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RESEARCH ARTICLE

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Knockout of NMDARs in CA1 and dentate gyrus fails to impair temporal control of conditioned behavior in mice

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Abstract

The hippocampus has been implicated in temporal learning. Plasticity within the hippocampus requires NMDA receptor-dependent glutamatergic neurotransmission. We tested the prediction that hippocampal NMDA receptors are required for learning about time by testing mice that lack postembryonal NMDARs in the CA1 and dentate gyrus (DG) hippocampal subfields on three different appetitive temporal learning procedures. The conditional knockout mice (*Grin1*$^{\Delta DCA1}$) showed normal sensitivity to cue duration, responding at a higher level to a short duration cue than compared to a long duration cue. Knockout mice also showed normal precision and accuracy of response timing in the peak procedure in which reinforcement occurred after 10 s delay within a 30 s cue presentation. Mice were tested on the matching of response rates to reinforcement rates on instrumental conditioning with two levers reinforced on a concurrent variable interval schedule. Pressing on one lever was reinforced at a higher rate than the other lever. *Grin1*$^{\Delta DGCA1}$ mice showed normal sensitivity to the relative reinforcement rates of the levers. In contrast to the lack of effect of hippocampal NMDAR deletion on measures of temporal sensitivity, *Grin1*$^{\Delta DGCA1}$ mice showed increased baseline measures of magazine activity and lever pressing. Furthermore, reversal learning was enhanced when the reward contingencies were switched in the lever pressing task, but this was true only for mice trained with a large difference between relative reinforcement rates between the levers. The results failed to demonstrate a role for NMDARs in excitatory CA1 and DG neurons in learning about temporal information.

KEYWORDS
hippocampus, knock out mice, learning, memory, NMDARs, timing

1 | INTRODUCTION

The hippocampus has long been implicated in aspects of timing behavior. Damage to the hippocampus has a number of effects on the learning and memory of temporal information. Meck et al. (1984) showed that fornix lesions that disconnect the hippocampus from other structures reduced the remembered time of reinforcement. Rats were reinforced for pressing a lever 20 s after the onset of a cue. On probe trials, no reinforcement was given and the cue was presented for 50 s. On probe trials, whereas control rats showed a peak in responding approximately 20 s after the cue onset, responding in fornix lesioned rats peaked significantly earlier. Similar findings have been found with lesions of the dorsal hippocampus in rats using

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Pavlovian procedures (Tam et al., 2013, 2015; Tam & Bonardi, 2012a). This suggests that lesioned rats underestimated the time of reinforcement and that the retrieved memory of the reinforced duration was shorter than the actual duration. The results of a temporal bisection procedure were consistent with this conclusion (Meck et al., 1984). Rats were trained to make a left lever response after a short duration signal of 2 s and a right lever response after a long duration signal of 8 s. On probe trials, rats were presented with intermediate durations and were not reinforced for either a left or right lever response. The proportion of right lever responses as a function of time indicated that fornix lesioned rats shifted to making right lever ("long duration") responses sooner than control rats. This suggests that the perceived midpoint between the durations was shorter for lesioned rats than for controls.

The hippocampus has also been implicated in maintaining memories over durations of time in order to allow temporally discontiguous events to be associated with one another. Whereas delay conditioning involves reinforcement being presented at the termination of a conditioned stimulus (CS), in trace conditioning, there is a temporal gap between the end of the CS and reinforcement. Typically, the longer the gap the weaker the strength of conditioned responding (e.g., Kaplan, 1984). Therefore, trace conditioning is sensitive to the temporal structure of events. Hippocampal damage has been found to impair trace conditioning procedures but spare delay conditioning (e.g., Bangasser et al., 2006; Beylin et al., 2001; McEchron et al., 1998; Solomon et al., 1986). However, the hippocampus may be necessary for delay conditioning when CS durations are relatively long (Tam & Bonardi, 2012b).

Further evidence for a role of the hippocampus in temporal learning and memory comes from the discovery of "time cells." These cells found in the hippocampus (although not exclusively; see Tiganj et al., 2017; Umbach et al., 2020) fire at particular timepoints within a temporal sequence of events (Eichenbaum, 2014). Time cells that fired later in the event had longer periods of activity than those that fired earlier (MacDonald et al., 2011). This is consistent with scalar variability in temporal encoding, such that error in timing scales with the timed duration (Gibson, 1977). This scalar property has been observed in the CA1 region of the hippocampus. The firing of CA1 cells scaled with increases and decreases in the timed duration in a temporal bisection task (Shimbo et al., 2021).

The hippocampus' role in temporal learning and memory depends on NMDA (N-methyl-D-aspartate) receptor-dependent synaptic plasticity. Infusion of the NMDA receptor (NMDAR) antagonist AP5 into the dorsal hippocampus selectively impaired trace conditioning (Quinn et al., 2005). Similarly, mice lacking the GluN1 subunit of the NMDAR in the CA1 subfield of the hippocampus showed impaired trace conditioning but normal delay conditioning (Huerta et al., 2000). This may suggest that hippocampal NMDARs are important for maintaining information over periods of time (Maccaferri & Dingledine, 2002) such that associations can form between events that are separated in time. An alternative explanation that may be more consistent with the finding that the relative temporal structure of events is encoded by cells in the hippocampus, is that NMDARs may be necessary for encoding a temporal map of events and that impaired NMDAR function results in a general deficit in timing. It is important to note that the results of Huerta et al. (2000) are confounded by the spread of NMDAR deletion to principal, cortical neurons, as evidenced in subsequent studies using this transgenic line (Brigman et al., 2010; Fukaya et al., 2003; Hoeffer et al., 2008; Rondi-Reig et al., 2006; Wiltgen et al., 2010).

The purpose of the present study was to test the role of hippocampal NMDARs in behavioral procedures that assess sensitivity to temporal information and timing of conditioned responding. We tested mice that lack NMDARs on the principal cells of the CA1 and dentate gyrus (DG) regions of the hippocampus as a consequence of knockout of *Grin1* (Bannerman et al., 2012), the gene that encodes for the obligatory GluN1 subunit of the NMDAR (Monyer et al., 1992; Moriyoshi et al., 1991). These $Grin1^{\Delta DGCA1}$ mice show Cre recombinase expression, allowing for the selective deletion of *Grin1*, throughout the DG and mossy fibers. In CA1, the expression included dorsal and ventral regions, although it was greater in the dorsal part. The expression was negligible in other hippocampal regions. Cre expression was restricted to the hippocampus except for olfactory bulb granule cells and some layer II piriform cortex cells. $Grin1^{\Delta DGCA1}$ mice show impaired synaptic plasticity in CA1, however, long-term plasticity in CA3 was preserved, demonstrating selective impairment of NMDA function within the CA1 subfields. $Grin1^{\Delta DGCA1}$ mice show a selective deficit in using spatial cues to inhibit responding to ambiguous cues (Bannerman et al., 2012).

In the present experiments, mice were tested on three procedures. The first was sensitivity to cue duration. Animals show greater conditioned responding to short duration cues for reinforcement than long duration cues (Austen et al., 2021; Austen & Sanderson, 2019, 2020; Holland, 2000; Lattal, 1999). This effect is impaired in mice that lack the GluA1 subunit of the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor (Austen et al., 2021). GluA1 is a key mediator of hippocampal synaptic plasticity (Zamanillo et al., 1999) and is necessary for hippocampus-dependent spatial working memory (Reisel et al., 2002; Sanderson et al., 2009; Schmitt et al., 2003). The second procedure was a Pavlovian peak procedure. Mice were trained with a 30 s cue that was reinforced 10 s after its onset on 75% trials. Timing of conditioned responding was examined by measurements of the peak in responding at the expected time point of reinforcement on the nonreinforced probe trials. In contrast to other studies that introduce nonreinforced probe trials after acquisition of conditioned responding (e.g., Tam et al., 2013), mice were partially reinforced from the start of training. This was done in an attempt to avoid the introduction of nonreinforcement disrupting performance of conditioned responding and to increase the overall number of probe trials. The third procedure was matching of lever press response rates to reinforcement rates in a concurrent variable interval (VI) schedule. Matching of response rates to reinforcement rates has been proposed as a measure of sensitivity to average time between successive reinforcements (Gallistel & Gibbon, 2000). After acquisition of lever pressing, the mice were also tested on reversal learning of the reinforcement contingencies. Hippocampal NMDA receptors have been found to be necessary for reversal learning of spatial information (Bannerman et al., 2012; Morris et al., 1990). Therefore, the reversal phase allowed

testing of the hypothesis that CA1 and DG NMDA receptors are necessary for reversal of lever press learning. If hippocampal NMDARs are necessary for timing, then knockout of *Grin1* will impair timing behavior as measured by the three procedures.

2 | METHODS

2.1 | Subjects

Mice were bred in the Life Sciences Support Unit, Durham University. The details of the generation of the mice and subsequent histological and electrophysiological analyses are reported in Bannerman et al. (2012). *Grin1*$^{\Delta DGCA1}$ mice and controls were generated by using a mouse line that was gene-targeted for both *lox*P-tagged *Grin1* alleles (*Grin1*^{tm1Rsp}) and that carried the *Tg*^{LC1} and *Tg*^{CN12} transgenes for doxycycline (Dox)-regulated and CamkIIα-promoter-controlled Cre expression. Pregnant females received drinking water with Dox supplement (50 mg Dox/l) until the pups were born. Control mice were a mixture of littermates from the Dox-treated dams that carried either one of the transgenes or neither. For each experiment, preliminary analyses failed to reveal any significant difference between the control subgroups and, therefore, the subgroups were combined to form one control group. Mice were housed in a temperature-controlled holding room on a 12-h light–dark cycle (light period: 8 a.m. to 8 p.m.). For several days prior to the start of testing, the weights of the mice were reduced by restricting access to food and they were maintained at 85% of their free-feeding weights throughout the experiment. Mice had ad libitum access to water in their home cages. All procedures were in accordance with the United Kingdom Animals Scientific Procedures Act (1986); under project license number PPL 70/7785.

For the cue duration experiment, 13 *Grin1*$^{\Delta DGCA1}$ (six female, seven male) and 34 control (11 *Tg*^{CN12}: four female, seven male; 12 *Tg*^{LC1}: six female, six male; 11 *Grin1*^{tm1Rsp}: four female, seven male) mice that were experimentally naïve were used. Mice were housed in groups of two to eight. They were between 30 and 37 weeks old at the start of testing and their free-feeding weights ranged from 21.9 to 42.1 g. For the peak procedure experiment, 16 *Grin1*$^{\Delta DGCA1}$ (eight female, eight male) and 23 control (seven *Tg*^{CN12}: four female, three male; eight *Tg*^{LC1}: four female, four male; eight *Grin1*^{tm1Rsp}: four female, four male) mice were used. These mice had previously been used in an unrelated experiment in which they consumed sucrose solutions but were naïve to the peak procedure testing conditions. Mice were housed in groups of one to seven. They were between 32 and 51 weeks old at the start of testing and their free-feeding weights ranged from 22.5 to 48.8 g. The same mice were subsequently used for the matching behavior experiment. The mice were naïve to lever pressing. Mice were between 51 and 70 weeks old at the start of testing on the matching procedure and their free-feeding weights ranged from 26.1 to 49.7 g. In between the peak procedure and the matching procedure, mice returned to free-feeding conditions.

The number of mice bred for each experiment was planned to be similar to those used in Austen et al. (2021) in which a significant effect of GluA1 AMPA subunit deletion on the cue duration procedure (total $N = 27$) and Pavlovian peak procedure (total $N = 37$) was found with observed power greater than 0.8. In addition, the sample sizes were larger than those used in Bannerman et al. (2012) in which a significant effect of CA1 and DG NMDAR knockout was observed on inhibition of spatial choice behavior (total $N = 23$) with observed power greater than 0.8.

2.2 | Apparatus

A set of eight identical operant chambers (interior dimensions: 15.9 × 14.0 × 12.7 cm; ENV-307A, Med Associates), enclosed in sound-attenuating cubicles (ENV-022V) were used. The operant chambers were controlled by Med-PC IV software (SOF-735). The side walls were made from aluminum, and the front and back walls and the ceiling were made from clear Perspex. The chamber floors each comprised a grid of stainless-steel rods (0.32 cm diameter), spaced 0.79 cm apart, running perpendicular to the front of the chamber (ENV-307A-GFW). A food magazine (2.9 × 2.5 × 1.9 cm; ENV-303M) was situated in the center of one of the sidewalls of the chamber, into which sucrose pellets (14 mg, TestDiet) could be delivered from a pellet dispenser (ENV-203-14P). An infrared beam (ENV-303HDA) across the entrance of the magazine recorded head entries at a resolution of 0.1 s. A fan (ENV-025F) was located within each of the sound-attenuating cubicles and was turned on during sessions, providing a background sound level of approximately 65 dB. Auditory stimuli were provided by a white noise generator (ENV-325SM) outputting a flat frequency response from 10 to 25,000 Hz at 80 dB and a clicker (ENV-335M) operating at a frequency of 4 Hz at 80 dB. Visual stimuli were a 2.8 W house light (ENV-315M) which could illuminate the entire chamber, and two LEDs (light emitting diodes; ENV-321M) positioned to the left and right of the magazine, which provided more localized illumination. Two retractable levers (ENV-312-2M), protruding 2.2 cm above the grid floor, were located on the same wall as the magazine, one to either side of the magazine. Levers were retracted into the wall of the chamber during the cue duration and peak procedure experiments and were used only for the matching behavior experiment.

2.3 | Procedure

2.3.1 | Cue duration experiment

The procedure was identical to that reported for Experiment 1 in Austen et al. (2021). Experimentally naïve mice received 12 sessions of training that consisted of presentations of four different cues. Two cues were 10 s in duration and the other two were 40 s. One cue of each duration terminated with the presentation of a sucrose pellet (10+, 40+). The remaining cues were nonreinforced (10–, 40–). For approximately half of the mice within each genotype, the 10 s cues were visual cues (house light/flashing LEDs illuminated alternately

every 0.5 s) and the 40 s cues were auditory (noise/clicker). For the remaining mice, the opposite was true. Within each of these subgroups the allocation of reinforced and nonreinforced trial types was approximately balanced across the stimuli. These counterbalancing contingencies were also applied to each sex within genotype as far as possible given the numbers of mice. Each session consisted of six trials of each trial type (24 trials in total). The order of cue presentations was random with the constraint that two presentations of each cue occurred within each block of eight trials. The inter-trial interval (ITI) was 120 s from the offset of one cue to the onset of the subsequent cue.

2.3.2 | Peak procedure experiment

Experimentally naïve mice (see Section 2.1) received 12 sessions in which two 30 s duration cues (white noise and clicker) were presented. Each cue was presented 12 times per session. The ITI was the same as the cue duration experiment. One of the cues (CS+) was reinforced with a sucrose pellet 10 s after cue onset on 75% of trials, but non-reinforced on the remaining 25% of trials. The other cue (CS−) was non-reinforced. The trials were presented in a random order with the constraint that there was an equal number of each trial type every eight trials and that the CS+ was reinforced on three of its four trials within each eight-trial block. The allocation of stimuli to trial types was approximately counterbalanced within genotype and sex.

2.3.3 | Matching behavior experiment

In the pre-training stage, at the start of each session both levers were inserted into the chamber. For the first five sessions, each lasting a maximum of 30 min, mice were pre-trained to lever press for sucrose pellets. In the first two of these sessions, each lever press, to either of the two levers, resulted in a pellet being delivered into the magazine, with a maximum of 15 pellets per session. If this limit was reached then the session ended and the levers were retracted from the chamber. In the third session of pre-training, lever presses were reinforced on a 30 s (VI) schedule, with both levers on the same VI schedule of reinforcement. The session ended once 16 rewards had been obtained or once 30 min had passed. On the fourth session, this schedule was increased to a VI 60 s schedule and then again increased to a VI 90 s schedule for the fifth session. Throughout the experiment all VI schedules were determined using a Fleshler and Hoffman (1962) distribution in which intervals were sampled from an exponential distribution.

In the acquisition stage, after pre-training mice were split into two groups, the 3:1 group and the 1.5:1 group, counterbalanced as far as possible with respect to genotype and sex (3:1 group: 12 control mice, 6 male, 6 female and 8 *Grin1*$^{\Delta DGCA1}$ mice, 4 female, 4 male; 1.5:1 group: 11 control type mice, 7 female, 4 male and 8 *Grin1*$^{\Delta DGCA1}$ mice, 4 female, 4 male). The allocation of the left and right levers to a high or low reinforcement rate was approximately balanced within each group, genotype, and sex. Both groups had the same overall reinforcement rate across the two levers (one pellet every 90 s) but the relative rates at which the two levers were reinforced differed. For the 3:1 group, the high lever was reinforced on a VI 120 s schedule and the low lever on a VI 360 s schedule. For the 1.5:1 group, the high lever was reinforced on a VI 150 s schedule and the low lever on a VI schedule of 225 s. The acquisition stage consisted of 12 daily testing sessions lasting 24 min. A maximum of 16 pellets could be obtained per session.

After the acquisition stage, for a further 12 sessions, the lever contingencies were reversed for both the 3:1 and 1.5:1 groups. The lever with a previously high reinforcement rate was now reinforced on the lower VI schedule, and vice versa for the low rate lever. The groups were kept the same, so the 3:1 group still had a 3:1 difference between the levers and the 1.5:1 group had a 1.5:1 difference.

2.4 | Data and statistical analyses

2.4.1 | Cue duration procedure

The number of head entries made to the food magazine during the presentation of each cue was recorded. In order to simplify analysis of performance, responding was converted to a difference score in which the response rates during each nonreinforced cue were subtracted from the response rates for the reinforced cue of the same modality. The difference scores provide a measure of conditioned responding above baseline for each condition. To determine, however, whether any effect of cue duration on differences scores reflected differences in responding to the nonreinforced cues, an additional analysis of the responding to the 10– and 40– cues was conducted. Responding was also measured for 10 s before each cue onset (pre-CS responding) as an additional baseline measure (see Supporting information).

In order to assess timing of responding within cues, responding during the last six sessions of training, during each reinforced cue (prior to food delivery), was divided into 10 equal time periods (i.e., ten 1-s time bins for the 10 s cue and ten 4-s time bins for the 40 s cue). These data were then normalized to show the proportion of responding an animal made during each of the 10 time bins throughout training. The linear gradients of these normalized data were then calculated to provide an indication of the extent that responding to cues was being timed (i.e., the steeper the gradient the more the responses were timed to the delivery of the US).

All data were analyzed using multifactorial analysis of variance (ANOVA). Responding to auditory cues was higher than to visual cues. Because of this, a counterbalancing factor of cue modality was included in the analyses for this experiment to account for this additional variance, but we do not report any main effects or interactions involving the factor of modality. Interactions were analyzed with simple main effects analysis using the pooled error term from the original ANOVA. Where sphericity of within-subjects variables could not be assumed, a Greenhouse–Geisser correction was applied to produce

more conservative *p*-values. The non-corrected degrees of freedom are reported for all statistics.

2.4.2 | Peak procedure

Analyses of timing focussed on performance during the last six sessions of training. The linear slopes were calculated for responding during the first 10 s of the reinforced cue (i.e., prior to the delivery of reinforcement), regardless of whether it was a reinforced or nonreinforced probe trial. Calculation of the slopes was the same as for the cue duration experiment. For the analysis of the response curves for individual mice, responding per second was calculated for the 30 s probe trials. From the curves, several measures were taken: the peak response (i.e., the maximum response rate that occurred on a given second), the peak response time, the start time (defined as the first time responding occurred at least 75% of the peak response), the stop time (defined as the last time responding occurred at least 75% of the peak response), peak time error (i.e., the unsigned difference between the peak time and 10 s, the time of reinforcement). The different measures of timing were analyzed using Mann-Whitney *U* tests and *p*-values were compared to Holm–Bonferroni adjusted alpha values in order to correct for the number of comparisons.

2.4.3 | Matching behavior experiment

The total numbers of presses made on each lever per session were recorded and the proportion of presses on the high lever was calculated. The number of lever presses on the same lever before switching to the other lever (lever press bout) was recorded as well as the bout duration (time from first lever press of the bout to the first lever press on the other lever, see Supporting information). The number of pellets obtained from each lever was also recorded.

In the reversal stage, there were two sessions in which one of the levers failed to operate. This affected one mouse in session 10 (a control mouse in the 3:1 group) and one mouse in session 12 (a *Grin1*$^{\Delta DGCA1}$ mouse in the 1.5:1 group). In order to avoid excluding mice on the basis of missing data, the mouse that was affected in session 10 was allocated the mean of their lever presses (on the affected lever) on sessions 9 and 11. Because the 12th session was the last session of reversal training, the mouse affected in session 12 was allocated the number of the lever presses they made on session 11. The proportion of presses on the high lever was then calculated as normal. A similar procedure was used for the lever press bout data. Data for the number of pellets remained unchanged in order to reflect that no pellets were received from pressing the malfunctioning lever on those sessions.

To determine the degree of matching behavior, the proportion of rewards earned on the high reward lever was subtracted from the proportion of responding on the high reward lever. A score of 0 indicates perfect matching, whereas scores below or above 0 indicate under-matching and over-matching, respectively.

3 | RESULTS

3.1 | Cue duration procedure

3.1.1 | Difference scores

The difference scores for the 10 and 40 s cues across the 12 sessions of training are shown in Figure 1a. Over the course of training, Grin1$^{\Delta DGCA1}$ and control mice showed greater difference scores for the 10 s cue than the 40 s cue and the levels of difference scores were similar between the genotypes. An ANOVA of cue (10 s/40 s) × genotype (*Grin1*$^{\Delta DGCA1}$/control) × modality of 10 s cue (auditory/visual; nuisance factor) × session showed significant main effects of cue ($F(1, 43) = 48.1$, $p < .001$, $\eta_p^2 = 0.53$, 90% CI [0.34, 0.64]) and session ($F(11, 473) = 35.5$, $p < .001$, $\eta_p^2 = 0.45$, 90% CI [0.39, 0.49]), and a significant cue × session interaction ($F(11, 473) = 13.1$, $p < .001$, $\eta_p^2 = 0.23$, 90% CI [0.16, 0.27]). All other main effects and interactions were nonsignificant (*p-values* ≥ .14). Simple main effects analysis of the cue by session interaction revealed that mice had significantly greater difference scores for the 10 s cue than the 40 s cue from session 5 onwards (largest *p*-value = .007). Prior to session 5, the effect of cue was not significant (*F*-values <1). There was a significant effect of session for both cues (*p*-values <.001).

3.1.2 | CS− Responding

The rates of responding to the nonreinforced 10 s and 40 s cues across the 12 sessions of training are shown in Figure 1b. *Grin1*$^{\Delta DGCA1}$ and control mice came to respond more to the 10 s non-reinforced cue than the 40 s non-reinforced cue but this difference decreased with training and eventually mice responded at a low rate to both cues. An ANOVA of cue (10 s/40 s) × genotype (*Grin1*$^{\Delta DGCA1}$/control) × modality of 10 s cue (auditory/visual; nuisance factor) × session on responding to the nonreinforced CS− cues showed significant main effects of cue ($F(1, 43) = 8.81$, $p = .005$, $\eta_p^2 = 0.17$, 90% CI [0.03, 0.33]) and session ($F(11, 473) = 16.4$, $p < .001$, $\eta_p^2 = 0.28$, 90% CI [0.21, 0.31]) and a significant cue × session interaction ($F(11, 473) = 6.26$, $p < .001$, $\eta_p^2 = 0.13$, 90% CI [0.07, 0.16]). All other main effects and interactions were nonsignificant (*p*-values ≥.12). Simple main effects analysis of the cue by session interaction showed that mice responded more to the 10 s CS− than 40 s CS− on sessions 2 and 4–8 (largest *p*-value = .016). There was no significant difference on the remaining sessions (largest $F(1, 43) = 3.07$, $p = .09$). There was a significant effect of session for both cues (smallest $F(11, 33) = 3.97$, $p = .001$).

3.1.3 | Timing of responding within trials

The rate of responding to the 10 and 40 s CS+ cues is shown as a function of time (s) within the trial in Figure 1c. Responding increased

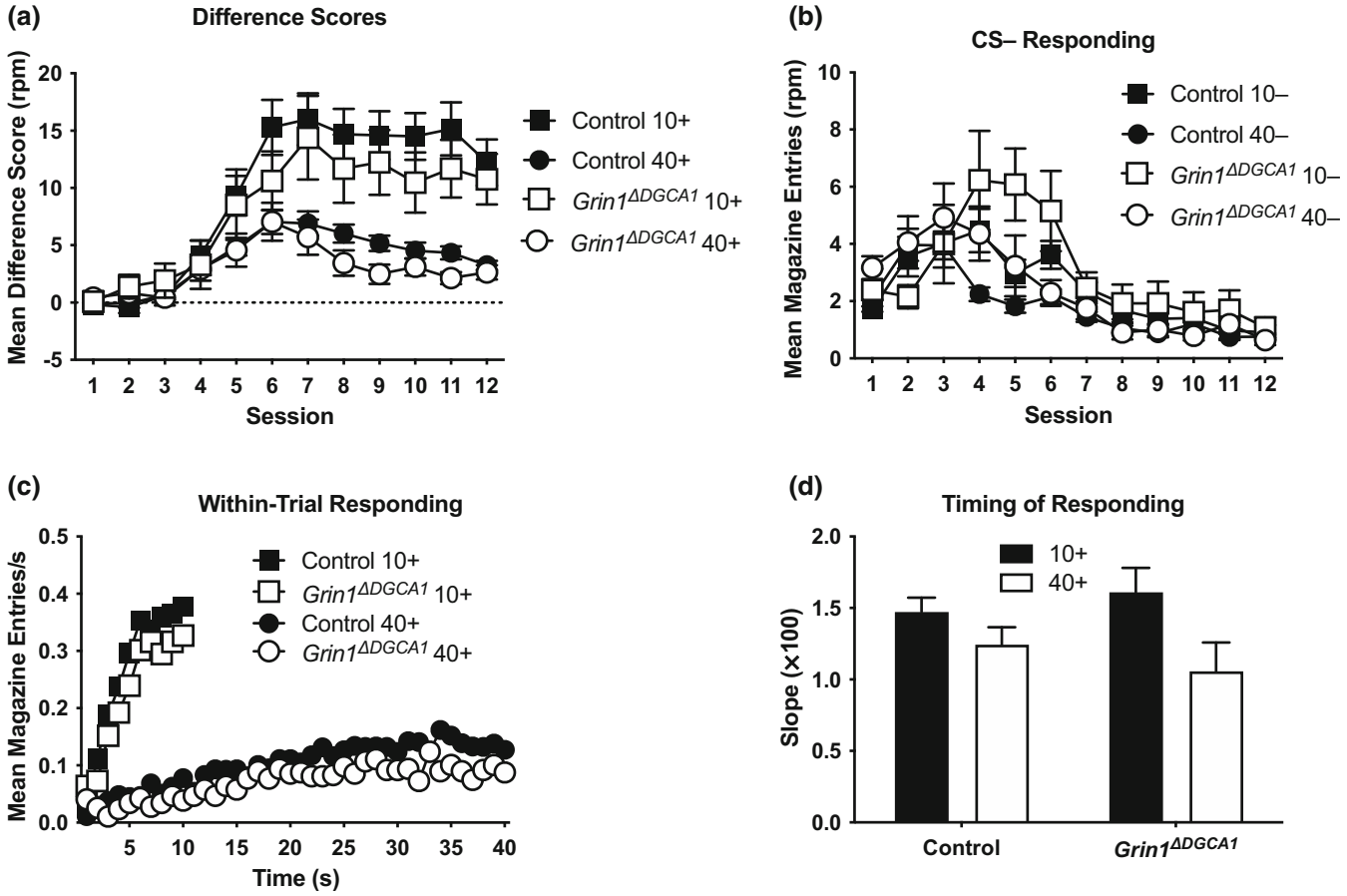


FIGURE 1 *Grin1*$^{\Delta DGCA1}$ mice show normal sensitivity to cue duration. Panel (a): Mice acquired greater difference scores (CS+ responding minus CS− responding; shown as rate per minute, rpm) across the 12 sessions of training for the 10+ and 40+ cues. Panel (b): Mean magazine entries (rpm) for the nonreinforced cues (10– and 40–) across sessions. Mice initially acquired greater conditioned responding to the 10– cue than 40– cue, but this effect reduced with training. Error bars indicate ±SEM (standard error of the mean). Panel (c): Mean magazine entries per second during the 10+ and 40+ cues, across the last six sessions of training. Responding increased over time for both cues. Panel (d): The mean slope (multiplied by 100) fitted to the distribution of responding (normalized for overall response levels and CS duration) within cues. Mean slopes were greater for the 10+ cue than 40+ cue. Error bars indicate SEM.

within trials for both the 10+ and 40+ cues. Linear gradients fitted to the normalized response rates are shown in Figure 1d. *Grin1*$^{\Delta DGCA1}$ and control mice showed steeper linear gradients for the 10 s cue than for the 40 s cue. An ANOVA of cue × genotype × modality of 10 s cue (auditory/visual; nuisance factor) for the gradients showed that gradients were significantly higher for the 10 s cue than 40 s cue ($F(1, 43) = 9.19$, $p = .004$, $\eta_p^2 = 0.18$, 90% CI [0.04, 0.33]). The effect of genotype was not significant, $F < 1$, and there was no significant interaction between factors, $F(1, 43) = 1.61$, $p = .21$.

3.2 | Peak procedure

Responding within probe trials is shown in Figure 2a. Both genotypes showed an initial increase in responding that peaked close to the expected time of reinforcement and then subsequently reduced. *Grin1*$^{\Delta DGCA1}$ and control mice showed similar gradients of normalized responding (see Figure 2b). There was no significant difference between genotypes ($t(37) = 1.21$, $p = .24$). Figure 3 shows measures extracted from the mean rate of responding across probe trials for individual mice: peak response, peak time, peak error, start time, stop time, and spread. All Mann-Whitney *U* test comparisons were not significant (smallest *p*-value = .47; Holm–Bonferroni corrected alpha value = 0.0083).

3.3 | Matching behavior

3.3.1 | Acquisition

Lever presses

The number of lever presses on the high and low reward levers during the acquisition phase is shown in Figure 4a for group 3:1 and Figure 4b for group 1.5:1. *Grin1*$^{\Delta DGCA1}$ mice made a greater number of presses than control mice in both groups. Both genotypes acquired the discrimination and made greater numbers of presses on the high

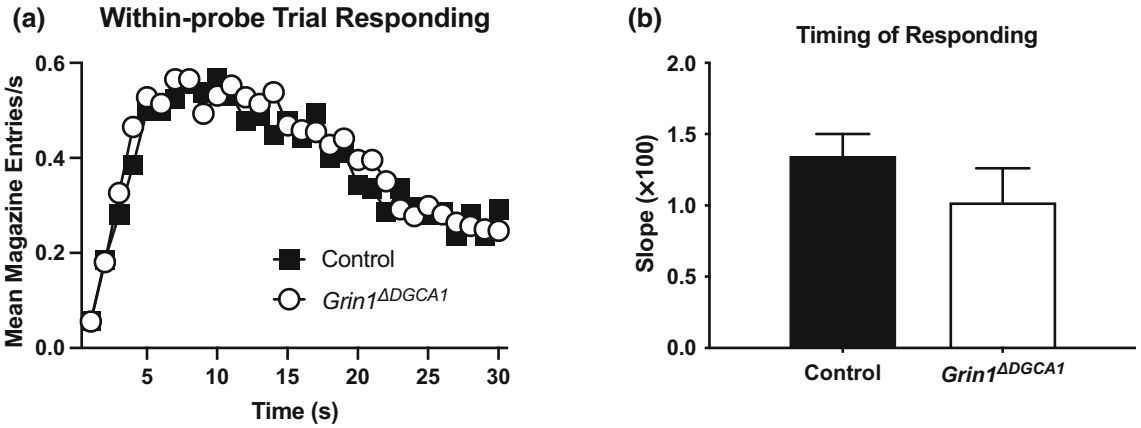


FIGURE 2 *Grin1*$^{\Delta DGCA1}$ mice show normal timing of conditioned responding in the peak procedure. Panel (a): Mean magazine entries per second within probe trials, across the last six sessions of training. For both genotypes, responding peaked close to the time of reinforcement (10 s). Panel (b): The mean slope (multiplied by 100) fitted to the distribution of responding (normalized for overall response levels) across the first 10 s of the probe trials. Slopes did not significantly differ between genotypes. Error bars indicate SEM.

than the low lever. An ANOVA of group (3:1/1.5:1) × genotype (*Grin1*$^{\Delta DGCA1}$/control) × lever (high/low) × session showed that there were significant effects of lever ($F(1, 35) = 119.5$, $p < .001$, $\eta_p^2 = 0.77$, 90% CI [0.64, 0.83]) and genotype ($F(1, 35) = 9.9$, $p = .003$, $\eta_p^2 = 0.22$, 90% CI [0.05, 0.39]). The effect of group was not significant ($F < 1$), but it significantly interacted with the effect of lever ($F(1, 35) = 9.5$, $p = .004$, $\eta_p^2 = 0.21$, 90% CI [0.04, 0.39]). There was a significant effect of session ($F(11, 385) = 4.5$, $p < .001$, $\eta_p^2 = 0.11$, 90% CI [0.05, 0.14]). All other interactions between factors were not significant (smallest p-value = .12). Simple main effects analysis of the lever by group interaction confirmed that although both groups made significantly more responses on the high lever than the low lever, the effect of lever was significantly greater for the 3:1 group ($F(1, 35) = 99.9$, $p < .001$) than the 1.5:1 group ($F(1, 35) = 30.3$, $p < .001$). The effect of group was not significant for either lever (largest $F(1, 35) = 2.6$, $p = .12$).

Proportion of high lever responses

The proportion of high lever responses across sessions in the acquisition phase is shown in Figure 4c. For both genotypes, the proportion of responses on the high lever remained relatively stable over sessions, with higher ratios in the 3:1 group than the 1.5:1 group, and little difference between the genotypes. An ANOVA of group (3:1/1.5:1) × genotype (*Grin1*$^{\Delta DGCA1}$/control) × session showed that the proportion of high lever responses was significant greater for the 3:1 group than 1.5:1 group ($F(1, 35) = 10.9$, $p = .002$, $\eta_p^2 = 0.24$, 90% CI [0.06, 0.41]). All other main effects and interactions were not significant (smallest p-value = .22).

Matching scores

The matching scores across sessions in the acquisition phase are shown in Figure 4d. For the 3:1 group, both genotypes showed a tendency to undermatch. For the 1.5:1 group, both genotypes showed a high degree of matching. An ANOVA of group (3:1/1.5:) × genotype (*Grin1*$^{\Delta DGCA1}$/control) × session showed that group 3:1 significantly undermatched relative response rates to relative reinforcement rates in comparison to group 1.5:1 ($F(1, 35) = 18.72$, $p < .001$, $\eta_p^2 = 0.35$, 90% CI [0.14, 0.51]). There was no other significant effects or interactions (smallest p value = .12).

Lever press bouts

The number of lever presses in a bout is shown for both levers across the acquisition stage in Figure 5, left panel. For both genotypes, mice in the 3:1 group made more lever presses per bout than in the 1.5:1 group. Lever press bouts were larger for the high lever than the low lever. An ANOVA of lever (high/low) × group (3:1/1.5:1) × genotype (*Grin1*$^{\Delta DGCA1}$/control) × session showed significant main effects of lever ($F(1, 35) = 69.8$, $p < .001$, $\eta_p^2 = 0.66$, 90% CI [0.49, 0.75]), group ($F(1, 35) = 7.92$, $p = .008$, $\eta_p^2 = 0.18$, 90% CI [0.03, 0.36]), and session ($F(11, 385) = 6.11$, $p = .002$, $\eta_p^2 = 0.15$, 90% CI [0.08, 0.18]). The main effect of genotype was not significant ($F(1, 35) = 3.03$, $p = .090$). There was a significant lever by group interaction ($F(1,35) = 11.2$, $p = .002$ $\eta_p^2 = 0.24$, 90% CI [0.06, 0.41]. All other interactions were not significant (p-values ≥ .15).

Number of pellets acquired

The mean number of pellets obtained, collapsed across acquisition sessions, is shown in Figure 6, left panel. *Grin1*$^{\Delta DGCA1}$ mice acquired more pellets than control mice. An ANOVA of group (3:1/1.5:1) × genotype (*Grin1*$^{\Delta DGCA1}$/control) × session showed that the difference between genotypes was significant ($F(1, 35) = 5.7$, $p = .022$, $\eta_p^2 = 0.14$, 90% CI [0.01, 0.31]). No other main effects of interactions were significant (smallest p-value = .18).

3.3.2 | Reversal learning

Lever presses

The number of lever presses on the high and low reward levers during the reversal stage is shown in Figure 4e for group 3:1 and Figure 4f

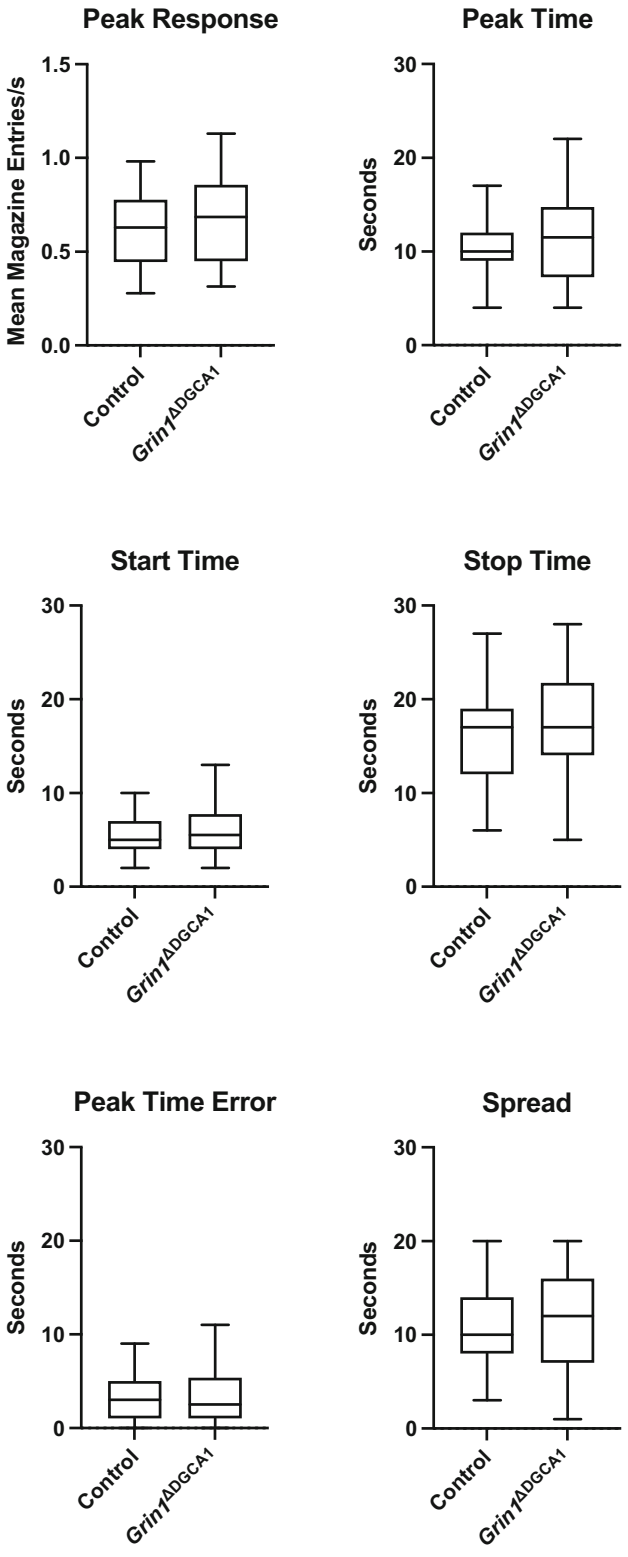


FIGURE 3 *Grin1*$^{\Delta DGCA1}$ mice show normal timing precision and accuracy in the peak procedure. Measures are taken from individual response curves averaged across probe trials in the latter half of training. The box plots show the median, inter-quartile range, and minimum and maximum values for the following measures: peak response, peak time, start time (first occurrence of responding at 75% of peak response), stop time (last occurrence of responding at 75% of peak response), peak time error, and spread (duration between the start and stop times).

for group 1.5:1. The reversal discrimination was acquired to a greater extent by the 3:1 group than the 1.5:1 group. Reversal acquisition was enhanced in *Grin1*$^{\Delta DGCA1}$ mice compared to control mice in the 3:1 group, but performance was similar between the genotypes in the 1.5:1 group. An ANOVA of group (3:1/1.5:1) × genotype (*Grin1*$^{\Delta DGCA1}$/control) × lever (high/low) × session revealed a significant interaction between lever, genotype and group ($F(1, 35) = 10.1$, $p = .003$, $\eta_p^2 = 0.22$, 90% CI [0.05, 0.40]), as well as a four-way interaction between these factors and the effect of session ($F(11, 385) = 2.6$, $p = .022$, $\eta_p^2 = 0.07$, 90% CI [0.01, 0.09]). The interaction between lever, group, and genotype was analyzed by conducting separate lever by genotype ANOVAs for each group. The lever by genotype interaction was not significant for the 1.5:1 group ($F < 1$). There was a significant lever by genotype interaction for the 3:1 group ($F(1, 18) = 12.6$, $p = .002$). Simple main effects analysis showed that *Grin1*$^{\Delta DGCA1}$ mice made significantly more high lever presses than control mice ($F(1, 18) = 12.5$, $p = .002$) but the effect of genotype was not significant for the low lever ($F(1, 18) = 3.12$, $p = .09$). Furthermore, the difference in response rates for the high lever compared to the low lever was significantly greater for the *Grin1*$^{\Delta DGCA1}$ mice ($F(1, 18) = 43.6$, $p < .001$) than the control mice ($F(1, 18) = 6.1$, $p = .02$).

Proportion of high lever responses

The proportion of high lever responses across the reversal stage is shown in Figure 4g. The ratios were initially below 0.5, chance level and then increased over the course of reversal training. The proportion of high lever responses was greater in group 3:1 than group 1.5:1. *Grin1*$^{\Delta DGCA1}$ mice made a greater proportion of high lever responses than controls in group 3:1, but the high lever ratios were similar between genotypes in group 1.5:1. An ANOVA of group (3:1/1.5:1) × genotype (*Grin1*$^{\Delta DGCA1}$/control) × session showed that there was a significant effect of session ($F(11, 385) = 34.1$, $p < .001$, $\eta_p^2 = 0.49$, 90% CI [0.42, 0.53]) and group ($F(1, 35) = 23.9$, $p < .001$, $\eta_p^2 = 0.41$, 90% CI [0.19, 0.55]). There was a significant genotype by group interaction ($F(1, 35) = 5.09$, $p = .030$, $\eta_p^2 = 0.13$, 90% CI [0.007, 0.30]). No other main effects or interactions of factors were significant (smallest p-value = .15). Simple main effects analysis of the interaction between genotype and group showed that for the 3:1 group, *Grin1*$^{\Delta DGCA1}$ mice made a significantly greater proportion of high lever responses than control mice ($F(1, 35) = 8.19$, $p = .007$). There was no significant difference between the two genotypes in the 1.5:1 group ($F < 1$).

Matching scores

The matching scores across the reversal stage are shown in Figure 4h. Scores started below zero and although they increased over time, both groups tended to undermatch by the end of reversal training. In group 3:1, *Grin1*$^{\Delta DGCA1}$ mice showed greater matching than controls. An ANOVA of group (3:1/1.5:1) × genotype (*Grin1*$^{\Delta DGCA1}$/control) × session showed that there was a significant effect of group ($F(1, 35) = 18.94$, $p < .001$, $\eta_p^2 = 0.35$, 90% CI [0.14, 0.51]), genotype ($F(1, 35) = 4.60$, $p = .039$, $\eta_p^2 = 0.12$, 90% CI [0.003, 0.28]) and session

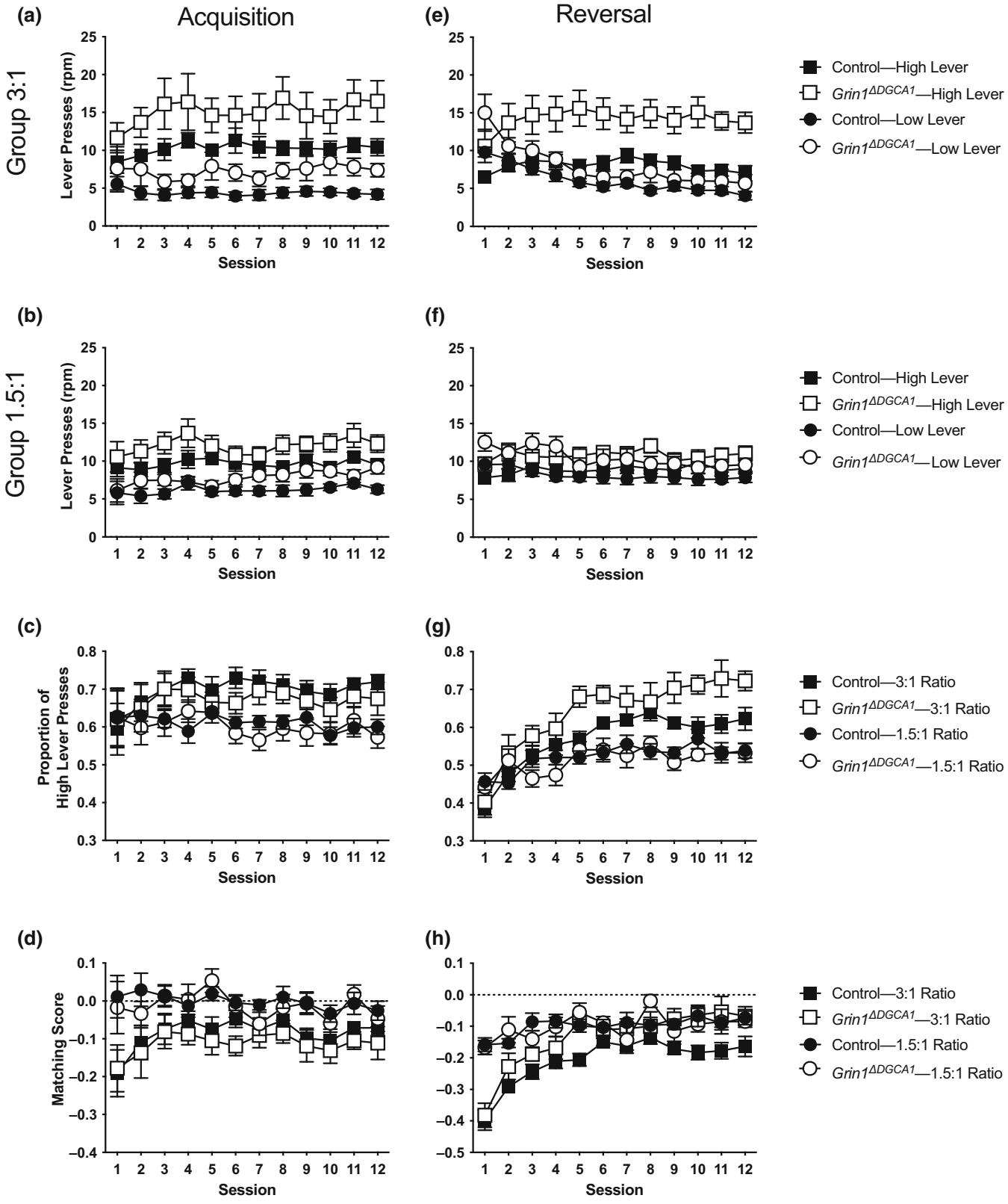


FIGURE 4 *Grin1*$^{\Delta DGCA1}$ mice show normal acquisition of matching behavior and enhanced reversal learning in the 3:1 condition. Panels (a) and (b) show acquisition of lever pressing on the high and low reinforcement rate levers for Group 3:1 and Group 1.5:1, respectively. Panels (e) and (f) show reversal of lever press responding. Panels (c) and (g) show the high lever responses as a proportion of total responding for the acquisition and reversal stages, respectively. Panels (d) and (h) show the proportion of high lever responses minus the proportion of rewards earned on the high lever (matching score) for the acquisition and reversal stages, respectively. The dashed line indicates perfect matching performance (i.e., relative response rates = relative reinforcement rates). Error bars indicate ±SEM.

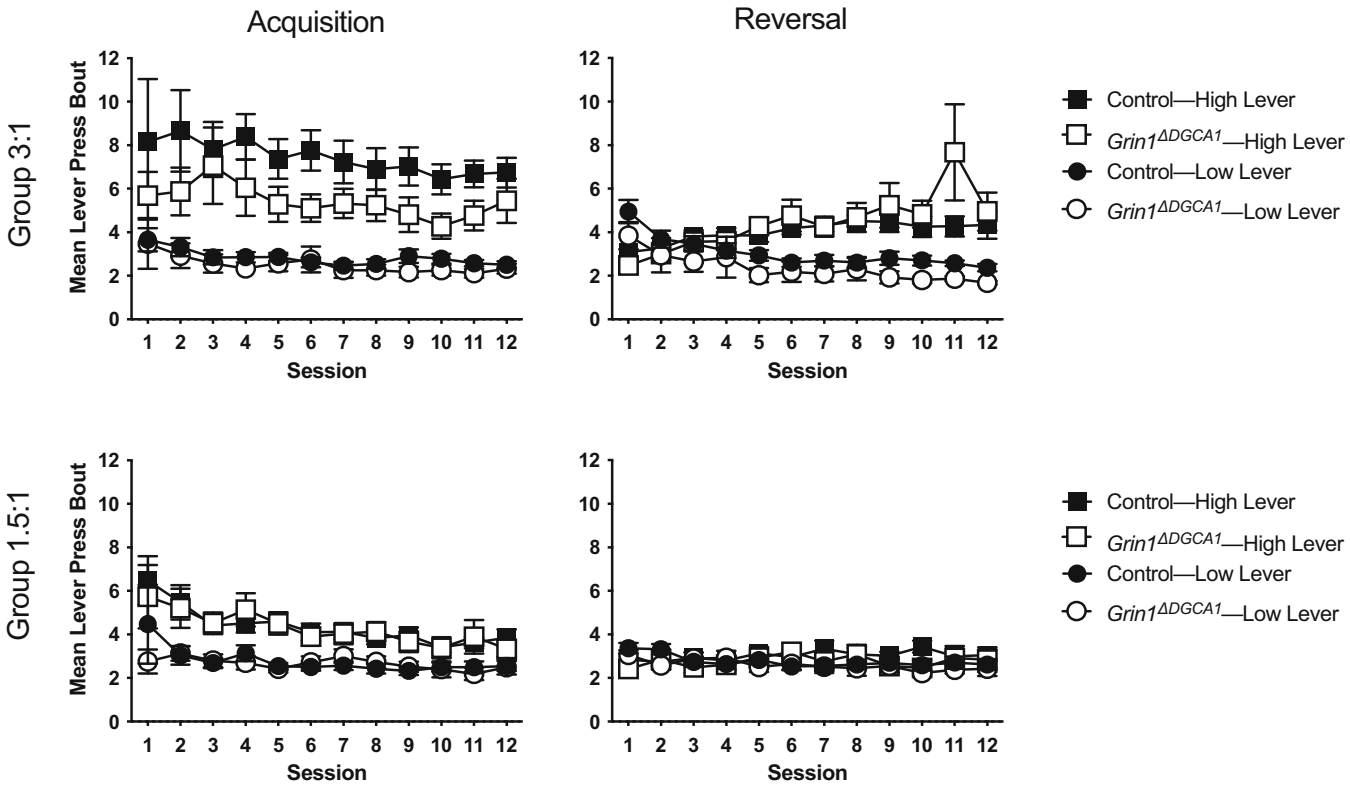
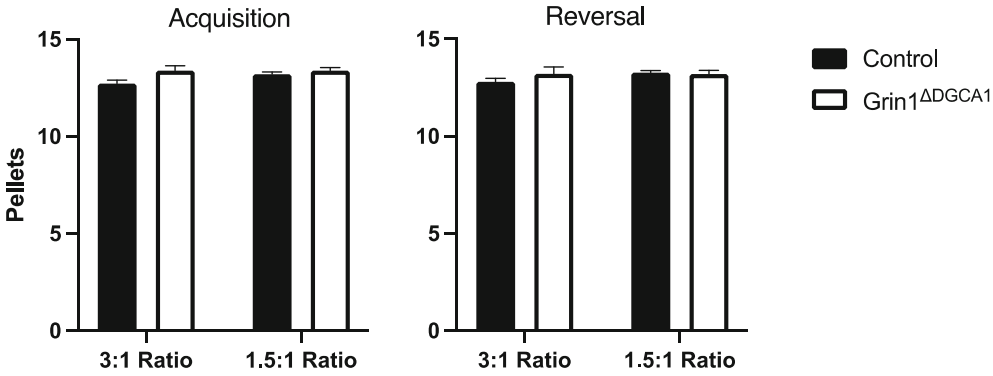


FIGURE 5 *Grin1*$^{\Delta DGCA1}$ mice and control mice show a similar number of lever presses on a given lever before switching to the other lever. Mice made a greater number of responses per bout on the high lever compared to the low lever. Mean lever press bout is shown for the acquisition and reversal stages for Group 3:1 and Group 1.5:1. Error bars indicate ±SEM.

FIGURE 6 *Grin1*$^{\Delta DGCA1}$ mice obtained a significantly greater number of pellets than control mice in the acquisition stage, but the amounts did not significantly differ in the reversal stage. Error bars indicate ±SEM.



($F(11, 385) = 22.82$, $p < .001$, $\eta_p^2 = 0.39$, 90% CI [0.32, 0.43]). The effect of group significantly interacted with session ($F(11, 385) = 7.13$, $p < .001$, $\eta_p^2 = 0.17$, 90% CI [0.10, 0.20]) and genotype ($F(1, 35) = 4.90$, $p = .034$, $\eta_p^2 = 0.12$, 90% CI [0.005, 0.29]). The remaining interactions were not significant (smallest p value = .14). Simple main effects analysis of group by genotype interaction revealed that *Grin1*$^{\Delta DGCA1}$ showed significantly greater matching than controls in group 3:1 ($F(1, 35) = 9.67$, $p = .004$), but there was no effect of genotype for group 1.5:1 ($F < 1$). *Grin1*$^{\Delta DGCA1}$ mice showed greater matching in group 3:1 than group 1.5:1 ($F(1, 35) = 26.23$, $p < .001$), but there was no significant effect of group for control mice ($F(1, 35) = 1.94$, $p = .17$).

Lever press bouts

The number lever presses per bout during the reversal stage is shown in Figure 5, right panel. The 3:1 group made larger lever press bouts than the 1.5:1 group. Mice made larger lever press bouts on the high lever than the low lever. An ANOVA of lever (high/low), group (3:1/1.5:1) × genotype (*Grin1*$^{\Delta DGCA1}$/control) × session showed that the difference between the high and low lever was significant ($F(1, 35) = 36.6$, $p < .001$, $\eta_p^2 = 0.51$, 90% CI [0.20, 0.64]). There was a significant effect of group ($F(1, 35) = 6.62$, $p = .014$, $\eta_p^2 = 0.16$, 90% CI [0.02, 0.33]), but no significant main effects of session ($F(11, 385) = 1.36$, $p = .26$) or genotype ($F < 1$). All interactions were nonsignificant (p-values >.08).

Number of pellets acquired

The number of pellets acquired during the reversal stage collapsed across session is shown in Figure 6, right panel. The number of pellets acquired was similar between the genotypes and the 3:1 and 1.5:1 groups. An ANOVA of group (3:1/1.5:1) × genotype (*Grin1*$^{\Delta DGCA1}$/control) × session showed that there were no significant main effects of genotype ($F < 1$), or group, $F(1, 35) = 1.2$, $p = .28$), or session ($F < 1$). The interaction between genotype and group was not significant ($F < 1$). All other interactions were not significant (F-values <1.8, p-values > .11).

4 | DISCUSSION

Across three experiments, we assessed the role of NMDARs in the CA1 and DG regions of the hippocampus in learning about temporal information. Deletion of hippocampal NMDARs consistently failed to affect behavioral performance on procedures that assessed sensitivity to time. Therefore, the experiments failed to provide evidence to support the hypothesis that hippocampal NMDARs are required for temporal learning.

Grin1$^{\Delta DGCA1}$ mice showed normal sensitivity to time in the cue duration procedure. Both genotypes responded more to the reinforced 10 s cue than the reinforced 40 s cue. Furthermore, both genotypes discriminated between the reinforced cues and the nonreinforced cues. Discrimination was measured using difference scores. It is possible that these scores may reflect differences between the nonreinforced cues rather than differences in conditioned responding to the reinforced cues. It was found that, early on in training, mice responded more to the 10 s nonreinforced cue than the 40 s nonreinforced. This likely reflects generalization of conditioned responding from the 10+ cue to the 10− cue, because the discrimination was between cues within the same modality. The greater responding to the 10 s nonreinforced cue than 40 s nonreinforced cue cannot account for the effect of cue duration observed with the difference scores and, instead, the effect of cue duration on difference scores is a result of greater responding to the reinforced 10 s cue than the reinforced 40 s cue.

Timing of responding did not differ between the genotypes in the cue duration experiment. Both *Grin1*$^{\Delta DGCA1}$ and wild-type mice showed greater timing, as indicated by slopes fitted to the change in response rates within a trial, for the 10+ cue than 40+ cue.

The differences in responding to the 10 and 40 s cues did not reflect a performance effect caused by the differences in the manner of their presentation, but instead reflected a learning effect (see also Austen et al., 2021; Austen & Sanderson, 2020). When tested under matched conditions, mice responded more to the 10+ cue than the 40+ cue (see Figure S2). The effect of cue duration was evident in the first 10 s of presentation of each trial within a session suggesting that it reflected learning as a consequence of prior experience. Furthermore, the difference in overall response rates was not due to withholding of responding until the latter portions of the 40 s cue that might be expected to occur if mice responded only within a particular time range before the expected time of reinforcement. Thus, a comparison of the last 10 s of the 40+ cue with the whole duration of the 10+ cue, in the first trials of each trial type per session, demonstrated that mice still responded at a lower level for the 40+ cue compared to the 10+ cue.

The cue duration effect may be a consequence of a number of potential processes. There is evidence, however, that it reflects sensitivity to reinforcement rate (Austen et al., 2021; Harris et al., 2015). The cue duration effect is abolished if the short duration cue is reinforced at the same overall reinforcement rate as the long duration cue. For example, we have shown that mice show similar levels of responding to a 40 s cue that is reinforced on 100% of trials and a 10 s cue that is presented four times as often as the 40 s cue, but is reinforced on only 25% of trials (Austen et al., 2021). Therefore, when the two cues are matched for reinforcement rate and the number of reinforcements, there is no effect of cue duration. Deletion of the GluA1 subunit of the AMPA receptor selectively impairs sensitivity to reinforcement rate in the cue duration effect and sensitivity to number of reinforcements is preserved (Austen et al., 2021, 2022). This suggests that GluA1 is necessary for weighting the numerical information by temporal information in order for sensitivity to reinforcement rate to be achieved. The present results suggest that NMDARs in CA1 and DG are not required for these processes.

The peak procedure was used to assess the distribution of responding before and after the time of reinforcement, which provides detailed measures of timing precision and accuracy. *Grin1*$^{\Delta DGCA1}$ and control mice showed similar acquisition of the discrimination between the reinforced and non-reinforced cues (see Figure S3). On nonreinforced probe trials, responding peaked close to the timepoint that reinforcement normally occurred. Both genotypes showed similar accuracy (in terms of time at which the peak occurred) and precision (in terms of the spread of responding around the peak). Analysis of individual trials demonstrated that *Grin1*$^{\Delta DGCA1}$ and wild-type mice showed similar start and stop times for responding and variation in start and stop times were similar across the genotypes (see Figure S4) suggesting that sensitivity of decision thresholds for responding were similar between genotypes.

Although the *Grin1*$^{\Delta DGCA1}$ and control mice showed similar cue duration effects and timing of responding, the genotypes did significantly differ in initial levels of baseline magazine entries (see Figures S1 and S3). Mice in both the cue duration and peak procedure experiments were naïve to the apparatus at the start of training. In both procedures, *Grin1*$^{\Delta DGCA1}$ mice initially showed a higher level of pre-CS magazine responding than control mice, but this difference reduced over sessions and the levels of baseline responding were then similar between the genotypes for the rest of training. Increased baseline responding may reflect increased locomotor activity in response to novelty as a consequence of impaired hippocampal function (Jarrard, 1968).

Grin1$^{\Delta DGCA1}$ and wild-type mice showed similar levels of matching behavior. Both groups showed approximate matching of relative response rates to relative reinforcement rates and were similarly sensitive to the manipulation of the ratio of high/low lever reinforcement

rates. Although both genotypes showed similar levels of relative response rates, *Grin1*$^{\Delta DGCA1}$ mice responded at an overall significantly higher level than control mice. This effect is similar to the increase in baseline levels of magazine entries seen in the Pavlovian cue duration and peak procedure experiments. In contrast, whereas the increase in baseline head entries reduced over training, the increased levels of lever pressing remained constant in the matching experiment. Despite the difference in overall levels, *Grin1*$^{\Delta DGCA1}$ mice and controls showed similar sized lever press bouts, which were larger for the high reward lever than the low reward lever. Therefore, the pattern of responding was similar between the genotypes, except that rates of pressing were faster for *Grin1*$^{\Delta DGCA1}$ mice such that they completed more bouts on each lever and switched between them more frequently.

An analysis of bout durations demonstrated that the cumulative probability of abandoning pressing on the high lever and switching to the low lever increased as a logarithmic function of bout duration (see Supporting information). This is consistent with the probability of switching at any given moment being a constant probability (Heyman, 1979) and the distribution of bout durations following a Bernoulli distribution. The logarithmic function is in contrast to that which would be expected if mice learnt that the probability of reward on the alternative lever increased as a function of time on the current lever. In that situation, the probability of leaving at any given moment would increase as a function of bout duration and thus the cumulative probability would exponentially increase. *Grin1*$^{\Delta DGCA1}$ mice showed similar distributions to controls suggesting that switching behavior was qualitatively similar between the genotypes.

During the initial acquisition phase, *Grin1*$^{\Delta DGCA1}$ mice also acquired significantly more pellets than control mice. This may simply be a consequence of the greater number of lever presses made by *Grin1*$^{\Delta DGCA1}$ mice, which reduced the latency between a scheduled reinforcement and the reinforced lever press. Although the difference in number of pellets obtained was significant, the advantage that *Grin1*$^{\Delta DGCA1}$ mice had over control mice was numerically small. Despite the difference in the number of pellets obtained, both genotypes showed similar levels of matching relative response rates to relative reinforcement rates.

When the reinforcement contingencies on the levers were reversed, *Grin1*$^{\Delta DGCA1}$ mice showed greater reversal learning than control mice, but this effect was restricted to the 3:1 group. The enhanced reversal learning in *Grin1*$^{\Delta DGCA1}$ mice was not a consequence of greater sampling of the new reinforcement contingencies. Both genotypes obtained a similar number of reinforcements over reversal training. Furthermore, when relative response rates were corrected for relative reinforcement rates experienced across levers, *Grin1*$^{\Delta DGCA1}$ mice in group 3:1 showed superior matching behavior during the reversal phase. These results suggest that deletion of NMDARs in CA1 and DG increased sensitivity to the current reinforcement rates during reversal learning. It is possible that NMDARs in CA1 and DG are necessary for the maintenance of associations. This idea is, however, in contrast to the findings that hippocampal lesions cause a lack of sensitivity to reinforcement contingencies under conditions in which previous acquired response-reward contingencies are degraded (Corbit & Balleine, 2000). Furthermore, deletion of NMDARs in CA1 and DG results in impaired, rather than enhanced, reversal of spatial learning in the Morris water maze (Bannerman et al., 2012). Therefore, the enhanced reversal learning observed in the matching behavior procedure may be specific to that procedure and is unlikely to reflect a general effect on reversal learning.

It is not clear why reversal learning was enhanced in the 3:1 group but not the 1.5:1 group. It is possible that the lack of effect in the 1.5:1 group was due to a ceiling effect. Indeed, matching was more accurate for the 1.5:1 group than the 3:1 group, which, instead, showed under-matching of relative response rates to relative reinforcement rates. Therefore, it is possible that the opportunity for *Grin1*$^{\Delta DGCA1}$ to enhance reversal learning is related to the degree of under-matching observed in control mice. Nevertheless, the precise conditions under which *Grin1*$^{\Delta DGCA1}$ enhances reversal learning remains to be determined.

Timing was examined using intervals that in comparison to other studies may be considered relatively short. The cue duration experiment examined 10 and 40 s durations. The peak procedure examined timing of 10 s. The average rate of reinforcement in the matching experiment, however, was some magnitudes larger, ranging from 120 to 360 s. The shortness of the intervals used in the cue duration and peak procedure experiments may be a factor in the reason for failing to find an effect of hippocampal *Grin1* knockout on timing behavior. Some studies have found that hippocampal manipulations affect temporally controlled behavior only when the durations are relatively long. For example, dorsal hippocampal lesions impaired trace fear conditioning after a 20 s trace interval, but not after 1 or 3 s (Chowdhury et al., 2005). Similarly, in an odor discrimination task, temporary inactivation impaired discrimination of long durations (8 vs. 12 min, but paradoxically enhanced discrimination of short (1 vs. 1.5 min) durations (Jacobs et al., 2013). It is possible that timing of the durations that we examined were not long enough to require the hippocampus. The role of the hippocampus in timing of relatively long durations, however, is not clear cut. Lin and Honey (2011) found that hippocampal lesions impaired a discrimination of trace appetitively conditioned cues when the trace interval was 10 s but not when it was 40 s. Therefore, although the hippocampus plays a role in temporally controlled behavior, it is not clear what timescale requires the hippocampus. At the very least, the lack of effect of hippocampal *Grin1* deletion on the cue duration and peak procedure experiments is in contrast to the effect of GluA1 deletion (Austen et al., 2021), a manipulation that selectively impairs hippocampus-dependent spatial working memory (Reisel et al., 2002; Schmitt et al., 2003) and hippocampus-dependent short-term habituation (Sanderson et al., 2009; Sanderson, Hindley, et al., 2011; Sanderson, Sprengel, et al., 2011).

The collective results fail to demonstrate a role of DG and CA1 NMDARs in temporal learning. Despite a lack of effect on timing, deletion of DG and CA1 NMDARs had a number of significant effects. It increased initial baseline levels of magazine entries, increased overall levels of lever pressing and, also, enhanced reversal learning of

lever pressing when the ratio of reinforcements for the high and low rewarded levers was 3:1. The lack of effect on timing behavior of NMDAR deletion in CA1 and DG may suggest that NMDAR-dependent plasticity in these subregions does not underlie representation of the temporal properties of events. This is in contrast to the suggested role of "time cells" that have been found in CA1 (e.g., MacDonald & Tonegawa, 2021) in encoding the temporal properties of remembered events. It is not clear whether "time cells" require NMDAR-dependent plasticity in order to show sensitivity to temporal information, but at the very least, the current results fail to provide evidence that behavioral expression of temporal learning requires NMDAR-dependent plasticity within CA1. Furthermore, in contrast to the effects of hippocampal lesions (Meck et al., 1984), the results failed to provide evidence that NMDARs in CA1 and DG underlie the encoding and retrieval of encoded durations or the speed of an internal clock that would be necessary for these processes.

The results are also in contrast to those demonstrating a role for hippocampal NMDAR-dependent plasticity in trace conditioning (Huerta et al., 2000; Quinn et al., 2005). The lack of effect of CA1 and DG NMDAR deletion on timing behavior suggests that the role of hippocampal NMDARs in trace conditioning is unlikely to reflect deficits in timing of the CS and reinforcement events. Therefore, trace conditioning may not be the result of timing per se (e.g., Gallistel & Gibbon, 2000), but the result of other factors that come into play when learning about discontiguous events. For example, trace conditioning likely reflects weakened excitatory learning through the development of inhibitory associations that may arise from associative and nonassociative mechanisms (Sanderson et al., 2017).

It is important to note that the current findings examining temporal learning are different from those found in spatial tasks in the water maze. Previous work with *Grin1*$^{\Delta DGCA1}$ mice suggests that CA1 and DG NMDARs are necessary for inhibiting incorrect spatial choices when mice are placed near to incorrect cues (Bannerman et al., 2012; Taylor et al., 2014). This effect is specific to inhibition rather than learning per se because *Grin1*$^{\Delta DGCA1}$ mice showed normal accuracy of responding when placed an equal distance from correct and incorrect spatial cues. Furthermore, the effect is specific to spatial cues, because *Grin1*$^{\Delta DGCA1}$ mice were able to inhibit responding to nonspatial cues. While we did find an increase in baseline responding in *Grin1*$^{\Delta DGCA1}$ mice, which may be consistent with a general form of disinhibition, discrimination learning and accuracy of responding was normal on the temporal procedures. Therefore, the role of hippocampal NMDARs in spatial choice behavior does not appear to extend to time-sensitive behavior. It is also important to note that Bannerman et al. (2012) suggested that hippocampal NMDA receptors were necessary for discriminating between ambiguous spatial cues, such as identical beacons distinguishable only by extra maze cues in the water maze task. Despite similarities with the matching procedure that we used, we failed to find an impairment and instead found a facilitatory effect of DG and CA1 NMDAR ablation. In the matching experiment, mice had to discriminate between identical levers that were distinguishable by their spatial location. *Grin1*$^{\Delta DGCA1}$ mice showed successful discrimination and enhanced reversal learning when the difference in reinforcement rate of the levers was 3:1. One possible explanation for the lack of effect that we observed may be that the spatial cues in the matching experiment were not allocentric but may have reflected egocentric encoding. While this may explain the lack of impairment it still does not explain the enhanced reversal learning. It is possible that reduced use of allocentric cues to inhibit responding to ambiguous cues in *Grin1*$^{\Delta DGCA1}$ mice may have facilitated reversal learning using other cues. Without identifying the precise cues used by mice to discriminate between the levers, however, it is not possible to make claims about the rate of learning or reversal learning with particular cues.

The three experiments used different procedures for assessing sensitivity to temporal information. The cue duration procedure assessed the effect of cue duration on learning and the strength of conditioned responding. The peak procedure assessed accuracy and precision of timing of conditioned responding. The matching procedure assessed the sensitivity of instrumental conditioned responding to differences in average reinforcement rates. *Grin1* deletion in the DG and CA1 failed to impair performance on these different procedures. While this may suggest that DG and CA1 NMDA receptors are not necessary for these forms of temporal learning we cannot rule out that effects of NMDAR deletion may be found with a wider range of temporal intervals or with other procedures for assessing aspects of timing or with other methods for manipulating NMDAR function. Future work will need to determine the temporal parameters and underlying psychological processes of the procedures that may require hippocampal NMDA receptors.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing financial interests.

DATA AVAILABILITY STATEMENT

The data are available upon request from the authors.

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