)** during training protocol, **(B)** during context recall, **(C)** with context recall isolated, **(D)** during cue recall. Data for Figure 5A is presented as mean ± SEM. Data for Figures 5B-D are presented as percent freezing per individual animal with a mean per experimental group. In Figure 5A, dashed lines indicate tone onset. * signifies $p < 0.05$; ** signifies $p < 0.01$.

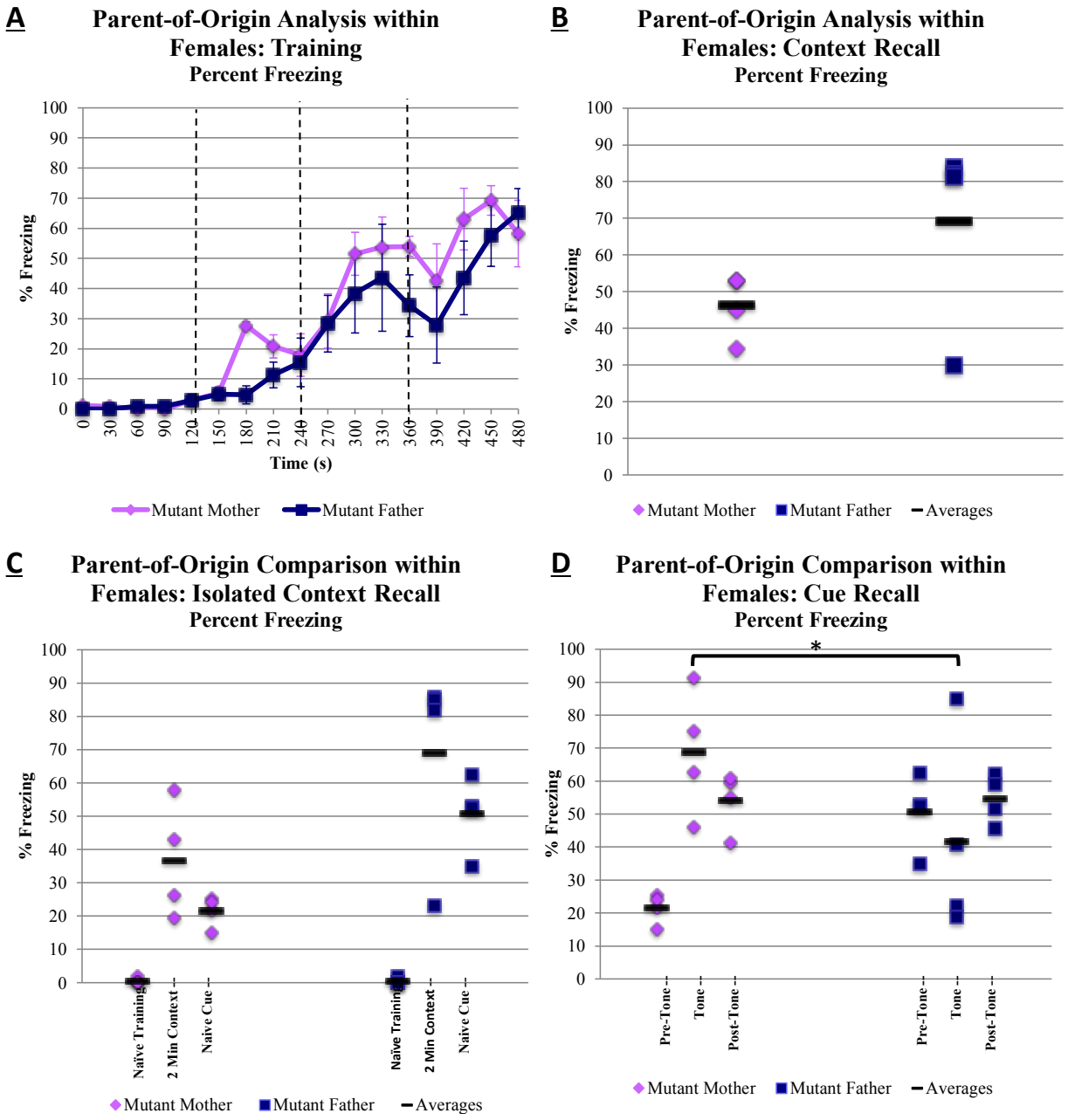

Figure 6. Female Percent Freezing by MM and MF. Percent freezing in female heterozygous mice with MMs and MFs **(A)** during training protocol, **(B)** during context recall, **(C)** with context recall isolated, **(D)** during cue recall. Data for Figure 6A is presented as mean ± SEM. Data for Figures 6B-D are presented as percent freezing per individual animal with a mean per experimental group. In Figure 6A, dashed lines indicate tone. * signifies $p < 0.05$.

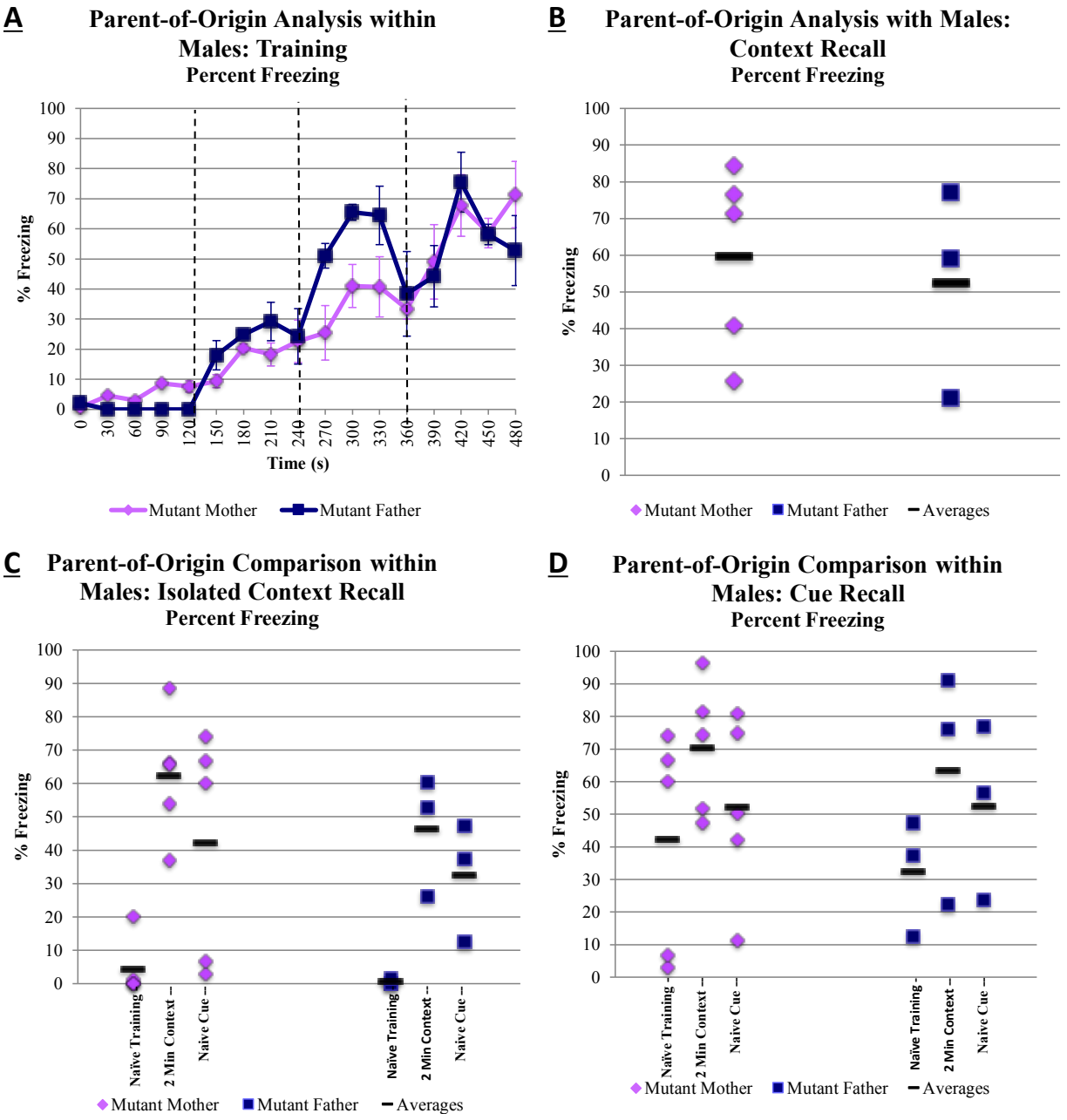

Figure 7. Male Percent Freezing by MM and MF. Percent freezing in male heterozygous mice with MMs and MFs **(A)** during training protocol, **(B)** during context recall, **(C)** with context recall isolated, **(D)** during cue recall. Data for Figure 7A is presented as mean ± SEM. Data for Figures 7B-D are presented as percent freezing per individual animal with a mean per experimental group. In Figure 7A, dashed lines indicate tone onset.

4. DISCUSSION

This study examined genotypic and parent-of-origin effects on both contextual and cued conditioned threat learning behaviors in Nrg1 mutant mice with a missense mutation in the transmembrane domain. During training and cue recall there were no significant differences between wild type, heterozygous, and mutant mice. However, male mutant exhibited reduced contextual associative learning as compared to wild type and heterozygous male mice. Additionally, MF female mice during cue recall exhibited reduced cue associative learning as compared to MM female mice.

This study indicates that Nrg1 influences contextual learning. Although these findings must be considered preliminary because of the small sample sizes, they support previous findings of Shang et al. that contextual memory is influenced by Type III Nrg1 in female mice heterozygous for a mutation that eliminates expression of Type III Nrg1 (Shang et al., 2017). In this study, a clear phenotype was observed with the male heterozygous when compared to mutant animals. However, no clear phenotype was observed with the female heterozygous animals. It is possible that the sample sizes for the female cohort in this study masked the heterozygous phenotypes.

A clear phenotype with the male heterozygous animals was observed when compared based on the parent-of-origin of the leucine allele. However, this phenotype was only exhibited when comparing cue associative learning. This conflicts with the parent-of-origin effect reported by Shang et al. for the Type III Nrg1 heterozygotes. Shang et al. reported a difference within the contextual associative learning of male heterozygous mice when compared based on parent-of-origin. This difference might be due to the different mutations studied. While there are differences in the type of associative learning affected by parent-of-origin, both Shang et al. and this study reported differences between MF and MM mice with mutations in the Nrg1 gene. Because parent-of-origin effects were demonstrated, it could be hypothesized that Nrg1 interacts with imprinted genes. Many imprinted genes are located near, or within, genes that regulate transcription (Mozaffari et al., 2019). Because the Nrg1 ICD is suspected to regulate gene expression (Bao et al., 2003), its function might depend on the product of one of these imprinted genes.

It is possible that the sample sizes in this study masked the heterozygous phenotypes. Although this study did not demonstrate statistical significance between male MM and MF mice, a larger study with more mice might have replicated the results of Shang et al.. On the other hand, the greater severity of the total disruption of Type III Nrg1, as evidenced by the death of homozygous mutants at birth, could account for the different effect of heterozygosity (Wolpowitz et al., 2000).

This study did not analyze baseline anxiety or exploratory behaviors. Baseline behavior data was not quantified, but mice seemed to display differences in their baseline behavior. Therefore, this would be an interesting aspect to add to future studies. The elevated plus maze and open field tests could be

performed to evaluate these behaviors. Although previous studies of Nrg1 mutant mice did not demonstrate significant differences in anxiety-like behaviors between heterozygous and wild type mice (Shang et al., 2017), a mutation in the transmembrane domain may produce a different result.

In conclusion, mice with a missense mutation in the Nrg1 transmembrane domain show strong trends towards impairments in contextual associative learning. Additionally, when compared based on the parent-of-origin of the mutation, female MF mice exhibit reduced cue associative. Therefore, nuclear back signaling by Type III Nrg1 contributes to associative threat learning, but is not the only Type III Nrg1 function that is required. This study contributes to the wealth of investigations into the functions and mechanisms of Nrg1. Additionally, these findings have implications for GWAS. Because genome databases do not contain parent-of-origin information, a parent-of-origin effect for Nrg1 would impact any Nrg1 GWAS. It is evident that further investigation is needed to determine the interactions and signaling cascades related to the Type III Nrg1 intracellular domain.

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BIBLIOGRAPHY

Actimetrics Software. (2018). *FreezeFrame: User manual*. Retrieved from https://www.harvardapparatus.com/media/manuals/Product%20Manuals/ACT-100A%20FreezeFrame-FreezeView%20Manual.pdf

American Psychiatric Association. (2013). Diagnostic and statistical manual of mental disorders (DSM-5®). *American Psychiatric Pub*.

Bao, J., Wolpowitz, D., Role, L. W., & Talmage, D. A. (2003). Back signaling by the Nrg-1 intracellular domain. *The Journal of Cell Biology, 161*(6), 1133–1141. doi: 10.1083/jcb.200212085

Birchmeier, C., & Nave, K.-A. (2008). Neuregulin-1, a key axonal signal that drives Schwann cell growth and differentiation. *Glia, 56*(14), 1491–1497. doi: 10.1002/glia.20753

Chen, Y., Hancock, M. L., Role, L. W., & Talmage, D. A. (2010). Intramembranous Valine Linked to Schizophrenia Is Required for Neuregulin 1 Regulation of the Morphological Development of Cortical Neurons. *Journal of Neuroscience, 30*(27), 9199–9208. doi: 10.1523/jneurosci.0605-10.2010

Chen, Y. J., Johnson, M. A., Lieberman, M. D., Goodchild, R. E., Schobel, S., Lewandowski, N., … Role, L. W. (2008). Type III neuregulin-1 is required for normal sensorimotor gating, memory-related behaviors, and corticostriatal circuit components. *The Journal of neuroscience : the official journal of the Society for Neuroscience, 28*(27), 6872–6883. doi:10.1523/JNEUROSCI.1815-08.2008

Clark, R. E., & Squire, L. R. (1998). Classical Conditioning and Brain Systems: The Role of Awareness. *Science, 280*(5360), 77–81. doi: 10.1126/science.280.5360.77

Curzon, P., Rustay, N. R., & Browman, K. E. (2009). Cued and contextual fear conditioning for rodents. In *Methods of Behavior Analysis in Neuroscience. 2nd edition*. CRC Press/Taylor & Francis.

Ehrlichman, R. S., Luminais, S. N., White, S. L., Rudnick, N. D., Ma, N., Dow, H. C., … Siegel, S. J. (2009). Neuregulin 1 transgenic mice display reduced mismatch negativity, contextual fear conditioning and social interactions. *Brain research, 1294*, 116–127. doi:10.1016/j.brainres.2009.07.065

Fleck, D., Bebber, F. V., Colombo, A., Galante, C., Schwenk, B. M., Rabe, L., … Haass, C. (2013). Dual Cleavage of Neuregulin 1 Type III by BACE1 and ADAM17 Liberates Its EGF-Like Domain and Allows Paracrine Signaling. *Journal of Neuroscience, 33*(18), 7856–7869. doi: 10.1523/jneurosci.3372-12.2013

Francks, C., DeLisi, L. E., Shaw, S. H., Fisher, S. E., Richardson, A. J., Stein, J. F., & Monaco, A. P. (2003). Parent-of-origin effects on handedness and schizophrenia susceptibility on chromosome 2p12–q11. *Human Molecular Genetics, 12*(24), 3225-3230.

Gassmann, M., Casagranda, F., Orioli, D., Simon, H., Lai, C., Klein, R., & Lemke, G. (1995). Aberrant neural and cardiac development in mice lacking the ErbB4 neuregulin receptor. *Nature, 378*(6555), 390–394. doi: 10.1038/378390a0

Goosens, K. A., & Maren, S. (2001). Contextual and Auditory Fear Conditioning are Mediated by the Lateral, Basal, and Central Amygdaloid Nuclei in Rats. *Learning & Memory, 8*(3), 148–155. doi: 10.1101/lm.37601

Hancock, M. L., Nowakowski, D. W., Role, L. W., Talmage, D. A., & Flanagan, J. G. (2011). Type III neuregulin 1 regulates pathfinding of sensory axons in the developing spinal cord and periphery. *Development, 138*(22), 4887–4898. doi: 10.1242/dev.072306

Harrison, P. J., & Law, A. J. (2006). Neuregulin 1 and Schizophrenia: Genetics, Gene Expression, and Neurobiology. *Biological Psychiatry, 60*(2), 132–140. doi: 10.1016/j.biopsych.2005.11.002

Karl, T., Duffy, L., Scimone, A., Harvey, R. P., & Schofield, P. R. (2007). Altered motor activity, exploration and anxiety in heterozygous neuregulin 1 mutant mice: implications for understanding schizophrenia. *Genes, Brain and Behavior, 6*(7), 677-687.

Kim, J. J., & Jung, M. W. (2006). Neural circuits and mechanisms involved in Pavlovian fear conditioning: A critical review. *Neuroscience & Biobehavioral Reviews, 30*(2), 188–202. doi: 10.1016/j.neubiorev.2005.06.005

Ludwig, K. U., Mattheisen, M., Mühleisen, T. W., Roeske, D., Schmäl, C., Breuer, R., ... & Rietschel, M. (2009). Supporting evidence for LRRTM1 imprinting effects in schizophrenia. *Molecular psychiatry, 14*(8), 743.

Mozaffari, S. V., DeCara, J. M., Shah, S. J., Sidore, C., Fiorillo, E., Cucca, F., ... & Ober, C. (2019). Parent-of-origin effects on quantitative phenotypes in a large Hutterite pedigree. *Communications biology, 2*(1), 28.

Murray, R. M., & Lewis, S. W. (1987). Is schizophrenia a neurodevelopmental disorder?. *British medical journal (Clinical research ed.), 295*(6600), 681.

Shang, K., Talmage, D. A., & Karl, T. (2017). Parent-of-origin effects on schizophrenia-relevant behaviours of Type III neuregulin 1 mutant mice. *Behavioural Brain Research, 332*, 250–258. doi: 10.1016/j.bbr.2017.05.057

Stefansson H, Sigurdsson E, Steinthorsdottir V, et al. Neuregulin 1 and susceptibility to schizophrenia. *Am J Hum Genet. 2002*;71(4):877–892. doi:10.1086/342734

Sullivan, P. F., Kendler, K. S., & Neale, M. C. (2003). Schizophrenia as a Complex Trait Evidence From a Meta-analysis of Twin Studies. *Archives of General Psychiatry, 60*(12), 1181–1284. doi: 10.1001/archpsyc.60.12.1187

Qiagen Inc. (2006). *DNeasy: Blood & Tissue Handbook*. Retrieved from https://www.qiagen.com/us/resources/download.aspx?id=6b09dfb8-6319-464d-996c-79e8c7045a50&lang=en

Tsuang, M. (2000). Schizophrenia: genes and environment. *Biological psychiatry, 47*(3), 210-220.

Velakoulis, D., Wood, S. J., Wong, M. T., McGorry, P. D., Yung, A., Phillips, L., ... & Pantelis, C. (2006). Hippocampal and amygdala volumes according to psychosis stage and diagnosis: A magnetic resonance imaging study of chronic schizophrenia, first-episode psychosis, and ultra–high-risk individuals. *Archives of general psychiatry, 63*(2), 139-149.

Walsh, T., McClellan, J. M., McCarthy, S. E., Addington, A. M., Pierce, S. B., Cooper, G. M., ... & Stray, S. M. (2008). Rare structural variants disrupt multiple genes in neurodevelopmental pathways in schizophrenia. *Science, 320*(5875), 539-543.

Walss-Bass, C., Liu, W., Lew, D. F., Villegas, R., Montero, P., Dassori, A., ... & Raventos, H. (2006). A novel missense mutation in the transmembrane domain of neuregulin 1 is associated with schizophrenia. *Biological psychiatry, 60*(6), 548-553.

Wolpowitz, D., Mason, T. B., Dietrich, P., Mendelsohn, M., Talmage, D. A., & Role, L. W. (2000). Cysteine-rich domain isoforms of the neuregulin-1 gene are required for maintenance of peripheral synapses. *Neuron, 25*(1), 79-91.