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Interval Timing Is Altered in Male Nrxn1$^{+/-}$ Mice: A Model of Autism Spectrum Disorder

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ABSTRACT

Autism spectrum disorder (ASD) is characterized by impaired social interactions and communication, and increased repetitive and stereotypical behavior. Neuroimaging shows functional abnormalities in brain areas involved in temporal processing in autistic individuals, and they also show deficits in interval timing. Neurexin (NRXN) mutations have been identified in a wide variety of neuropsychiatric disorders, including ASD, and Nrxn1$^{+/-}$ mice possess a mutation that disrupts the α, β, and γ isoforms of Nrxn1, a gene involved in synapse structure. We investigated the interval timing abilities of the Nrxn1$^{+/-}$ mouse model of ASD in the peak interval procedure using a 15-s target interval and compared their performance with that of Nrxn1$^{+/+}$ and Nrxn1$^{\Delta S5/-}$ rescue mice. Two-month-old male Nrxn1$^{+/+}$ (C57BL/6J), Nrxn1$^{+/-}$, and Nrxn1$^{\Delta S5/-}$ mice were trained to obtain sucrose liquid rewards 15 s after the onset of a discriminative stimulus (discrete fixed-interval training), and their timing responses were tested in non-reinforced probe trials. Our analysis of responses across individual trials revealed that Nrxn1$^{+/-}$ mice had earlier timing responses overall. This difference was manifested as earlier termination of responding in terms of the response curves. These findings are consistent with leftward shifts observed with experimental animal models of ASD. In conclusion, we believe these results indicate a bias in long-term memory in the Nrxn1$^{+/-}$ mouse model of ASD and may capture the timing deficit observed in autistic individuals.

1 | Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental condition that is associated with a variety of deficits in social functions, cognition, sensory processing, and behavioral flexibility (American Psychiatric Association 2022). In light of anecdotal evidence (Allman and DeLeon 2009), it is possible that disrupted interval timing, namely the ability to keep track of time intervals in the seconds to minutes range, is at least partially responsible for ASD symptomatology, particularly in domains where temporal information is imperative (e.g., social cognition, communication, and predictive coding). Consequently, interval timing has become a topic of interest in the clinical domain (for review, see Allman and Meck 2012; Casassus et al. 2019).

Lu et al. (2023) had shown that exclusion of splice site 5 of Nrxn1 boosts hippocampal excitatory synapse density and transmission and results in behavioral changes opposite to those expected in ASD models, including increased pup ultrasonic vocalizations and decreased grooming, leading to the question of whether splice site 5 exclusion can be used as a genetic rescue of ASD. Lu et al. (2025) tested mice hemizygous for a knockout of Nrxn1α, β, and γ and found deficits in excitatory synaptic transmission in the hippocampus, lower protein levels of Nrxn1, and

Lay Summary

Neurexins help nerve cells connect and communicate with each other, and changes in these genes are often seen in people with autism. Mice with a mutation in the neurexin1 gene, called Nrxn1$^{+/-}$ mice, exhibit autism-like behaviors. In a time-judging test, these mice respond early, similar to some people with autism. This study helps develop our understanding of how interval timing is affected in ASD.

typical ASD-like behavioral deficits, including increased rearing and grooming and decreased nest quality, but no deficits in social behavior. They also tested mice with this Nrxn1 knockout and exclusion of splice site 5 from the remaining Nrxn1 gene as a therapeutic approach, which alleviated deficits in excitatory synaptic transmission and behavioral deficits and partially alleviated reduced Nrxn1 protein levels (Lu et al. 2025). The present study tested interval timing in this recently developed neurexin1 model of ASD and the corresponding genetic rescue mice.

Allman et al. (2011) tested autistic children in a temporal bisection task that required participants to compare test durations as short or long as their proximity to reference durations indicated. Children were tested with two different sets of reference durations (1–4 and 2–8 s). The psychometric functions of the autistic children were shifted leftward compared to those of the typically developing (TD) children for both reference duration sets. In addition, the psychometric functions were flatter in ASD children for the 2–8 s reference durations (see Karaminis et al. (2016) for reduced timing sensitivity in ASD). In another set of tasks in which participants were asked to reproduce target intervals, Szelag et al. (2004) found that the time reproductions of ASD children differed more from the target durations than those of TD children (see Brenner et al. (2015) and Maister and Plaisted-Grant (2011) for disrupted temporal accuracy). Despite findings indicating poor timing ability in autistic individuals, several studies failed to detect any difference. For instance, Gil et al. (2012) did not find any difference between ASD and TD children tested in the temporal bisection task across a wide range of reference interval pairs (also see Jones et al. (2017), for a lack of differences in temporal bisection in an adult sample). In addition, neither Wallace and Happé (2008) nor Doenyas et al. (2019) found any disruption in the timing performance of ASD children using a wider range of tasks (e.g., temporal reproduction and rhythmic timing). Overall, results from human participants are inconsistent regarding whether interval timing is affected in ASD (for a systematic review of studies on time perception in individuals with ASD, see Casassus et al. 2019).

There are multiple ways through which altered interval timing may contribute to specific changes in the behavior and function of autistic individuals. To this end, greater timing uncertainty would reduce the brain's predictive power, thereby increasing sensory ambiguity. Not being able to predict the timing of upcoming events may lead to sensory overload (overstimulation), as such events are perceived as "out of the blue" without a veridical temporal fabric. In fact, the routine-building and repetitive-action tendencies of autistic individuals may be a way to cope with higher uncertainty or violations of expectations arising from altered time perception. Higher timing uncertainty may even broaden the temporal binding window (the time window during which different sensory inputs are encoded as belonging to a single event) when it manifests at the perceptual level. This would, in turn, serve as an independent source of sensory overloading, often observed in autism (see Casassus et al. 2019). Higher timing uncertainty can also manifest as more variable movement timing, which could lead to motor impairments and dyspraxia, as typically observed in autism (da Silva et al. 2025; Miller et al. 2014). Finally, higher temporal uncertainty and/or biased estimates of elapsed time can also lead to social communication disruptions, including altered turn-taking during verbal communication (due to misperceived pauses), gesturing, and synchronizing with other members of a group, as well as delays in language acquisition (Allman et al. 2011; Falter et al. 2012). Briefly, a disrupted internal clock can affect functioning across many domains, from motor planning/execution to sensory gating to communication.

Based on an in-depth review of timing work in autistic individuals, Casassus et al. (2019) attributed differences in temporal sensitivity tasks to the use of different methodologies (e.g., temporal threshold, temporal synchrony, temporal binding window, and temporal order judgment), temporal sensitivity calculations, sample demographics (e.g., children vs. adolescents vs. adults), sensory modality (e.g., empty vs. filled interval, auditory vs. visual vs. audiovisual vs. tactile, temporal vs. gap detection threshold), measures, motor involvement versus not, task complexity in terms of cognitive demands (temporal sensitivity, interval timing, and high-level temporal processing), controls for chronometric counting and inconsistent taxonomy of timing abilities. For an in-depth review of all these potential factors underlying inconsistent results, we refer the readers to Casassus et al. (2019).

Few studies have investigated how interval timing is affected in animal models of ASD based on prenatal exposure to valproic acid (see Patterson 2011). In one study, male and female CF1 mice were prenatally exposed to valproic acid (VPA) and later tested using the dual peak procedure with 15-s and 45-s target intervals (Acosta et al. 2018). In the peak interval (PI) procedure, mice were trained to anticipate reward delivery contingent upon their first response after a fixed delay following the onset of a discriminative stimulus. In PI trials, the discriminative stimulus is presented for a longer duration; the reward is omitted, and the modulation of anticipatory responding as a function of stimulus duration is examined. The response rate is typically maximal at the time of reward availability. In the dual-PI procedure used by Acosta et al. (2018), two levers were associated with different delays to reinforcement: 15 and 45 s. They found that both male and female mice that were prenatally exposed to VPA (and weaned with other VPA mice) had leftward shifts in their timed responses; female VPA mice had a leftward shift for the 15-s interval only, while male VPA mice had a leftward shift in both 15-s and 45-s intervals. In addition, both male and female VPA mice had lower temporal precision and lower peak amplitude than control mice. DeCoteau and Fox (2021) tested male Wistar rats prenatally exposed to VPA on fixed-interval (FI) temporal bisection, PI, and intertemporal choice timing tasks. They found a leftward shift in the middle times of the VPA rats on the PI

task, and a trend towards a leftward shift in the FI temporal bisection task. In summary, these studies demonstrate a clear timing deficit in animal VPA models of ASD.

An alternative approach that offers higher construct validity than VPA exposure is the use of genetically modified animals that replicate the genetic mutations associated with the human condition. To this end, mutations to neurexin genes that control the production of presynaptic cell adhesion molecules may provide a better model of ASD. The neurexins play a critical role in synaptic development (Krueger et al. 2012) and are associated with a number of neuropsychiatric conditions, including ASD and schizophrenia (Gauthier et al. 2011; Gomez et al. 2021; Hu et al. 2019; Kasem et al. 2018; Reissner et al. 2013). Neurexin 1 is a molecule that acts to recruit or stabilize neurotransmitter receptors (Craig and Kang 2007). NRXN1 point mutations have been identified in ASD patients (Onay et al. 2016), and NRXN1 exon deletions are estimated to increase the risk of ASD by approximately 20-fold (Dabell et al. 2013). Several previous studies have examined the effect of Nrxn1α knockout in mice, and some have reported ASD-like phenotypes. For instance, Etherton et al. (2009) found impaired prepulse inhibition and altered nest-building behavior, as well as increased self-grooming, in Nrxn1α knockout mice. However, they did not observe differences in other tasks, including social behavior and anxiety. Grayton et al. (2013) found altered social behavior in male and female homozygous (but not hemizygous) Nrxn1α knockout mice, which displayed altered social approach and reduced social investigation, and males also showed increased aggressive behavior. Dachtler et al. (2015) also found decreased social behavior in heterozygous Nrxn1α knockout mice, which showed no preference for a novel mouse in a social approach task. Armstrong et al. (2020) examined Nrxn1α knockout mice across different ages and found that homozygous Nrxn1α knockout mouse pups produced shorter ultrasonic vocalizations than their hemizygous and wild-type (WT) littermates. Abnormal social behavior, including decreased investigative and affiliative behaviors and increased aggression, was observed in male homozygous and hemizygous Nrxn1α knockout mice, both as juveniles and as adults.

The neurexin1 mutation is expected to disrupt the "handshake" between neurexins and neuroligins, which is important for synaptic communication within neural networks (e.g., affecting cortico-striatal circuitry and long-term potentiation in the hippocampus), potentially leading to mistimed synaptic function. For instance, Davatolhagh and Fuccillo (2021) showed that Neurexin1α differentially regulates synaptic efficacy within striatal circuits, particularly decreasing synaptic strength in the prefrontal cortex-dorsomedial striatum circuits in the form of reduced synaptic strength in the spiny projection neurons in Nrxn1α$^{+/-}$ and Nrxn1α$^{-/-}$ mice. Importantly, these circuits have been implicated in interval timing and its behavioral manifestations (Balcı and Öztel 2025). Within the framework of the Striatal Beat Frequency model (Matell and Meck 2004), in which the striatum serves as a perceptron for the time-dependent patterning of phase-reset cortical oscillatory signals, the above-mentioned synaptic dysfunction can lead to biases in the coding used to recognize the cortical signaling. Furthermore, a Neurexin1 mutation is known to result in imbalanced excitation and inhibition, thereby altering the fidelity of memory traces formed during learning (Choi et al. 2011; Zhang et al. 2021). Assuming that temporal memories are encoded through synaptic modulation, such deficits would be expected to lead to altered memory for time intervals (akin to a bias in long-term memory for time intervals). Finally and interestingly, a significant elevation of oscillatory power in the gamma band (as a faster brain rhythm) have been observed across brain regions of Nrxn1α$^{-/-}$ rats (Janz et al. 2022), which may alter temporal binding and the underlying oscillatory basis functions for timing intervals (within the framework of SBF) possibly leading to a bias in temporal processing. This said, note that higher gamma-band activity would also predict more efficient temporal binding, which runs counter to the broader temporal binding windows attributed to ASD. These possibilities form an empirical framework for future work. These studies used knockouts that affected only Nrxn1α, leaving the β and γ isoforms of Nrxn1 unaffected. In contrast, the knockout used in the present study affects all three isoforms of Nrxn1.

To our knowledge, no study has investigated how interval timing, a potential biomarker of ASD, is affected in a genetic mouse model of ASD, and none has examined it in a rescue model. The current study bridges this gap by investigating the timing behavior of a recently developed mouse model of ASD, which is hemizygous for the α, β, and γ isoforms of neurexin 1, as well as genetic rescue, Nrxn1$^{\Delta S5/-}$, and WT mice on the PI timing task. We hypothesized that the Nrxn1$^{+/-}$ mice would have abnormal timing performance compared to the Nrxn1$^{+/+}$ mice, and that the Nrxn1$^{\Delta S5/-}$ mice would be similar to the Nrxn1$^{+/+}$ mice.

2 | Methods

2.1 | Subjects

Forty-eight male mice, 16 C57BL/6J (Nrxn1$^{+/+}$), 16 Nrxn1$^{+/-}$ mice, and 16 Nrxn1$^{\Delta S5/-}$ mice were tested at 2–3 months of age. The Nrxn1$^{+/-}$ mice are hemizygous for a 140-bp deletion that knocks out the α, β, and γ isoforms of Nrxn1, while the Nrxn1$^{\Delta S5/-}$ mice, a proposed genetic rescue of the Nrxn1$^{+/-}$ mice (Lu et al. 2025), have the same 140-bp deletion to one copy of Nrxn1, and the remaining copy has an exclusion of splice site 5, shown to upregulate Nrxn1 protein levels and alleviate synaptic transmission and behavioral deficits caused by the 140-bp deletion (Lu et al. 2023, 2025). Nrxn1$^{+/+}$ and Nrxn1$^{+/-}$ mice were bred by mating WT Nrxn1$^{+/+}$ females and transgenic Nrxn1$^{+/-}$ males, while Nrxn1$^{\Delta S5/-}$ mice were bred by mating Nrxn1$^{\Delta S5/-}$ and Nrxn1$^{\Delta S5/\Delta S5}$ mice. All mice were bred in-house at Dalhousie University from mice obtained from Dr. Ann Marie Craig at the University of British Columbia. Genotypes were determined using PCR with DNA from ear punches by Dr. Chris Sinal at Dalhousie University. The mice were weaned at 30 days of age and separated into same-sex groups of two to four siblings, housed in 30 cm × 18 cm × 12 cm polycarbonate cages with wire tops, and had ad lib access to food (Purina Rodent Laboratory Chow #5001) and water. The cages had wood chip bedding, a 5-cm-diameter by 7-cm-long PVC tube for enrichment, and paper strips for nesting material. The colony room was maintained at 20°C ± 2°C on a reversed 12-h light/12-h dark cycle, with lights off from 9:30 a.m. to 9:30 p.m. All testing

was performed during the dark phase of the cycle. All procedures were approved by the Dalhousie University Committee on Laboratory Animals and were conducted in accordance with the guidelines of the Canadian Council on Animal Care (protocol #18-096).

2.2 | Apparatus

The apparatus and procedures were the same as those described by Gür, Fertan, Kosel, et al. (2019). Testing was conducted in a mouse nine-hole box (Cambridge Cognition Ltd., England) with a lick tube attached to a peristaltic pump, house lights, and speakers (mounted on both sides of the inner walls). The box was placed in a sound- and light-attenuating chamber with a video camera mounted above, allowing the behavior of the mice to be observed without disrupting the test procedure. The test chamber contained a grid floor with a removable tray underneath. Six of nine holes (Holes 1–3 and 7–9) were plugged, and the remaining three holes in the middle (Holes 4–6) were left open. Each of the three holes and the reinforcement tube/tray could be illuminated, and nose pokes to the open holes were detected via infrared beams. Computer software (Cambridge Cognition Ltd., England) was used to control the test box and record time-stamped nose pokes.

2.3 | Procedure

2.3.1 | Water Restriction

Two days before the first training session started, the mice were separated into individual cages, and their water intake was restricted while maintaining each animal at 85% of its ad libitum weight. On the first day of water deprivation, the water bottles were removed from the cages, and each mouse was given powdered rodent chow that was mixed with tap water (mash). The weight of the mice was maintained at the desired level by providing mash (on average, 2.5 mL) after each test. Subjects also received a 5% sucrose solution as a reward during testing. During water restriction, subjects had ad lib access to food in their home cages.

2.3.2 | PI Procedure

2.3.2.1 | Magazine Training. Mice received one 20-min session of magazine training per day for two consecutive days. At the start of the sessions, 0.025 mL of 5% sucrose water was delivered via the peristaltic pump, followed by 0.02 mL every 40 s during the session (note that 0.7 mL indicated in Gür, Fertan, Alkins, et al. (2019) and Gür, Fertan, Kosel, et al. (2019) was the approximate total amount of water received during magazine training and not the amount delivered every 40 s). The reward tray was illuminated throughout the sessions.

2.3.2.2 | Fixed Interval Training. Mice received one 20-min session of FI training per day for four consecutive days. In each trial, reinforcement (0.03 mL of 5% sucrose water) was delivered contingent upon the first nose poke into the central nose poke hole after 15 s since the onset of the discriminative stimulus (i.e., the onset of the light in the central nose poke hole). Trials with no response after the fixed interval were terminated after 45 s. Following a nose poke response, the light was extinguished, and an inter-trial interval (ITI) of 20 s fixed plus a uniformly distributed random variable with a mean of 10 s commenced.

2.3.2.3 | PI Testing. Mice received one 20-min session of PI testing per day for 30 consecutive days. PI trials were randomly mixed with FI trials with a 1:2 ratio of PI:FI trials. The PI trials began with illumination of the central nose-poke hole and lasted 45 s. At the end of the PI trial, the light in the central nose-poke hole was turned off, and an ITI began. No reinforcement was given during PI trials. Due to a one-day interruption in the testing of one batch of mice (5 $Nrxn1^{+/-}$ mice and 2 $Nrxn1^{+/+}$ mice), the data collected from these mice the day after the interruption were excluded from the dataset prior to data analysis.

3 | Data Analysis

Data analysis was conducted with MATLAB (version 25.1 (R2025a); The MathWorks Inc. 2025) and R (version 4.5.2; R Core Team 2025).

3.1 | Analysis of the Individual Trial Data

In individual PI trials, subjects exhibit a response pattern that differs from the smooth bell-shaped average response curves (Church et al. 1994). In each trial, responding in the steady state occurs as a pattern composed of three stages. At the beginning of the trial, the response rate is low, and as the time of reinforcement availability approaches, it increases abruptly (start time). Since reinforcement is omitted in the PI trials, sometime after the time that the reinforcement should be available, the response rate abruptly declines (stop time). The period of high response rates is typically clustered around the time of reinforcement availability, indicating the period when there is a high expectancy of reinforcement delivery on that trial. The interval between the stop and start times is called the spread and reflects timing uncertainty. The average of the start and stop times reflects the targeted interval in that trial.

The primary aim of the individual-trial analysis was to estimate the trial time at which animals shift from a break to a run (start time) and the run back to a break (stop time). In other words, the time when the subject starts anticipating and stops anticipating the reward delivery (following its omission), respectively. The period before the run, the first break period prior to the subject initiating its high rate of anticipatory responding, should have lower-than-average response rates. The run should have higher-than-average response rates, and the second break period, following the subject's termination of its high rate of anticipatory responding, should again show lower-than-average response rates. To detect the start and stop times, we used a variant of the algorithm presented in Church et al. (1994), which assumed a second start time. Additionally, the coefficients of variation for the start, stop, and spread times were compared. Comparisons between genotypes for these measures were made using the

peak-interval trials of the last five sessions, performed with one-way ANOVA.

As recommended by Karson and Balcı (2021), based on Gibbon and Church's (1990) theoretical work, the correlation coefficients between the start and stop times, the start times and the spreads, and the middle times ([start time + stop time]/2 as the trial-based estimate of the target time) and spreads were calculated for each mouse using the data from the last five sessions. Comparisons between genotypes for each of these three correlation coefficients were performed by first applying the Fisher z transformation to the correlation coefficients, then analyzing them using one-way ANOVAs.

3.2 | Analysis of the Peak Response Curve

The average response curves in the PI procedure are nearly bell-shaped, with the peak located around the 15 s delay of reinforcement availability. The latency of the peak (i.e., peak time) reflects the time of maximum expectancy for the reinforcement delivery (timing accuracy), the width of the response curve (i.e., spread) measures timing variability, and the amplitude of the response gradient reflects the motivation level of the subjects, independent of the timing performance (Balcı 2014; Roberts 1981). For parameter estimations from the average response curve or gradient, we use a method previously applied by Balcı, Gallistel, et al. (2009). The peak response curve was determined using the trials from the final five sessions. Initially, the average response rate data of each mouse was expressed in 1-s bins. The overall response rate for each mouse was calculated as the mean of the binned average response rates that formed the average response curve. The amplitude was also determined from the non-normalized average response curve as the height of the curve at its peak. For the estimation of the peak time and response curve width, on the other hand, each response curve was normalized by its amplitude for each subject, then smoothed by a window of three bins, which replaces the average value in a 1-s bin with the average of the three bins surrounding it (except for the endpoints of the data). A moving-average smoothing method is applied to reduce noise in the individual response curves while preserving their shape. The global maximum of the normalized response curve is the peak time. The first point that exceeded the normalized response rate of 0.70 is determined as the start point. If there is a decrease below the normalized rate of 0.50 between this point and the peak time, the start point is determined after this point. The first point at which the normalized response rate falls below 0.70 after reaching its peak is determined as the stop point. The width of the response curve was defined as the difference between the average start and stop points derived from the response curve. Each parameter was compared between genotypes using one-way ANOVA.

3.3 | Analysis of Response Rates During ITI and the Discriminative Stimulus

The mean number of responses across all sessions was compared. To evaluate whether the mice learned to associate the discriminative stimulus (light) with reinforcement, the ratio of nose pokes during light presentations to the number of nose pokes during ITIs was calculated. A higher ratio is indicative of better associative learning (e.g., Papachristos and Gallistel 2006); however, this measure is confounded by timing imprecision (an aspect of poor timing performance). Finally, locomotor activity was quantified as the number of infrared beam breaks recorded in front of the choice wall. One-way ANOVA was used to examine genotype differences in these metrics.

4 | Results

4.1 | Well-Timed Trials

During the final block of testing, there was no significant genotype difference in terms of the number of poorly timed trials (i.e., start times > 15 s or stop times < 15 s; $F_{(2,45)} = 1.34$, $p = 0.273$, $\eta^2_G = 0.056$). Thus, all trials were included in the subsequent analyses.

4.2 | Single Trial Analysis

The single-trial parameters were analyzed using one-way ANOVA, followed by planned comparisons between Nrxn1$^{+/+}$ and Nrxn1$^{+/-}$, and between Nrxn1$^{+/-}$ and Nrxn1$^{\Delta S5/-}$ mice.

4.2.1 | Start, Middle, and Start Times

While there was no main effect of genotype on start times ($F_{(2,45)} = 2.68$, $p = 0.079$, $\eta^2_G = 0.107$; Figure 1A), the planned comparisons showed that the Nrxn1$^{+/-}$ mice (8.25 ± 2.03) had an earlier start time than the Nrxn1$^{\Delta S5/-}$ mice (10.2 ± 2.85; $t_{(45)} = -2.22$, $p = 0.0315$, $d = -0.782$). There was an effect of genotype on middle times ([start time + stop time]/2 as the trial-based estimate of the target time; $F_{(2,45)} = 3.86$, $p = 0.028$, $\eta^2_G = 0.147$), with the Nrxn1$^{+/-}$ mice (17.1 ± 1.97) having earlier middle times than the Nrxn1$^{\Delta S5/-}$ mice (19.4 ± 2.63; $t_{(45)} = -2.76$, $p = 0.008$, $d = 0.989$; Figure 1B). There was a main effect of genotype on stop times ($F_{(2,45)} = 4.26$, $p = 0.02$, $\eta^2_G = 0.159$; Figure 1C), with the Nrxn1$^{+/-}$ mice (25.9 ± 2.24) having earlier stop times than the Nrxn1$^{\Delta S5/-}$ mice (28.6 ± 2.78; $t_{(45)} = -2.92$, $p = 0.00548$, $d = 1.05$). There was no effect of genotype on spread ($F_{(2,45)} = 1.03$, $p = 0.366$, $\eta^2_G = 0.044$; Figure 1D).

In order to interpret the timing precision at a more granular level, we analyzed the coefficient of variation (CV) of start and stop times. There was an effect of genotype on the CV of the start times ($F_{(2,45)} = 4.09$, $p = 0.023$, $\eta^2_G = 0.154$), with the Nrxn1$^{+/+}$ mice (0.802 ± 0.117) having a lower CV values than the Nrxn1$^{+/-}$ mice (0.956 ± 0.214; $t_{(45)} = -2.57$, $p = 0.0136$, $d = -0.894$), and the Nrxn1$^{+/-}$ mice (0.956 ± 0.214) having greater CV values than the Nrxn1$^{\Delta S5/-}$ mice (0.814 ± 0.164; $t_{(45)} = 2.37$, $p = 0.022$, $d = 0.747$). There was no significant effect of genotype on the CV of stop times ($F_{(2,45)} = 0.865$, $p = 0.428$, $\eta^2_G = 0.037$; Nrxn1$^{+/+}$: 0.271 ± 0.0743; Nrxn1$^{+/-}$: 0.28 ± 0.0585; Nrxn1$^{\Delta S5/-}$: 0.298 ± 0.0367).

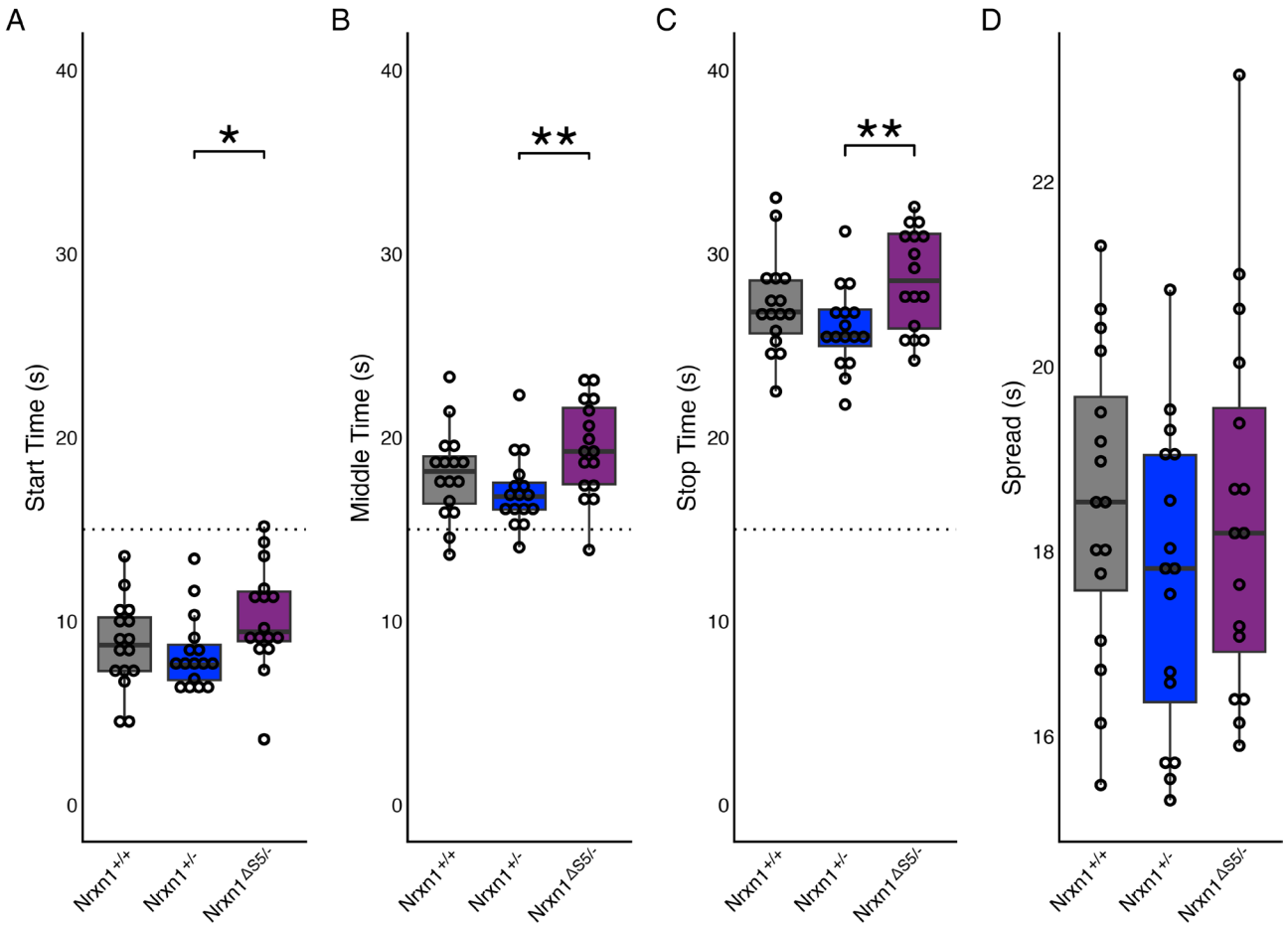

FIGURE 1 | The comparison of single-trial analysis between the Nrxn1$^{+/+}$, Nrxn1$^{+/-}$, and Nrxn1$^{\Delta S5/-}$ mice for (A) start times, (B) middle times (i.e., [start time + stop time]/2), (C) stop times, and (D) spread (i.e., stop time − start time). The visual inspection of these values shows an overall leftward shift in the timing responses of Nrxn1$^{+/-}$ mice, particularly compared to Nrxn1$^{\Delta S5/-}$ mice. These shifts were not accompanied by wider response periods that would result from higher timing uncertainty. The boxes indicate the interquartile ranges, with the medians indicated by the horizontal lines; whiskers extend to a maximum of 1.5 interquartile ranges; and the individual data points are plotted over the boxes (0.05 * 0.01 ** 0.001).

4.2.2 | Correlation Analysis

Correlational analyses are informative in terms of the sources of variability in timing behavior. Pearson's correlations between the start and stop times, the start time and the spread, and the spread and the middle time were also examined. Genotype differences in correlation coefficients were analyzed with ANOVAs. Start-stop time correlations (Nrxn1$^{+/+}$: $r_{(629)} = 0.7$, $p < 0.0001$; Nrxn1$^{+/-}$: $r_{(611)} = 0.72$, $p < 0.0001$; Nrxn1$^{\Delta S5/-}$: $r_{(576)} = 0.69$, $p < 0.0001$) and start time-spread correlations (Nrxn1$^{+/+}$: $r_{(629)} = -0.31$, $p < 0.0001$; Nrxn1$^{+/-}$: $r_{(611)} = -0.43$, $p < 0.0001$; Nrxn1$^{\Delta S5/-}$: $r_{(576)} = -0.32$, $p < 0.0001$) were significant for all genotypes, and different genotypes of mice did not differ on either start-stop time correlations ($F_{(2,45)} = 1.59$, $p = 0.216$, $\eta_G^2 = 0.066$; Figure S1A), or start-spread correlations ($F_{(2,45)} = 2.42$, $p = 0.1$, $\eta_G^2 = 0.097$; Figure S1B). While the middle-spread correlations were significant for the Nrxn1$^{+/+}$ mice ($r_{(629)} = 0.1$, $p = 0.0106$) and the Nrxn1$^{\Delta S5/-}$ mice ($r_{(576)} = 0.094$, $p = 0.0234$), they were not significant for the Nrxn1$^{+/-}$ mice ($r_{(611)} = -0.079$, $p = 0.052$), resulting in a significant difference in the middle-spread correlations between genotypes ($F_{(2,45)} = 3.26$, $p = 0.048$, $= 0.126$), with the Nrxn1$^{+/-}$ (-0.0996 ± 0.259) mice showing lower correlations than both the Nrxn1$^{+/+}$ mice (0.0909 ± 0.224; $t_{(45)} = 2.2$, $p = 0.0333$, $d = -0.777$) and the Nrxn1$^{\Delta S5/-}$ mice (0.0885 ± 0.242; $t_{(45)} = -2.22$, $p = 0.0312$, $d = -0.755$; Figure S1C).

4.3 | Response Curve Analysis

The parameters calculated from the average response curves (Figure 2A) were compared between genotypes using ANOVAs. While there were no effects of genotype on maximum amplitudes (Figure 2B), start times (Figure 2C), nor peak times (Figure 2D), there was an effect of genotype on stop times ($F_{(2,45)} = 3.26$, $p = 0.048$, $\eta_G^2 = 0.127$; Figure 2E), with planned comparisons showing the Nrxn1$^{+/-}$ mice (24.3 ± 3.66) had earlier peaks than the Nrxn1$^{\Delta S5/-}$ mice (30.2 ± 8.13; $t_{(45)} = -2.47$, $p = 0.0172$, $d = -0.932$). There was also an effect of genotype on the amplitude at the tail of the response curve (i.e., 30 s; $F_{(2,45)} = 3.37$, $p = 0.043$, $\eta_G^2 = 0.13$; Figure 2G), with planned comparisons showing the Nrxn1$^{+/+}$ mice (0.599 ± 0.249) had a greater amplitude than the Nrxn1$^{+/-}$ mice (0.407 ± 0.259; $t_{(45)} = 2.18$, $p = 0.0342$, $d = 0.757$), as well as the Nrxn1$^{\Delta S5/-}$ mice

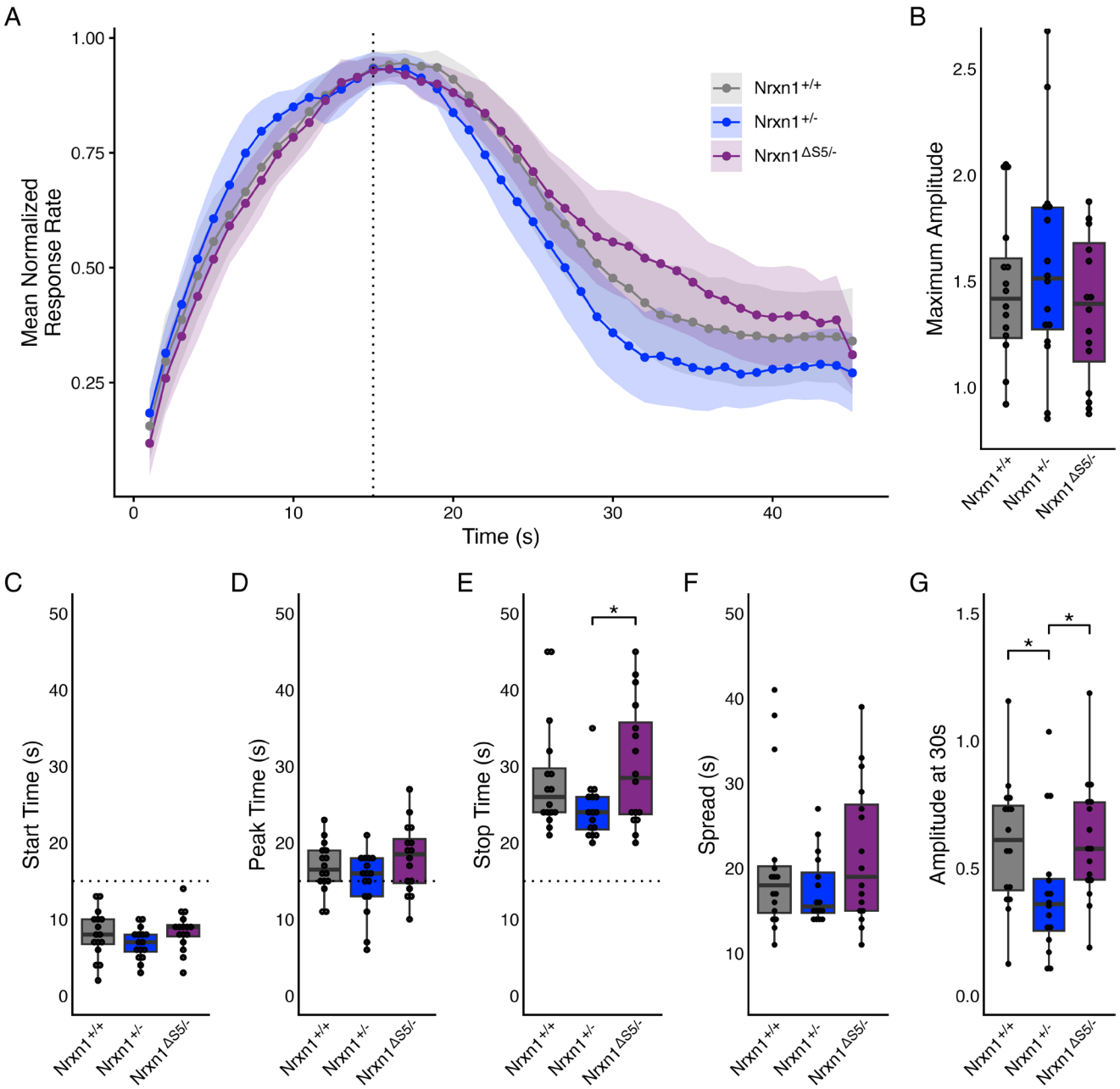

FIGURE 2 | (A) Average peak response curves of the Nrxn1$^{+/+}$, Nrxn1$^{+/-}$, and Nrxn1$^{\Delta S5/-}$ mice. The timing indices estimated from the average peak response curves are shown in the other panels for the same groups: (B) maximum amplitude of the non-normalized response rate of the average response curve, (C) start times, (D) peak times, (E) stop times, (F) spread (i.e., stop times − start times), and (G) response rate around the right-hand tail of the average response curve (i.e., 30s). For subplots (B) through (G), the boxes indicate the interquartile ranges, with the medians indicated by the horizontal lines; whiskers extend to a maximum of 1.5 interquartile ranges; and the individual data points are plotted over the boxes (0.05 * 0.01).

(0.61 ± 0.239) having a greater amplitude than the Nrxn1$^{+/-}$ mice ($t_{(45)} = -2.31$, $p = 0.0258$, $d = 0.815$). No other average response curve measures showed a significant difference between genotypes ($ps \geq 0.202$).

4.4 | Response Rates and Activity Throughout the Session

Response rates and activity around the choice wall (measured by the total number of a single infrared beam placed before the choice wall) were analyzed with ANOVAs. During the final block of testing, there were no genotype differences in the number of total nose pokes per session (Nrxn1$^{+/+}$: 6381 ± 2106, Nrxn1$^{+/-}$: 7111 ± 2225, and Nrxn1$^{\Delta S5/-}$: 6426 ± 2699; $F_{(2,45)} = 0.481$, $p = 0.621$, $\eta^2_G = 0.021$). This was true when examining both nose pokes made while the discriminative stimulus was on (Nrxn1$^{+/+}$: 4241 ± 1407, Nrxn1$^{+/-}$: 4726 ± 1488, and Nrxn1$^{\Delta S5/-}$: 4245 ± 1759; $F_{(2,45)} = 0.512$, $p = 0.603$, $\eta^2_G = 0.022$), and during the ITIs (Nrxn1$^{+/+}$: 2140 ± 700, Nrxn1$^{+/-}$: 2385 ± 738, and Nrxn1$^{\Delta S5/-}$: 2181 ± 941; $F_{(2,45)} = 0.430$, $p = 0.653$, $\eta^2_G = 0.019$). The ratio of the number of nose pokes made during the discriminative stimulus to the number of nose pokes during the ITIs did

not differ ($Nrxn1^{+/+}$: 1.98 ± 0.049, $Nrxn1^{+/-}$: 1.98 ± 0.043, and $Nrxn1^{\Delta S5/-}$: 1.95 ± 0.033; $F_{(2,45)} = 1.76$, $p = 0.183$, $\eta^2_G = 0.073$). The total amount of activity around the choice wall did not differ ($Nrxn1^{+/+}$: 4434 ± 1377, $Nrxn1^{+/-}$: 4220 ± 1312, and $Nrxn1^{\Delta S5/-}$: 3822 ± 530; $F_{(2,45)} = 1.19$, $p = 0.315$, $\eta^2_G = 0.05$), indicating similar levels of movement by each genotype in the testing apparatus.

5 | Discussion

The current study investigated how timing behavior is altered in male $Nrxn1^{+/-}$ mice, a novel genetic model of ASD, and compared their performance with that of WT and rescue mice $Nrxn1^{\Delta S5/-}$. Consistent with the findings of Allman et al. (2011), Acosta et al. (2018), and DeCoteau and Fox (2021), we observed that male $Nrxn1^{+/-}$ mice exhibited a leftward shift in their timing behavior, but interestingly, this difference was significant only with respect to the $Nrxn1^{\Delta S5/-}$ mice.

$Nrxn1^{\Delta S5/-}$ variant alleviates synaptic deficits associated with the Nrxn1 mutation and restores several synaptic properties (Lu et al. 2025). One would expect these rescued synaptic functions to restore the timing deficits predicted to occur in the Nrxn1 mutation. The timing behaviors in the $Nrxn1^{+/-}$ mice changed in the expected direction and in the opposite direction to the $Nrxn1^{\Delta S5/-}$. The interesting observation is that the statistically significant differences are between $Nrxn1^{\Delta S5/-}$ and $Nrxn1^{+/-}$, not between $Nrxn1^{\Delta S5/-}$ and WT mice, suggesting that WT manifests a phenotype between $Nrxn1^{\Delta S5/-}$ and $Nrxn1^{+/-}$. We believe that $Nrxn1^{\Delta S5/-}$ exhibits a phenotype that approximates a functional rescue of the $Nrxn1^{+/-}$ mice.

Acosta et al. (2018) found that female mice exhibited this effect only at the 15-s interval, both in the response curve and in single-trial analysis. Male VPA mice had leftward shifts for both the 15-s and 45-s intervals, observed only in single-trial analysis. These shifts were primarily due to changed start times, suggesting motivational mediation of the effect (see Balcı 2014). We observed a leftward shift in the middle times, accompanied by shifts in both start and stop times (suggesting a true timing phenotype). In contrast, in the response curve analysis, the most prominent shift was in the stop times, as in Acosta et al. (2018). Thus, despite minor differences overall, the timing behavior (e.g., peak times) is altered in the same direction in both mouse models of ASD.

Many of the measures are derived from the same underlying distribution of responses, resulting in high dependence between measures. For that reason, we have avoided emphasizing the individual differences in measures, instead focusing on the overall leftward shift in the response distribution.

Earlier start and stop times would create a biased mismatch between objective and subjective time for events, resulting in an unexpectedly delayed delivery of reward over time. In other words, the autistic agent would presumably experience a negative dopaminergic reward prediction error on all trials, even when the reward is delivered (i.e., FI trials). The recurrent prediction error signaling due to biased timing would require a continuous effort to change the temporal model of the world, which would be challenging with an altered reference point (presumably due to biased temporal memory). One can liken this to always pressing a button earlier than needed in a video game, thereby missing a target, even if one has a veridical perceptual representation of the target time.

Finally, Acosta et al. (2018) also observed that the peak amplitude was lower (also indicating a motivational effect) and the peak width was higher in mice prenatally exposed to VPA (prenatal environment) and weaned with other VPA mice (growing environment), particularly in female mice (only at 45 s). We did not observe any difference in the peak amplitudes between genotypes. However, consistent with the leftward shift in the timing function of $Nrxn1^{+/-}$, we observed a lower amplitude at 30 s for $Nrxn1^{+/-}$ compared to $Nrxn1^{+/+}$ and $Nrxn1^{\Delta S5/-}$ mice.

In specific experimental preparations, such as those in which researchers test the acute effects of a drug on timing behavior, the shifts in the timing function are explained in terms of the drug's effect on the speed of the internal clock. For instance, a leftward shift in the timing function would be attributed to faster clock speed (e.g., Meck 1996). However, internal clock speed-based explanations do not apply to our findings, since the clock speed by which long-term memory representations of time intervals are established and by which the animal times in the current trial is the same. The clock-speed effects would occur only when the clock speeds differ between these two conditions. In fact, the same drug that initially causes a shift in the timing behavior after acute administration would lose its effect after repeated administration since the long-term memory representation would be recalibrated according to the altered clock speed (Meck 1996).

Shifts in the timing behavior of a genetic model can be explained by the hypothesis of disrupted memory function rather than altered clock speed (see also Gür, Fertan, Alkins, et al. 2019; Gür, Fertan, Kosel, et al. 2019; Karson and Balcı 2021). For instance, if the consolidation of memory into long-term memory is altered such that some of the information is lost (which can be formally exemplified by multiplying the working memory time representation by a value lower than 1 when transferring it to long-term memory), then the timing behavior would be biased throughout testing. Thus, one explanation for our findings is that $Nrxn1^{+/-}$ mice encode a biased memory representation into long-term memory due to the loss of information (at least compared to $Nrxn1^{\Delta S5/-}$ mice). Note that such an account would predict leftward shifts in both start and stop times, which were confirmed by our results in the single-trial analysis. Another possibility is that the long-term memory representation in Nrxn1+/− mice activated by the presentation of a discriminative stimulus leaks over time, resulting in a stronger effect at stop times than at start times (as observed in the response curve analysis).

The same findings can also be explained by threshold modulation; however, the correlations between start and stop correlations that favored a single threshold do not support this account. Specifically, under the threshold account, if the threshold is raised, this would not lead to a change in peak time, earlier start times, or later stop times. On the other hand, if the threshold is reduced, this would not lead to a change in peak time, later start times, and earlier stop times, which does not explain our empirical observations. If the threshold for start times is increased

and the threshold for stop times is decreased, one can explain a leftward shift, but the correlation patterns do not favor independent thresholds, and modulation of two thresholds in different directions is not the most parsimonious explanation.

Based on the fact that VPA mice did not exhibit a novel object recognition deficit, Acosta et al. (2018) suggested the observed effects in their study were not due to memory deficits in the novel object recognition task; however, the task parameters are known to affect the hippocampal involvement in this task (Cohen and Stackman Jr 2015), and Acosta et al. (2018) did not provide sufficient information regarding the task parameters. Thus, we believe that a biased long-term memory representation remains the most parsimonious explanation of our findings. While deficits in the long-term memory of Nrxn1$^{+/-}$ mice have not been reported, timing deficits are reported in individuals with, and animal models of, conditions associated with long-term memory deficits, such as individuals with amnesic mild cognitive impairment (Gür, Fertan, Alkins, et al. 2019; Gür, Fertan, Kosel, et al. 2019; Larson et al. 2022).

Consistent with this explanation, the leftward shift seen in the Nrxn1$^{+/-}$ mice is similar to the effect seen in animals with hippocampal lesions (e.g., Balcı, Meck, et al. 2009; Olton et al. 1987; Yin and Meck 2014). Understanding how the hippocampus differs between the brains of individuals with ASD and TD individuals is a complex topic. Several studies have found differences in hippocampal shape between individuals with ASD and controls (Dager et al. 2007; Nicolson et al. 2006; Richards et al. 2020). Radiomic studies have found differences in the MRI texture of the hippocampus between individuals with ASD and controls (Chaddad, Desrosiers, and Toews 2017; Chaddad, Desrosiers, Hassan, et al. 2017), and decreased hippocampal density in ASD (Mei et al. 2020). Postmortem examinations of ASD hippocampal tissue have found decreased cell size, increased cell body packing, and decreased dendritic branching (Bauman and Kemper 1985, 2005; Kemper and Bauman 2002; Raymond et al. 1995). There is also evidence of abnormal neurogenesis and migration (Wegiel et al. 2010) and swollen axon terminals (Weidenheim et al. 2001) in the hippocampi of individuals with ASD. Blatt et al. (2001) found decreased density of GABAergic neurons in the hippocampus of ASD brains, while Lawrence et al. (2010) found increased density of GABAergic interneurons. Increased recruitment of the hippocampus during memory encoding (Hogeveen et al. 2020) and impaired performance on a hippocampal-dependent learning task (Ring et al. 2017) were observed in ASD patients.

Finally, this may be underlain by an increase in the transmission rate (i.e., signal propagation between subsequent time cells) within the time cell architecture observed in the hippocampus (Eichenbaum 2014, 2017). However, this account is inconsistent with the report of decreased excitatory transmission within CA1 pyramidal cells of this ASD model (Lu et al. 2025). Interestingly, the correlational patterns suggested lower relative memory variability or higher relative decision threshold variability in Nrxn1$^{+/-}$ mice.

Another possibility is that altered cortico-striatal circuit function, which is highly implicated in interval timing (Buhusi and Meck 2005), may underlie the timing deficits observed in our work. Consistent with this view, Acosta et al. (2018) reported higher striatal dopamine function and altered dopamine turnover in the dorsal striatum (lower in female and higher in male VPA mice) in the VPA model, along with leftward shifts in peak response curves similar to our findings. But note that at the information-processing level, the related deficits cannot be attributed to the resultant altered clock speed (c.f., Meck 1996). Testing the same mouse models of ASD in the differential reinforcement of low rates of responding (DRL) task may help distinguish between these possibilities, since performance in that task is known to be sensitive to hippocampal dysfunction but relatively less sensitive to striatal dysfunction (e.g., Cho and Jeantet 2010).

The results gathered in this study (at least the comparison of Nrxn1$^{-/-}$ and Nrxn1$^{\Delta S5/-}$ mice) also closely resembled the differences observed between the 5xFAD mice and their WT controls (compare Figure 2A in this paper with fig. 2a of Gür, Fertan, Alkins, et al. (2019)). This raises the question of whether these similar behavioral effects observed in two very different genetic mouse models of ASD and AD have a common underlying mechanism. In fact, several researchers have focused on the similarities between these two disease states (e.g., Alexiou et al. 2018; Khan et al. 2016; Lahiri et al. 2021; Sokol et al. 2011).

The fact that a single interval was used in the current study constitutes a limitation of our study. Consequently, we were unable to test whether the core psychophysical features of interval timing, such as the scalar property, are altered in Nrxn1$^{+/-}$ mice. Future studies can test Nrxn1$^{+/-}$ mice in the dual PI procedure (as in Acosta et al. 2018). Another limitation of the current study is that only male mice were tested at a single age. Future studies can also test both male and female Nrxn1$^{+/-}$ mice at different ages to better characterize the timing deficit found in Nrxn1$^{+/-}$ mice.

In conclusion, the Nrxn1$^{+/-}$ mice exhibited a leftward shift towards earlier responding on the PI task. As the leftward shift is seen in both the start and stop times in the single-trial analysis, we believe that a biased long-term memory representation is the most parsimonious explanation for this finding. These results are consistent with findings in mouse and rat VPA experimental (i.e., prenatal exposure) models of ASD and findings in children with ASD, providing further evidence of a timing deficit in ASD.

Author Contributions

Kyle M. Roddick: formal analysis, investigation, data curation, writing – original draft, writing – review and editing, visualization. **Elias B. Habib:** investigation, writing – review and editing. **Richard E. Brown:** conceptualization, resources, writing – review and editing, supervision, project administration, funding acquisition. **Fuat Balcı:** conceptualization, methodology, formal analysis, resources, writing – original draft, writing – review and editing, visualization, supervision, project administration, funding acquisition.

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Ethics Statement

All procedures were approved by the Dalhousie University Committee on Laboratory Animals and were conducted in accordance with the guidelines of the Canadian Council on Animal Care (protocol #18-096).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data and analysis code used in this study are available on Borealis, the Canadian Dataverse Repository, at http://doi.org/10.5683/SP3/LWXNZG.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Scatterplots with overlaid linear regression lines showing the correlations between the (A) start and stop, (B) start and spread, (C) middle and spread times from the single trial analysis.