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The T-type calcium channel antagonist Z944 rescues impairments in crossmodal and visual recognition memory in Genetic Absence Epilepsy Rats from Strasbourg

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ABSTRACT

Childhood absence epilepsy (CAE) is often comorbid with behavioral and cognitive symptoms, including impaired visual memory. Genetic Absence Epilepsy Rats from Strasbourg (GAERS) is an animal model closely resembling CAE; however, cognition in GAERS is poorly understood. Crossmodal object recognition (CMOR) is a recently developed memory task that examines not only purely visual and tactile memory, but also requires rodents to integrate sensory information about objects gained from tactile exploration to enable visual recognition. Both the visual and crossmodal variations of the CMOR task rely on the perirhinal cortex, an area with dense expression of T-type calcium channels. GAERS express a gain-in-function missense mutation in the Cav3.2 T-type calcium channel gene. Therefore, we tested whether the T-type calcium channel blocker Z944 dose dependently (1, 3, 10 mg/kg; i.p.) altered CMOR memory in GAERS compared to the non-epileptic control (NEC) strain. GAERS demonstrated recognition memory deficits in the visual and crossmodal variations of the CMOR task that were reversed by the highest dose of Z944. Electroencephalogram recordings determined that deficits in CMOR memory in GAERS were not the result of seizures during task performance. In contrast, NEC showed a decrease in CMOR memory following Z944 treatment. These findings suggest that T-type calcium channels mediate CMOR in both the GAERS and NEC strains. Future research into the therapeutic potential of T-type calcium channel regulation may be particularly fruitful for the treatment of CAE and other disorders characterized by visual memory deficits.

1. Introduction

Childhood absence epilepsy (CAE) accounts for approximately 8% of all epilepsies in school-aged children and is characterized by a disruption in consciousness sometimes with mild clonic movements and automatisms (Pavone et al., 2001). Although CAE was originally considered a benign disorder (Dieterich et al., 1985), recent research has shown that children with absence epilepsy have co-morbid behavioral (Caplan et al., 2009) and cognitive (Caplan et al., 2009; Henkin et al., 2005; Killory et al., 2011; Loughman et al., 2014; Mandelbaum and Burack, 1997; Pavone et al., 2001) symptoms. In particular, visual skill and visual memory deficits are consistently observed in CAE (Nolan et al., 2004; Pavone et al., 2001; Siren et al., 2007). Genetic Absence Epilepsy Rats from Strasbourg (GAERS) is an animal model closely resembling CAE. GAERS not only demonstrate recurrent non-convulsive seizures with bilateral and synchronous spike-and-wave discharges (SWD) characteristic of CAE (Marescaux et al., 1992) but also the anxiety and psychiatric-like phenotypes associated with epilepsy (Bouilleret et al., 2009; Dezsi et al., 2013; Jones et al., 2008, 2010; Marks et al., 2016; Powell et al., 2014; but see also Marques-Carneiro et al., 2014). Similar to CAE, GAERS exhibit altered cognition with increased performance observed in fear conditioning and two-way active avoidance tests (Getova et al., 1997; Marks et al., 2016), performance deficits observed for latent inhibition and extinction of conditioned fear (Marks et al., 2016), and delayed acquisition of spatial reference and working memory in a Morris water maze (Marques-Carneiro et al., 2016). Although progress has been made in characterizing cognitive alterations in GAERS, previous research has observed behavioral alterations using aversive Pavlovian and operant conditioning or stress-inducing tasks. Further, the mechanisms mediating alterations in cognition and behavior in CAE models are poorly understood.

Spontaneous object recognition tasks rely on the natural curiosity of rodents to explore novelty and are advantageous in that they measure learning and memory without prior training and are non-aversive (Cazakoff et al., 2010; Dere et al., 2007; Winters et al., 2008).

Abbreviation: CAE, childhood absence epilepsy; CMOR, crossmodal object recognition; DR, discrimination ratio; GAERS, Genetic Absence Epilepsy Rats from Strasbourg; NEC, non-epileptic control; PRh, perirhinal cortex; SWD, spike-and-wave discharge.

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Crossmodal object recognition (CMOR) is a recently developed memory task that examines not only purely visual and tactile memory, but also requires rodents to integrate sensory information about objects gained from tactile exploration to enable visual recognition (Ballendine et al., 2015; Winters and Reid, 2010; Reid et al., 2014). Both visual learning and memory deficits and sensory integration deficits are observed in CAE and psychiatric disorders that are highly comorbid with epilepsy (Heinrichs and Zakzanis, 1998; Stone et al., 2011; Williams et al., 2010; Wood et al., 2002). Given the specific learning and memory deficits observed in humans with epilepsy, examining CMOR performance in GAERS will broaden our understanding of the cognitive alterations associated with this absence epilepsy model.

In GAERS, a gain-of-function missense mutation in the Cav3.2 T-type calcium channel gene contributes to seizure activity (Powell et al., 2009). Further, administration of the pan-T-type calcium channel blocker, Z944, attenuates absence seizures in GAERS (Tringham et al., 2012). Previous research has shown that Cav3.2 T-type calcium channel deficient mice are impaired on novel and spatial object recognition tasks although working memory was unaffected (Gangarossa et al., 2014). Lesions of the perirhinal cortex (PRh) and posterior parietal cortex also disrupt CMOR with the PRh particularly relevant to visual recognition memory (Winters and Reid, 2010). Given that T-type calcium channels are expressed throughout the cerebral cortex with particularly dense expression in the PRh (Talley et al., 1999), investigating the role of T-type channels in mediating crossmodal recognition memory performance is warranted. We have recently demonstrated that Z944 administration has a profound effect on prepulse inhibition, a cognitive test that measures sensorimotor gating, in GAERS, NEC, and Wistar rats (Marks et al., in press). Taking into consideration the robust effects of Z944 on behavior, the objective of the present study was to examine the dose-dependent effect of Z944 on CMOR performance in GAERS and NEC rats. Experiments confirmed a deficit in both visual and crossmodal memory performance in GAERS compared to NECs. As anticipated, visual and crossmodal memory performance in GAERS was rescued through blockade of T-type calcium channels by Z944.

2. Materials and methods

2.1. Animals

Male and female GAERS and NEC (University of Saskatchewan Lab Animal Services Unit, Saskatoon, Canada) (Marks et al., 2016) were used. All rats were group housed (2 or 3 per cage) in standard polypropylene cages in a temperature controlled (21 °C) colony room on a 12/12 h light/dark cycle. Experimental procedures were carried out during the light phase (lights on at 07:00 h), and food (Purina Rat Chow) and water were available *ad libitum*. This work was approved by the University of Saskatchewan's Animal Research Ethics Board and adhered to the Canadian Council on Animal Care guidelines for humane animal use.

2.2. Electroencephalogram (EEG) recordings

To confirm the presence and absence of SWDs in adult GAERS and NEC animals prior to, and during the sample and test phases of crossmodal testing, EEG recordings were performed in freely moving rats. Two month old, male NEC ($n = 3$) and GAERS ($n = 4$) were implanted with a recording electrode skull screw (bregma, lateral from midline, depth from skull in mm) into the somatosensory cortex (S1Cx; AP +0.5 mm, ML 4.0, DV −1.2) and a reference electrode skull screw into the occipital cortex (−6.0, 5.0, −1.2) under isoflurane anesthesia. Electrodes were connected to a custom EEG interface fitted to the head and used to analyze SWD activity in NEC and GAERS. Following a surgical recovery period, freely-moving EEG recordings were performed using a wireless headstage and receiver (Multichannel Systems) during a 20 min habituation session prior to behavioral testing, a 3 min session during the sample phase, and a 2 min session during the test phase of the crossmodal test. SWDs were analyzed semi-automatically using a custom Matlab script developed by Dr. Stuart Cain and Jeff LeDue at the University of British Columbia.

2.3. Drug and drug preparation

The synthesis and initial characterization of Z944 is reported in Tringham et al. (2012). Z944 is a small organic molecule derived from the piperazine-based compound NP118809, a high affinity N-type Ca^{2+} channel blocker. *In vitro* assays indicate that Z944 inhibition of hCav3.1, hCav3.2, and hCav3.3 T-type channels to be submicromolar (IC_{50} values = 50 to 160 nM), 50–600 times higher than its affinity for N- and L-type Ca^{2+} channels, hERG potassium channel, and the cardiac $Na_V1.5$ sodium channel (Tringham et al., 2012).

Z944 was prepared fresh daily in a 0.2 mg/ml, 0.6 mg/ml, or 2 mg/ml solution of 10% dimethyl sulfoxide (DMSO; Sigma Aldrich, St. Louis, MO) and 90% sodium carboxymethyl cellulose (0.5% in saline, Sigma Aldrich). Z944 was administered intraperitoneally at a volume of 5 ml/kg to yield doses of 1 mg/kg, 3 mg/kg, or 10 mg/kg. Previous research has demonstrated significant blockade of T-type calcium channels without altering the state of alertness in the 10 mg/kg dose (Tringham et al., 2012) which provided the basis for the highest dose of Z944 used. Z944 or vehicle was administered 15 min prior to the sample phase of the crossmodal, visual, or tactile test.

2.4. Behavioral testing procedures

Rats were handled in small groups of 2 or 3 for 5 min/day at least 3 times before testing began. Testing equipment was cleaned with 70% ethanol between all trials. Drug naïve rats were initially run on the tactile (12 GAERS, 12 NEC), visual (12 GAERS, 12 NEC), and CMOR tests (12 GAERS, 12 NEC). Following these initial experiments, tests with Z944 treatment during CMOR memory were carried out using 54 GAERS (12 rats per dose, 18 vehicle injected rats) and 53 NECs (12 rats per dose, 17 vehicle injected rats). All rats that received Z944 treatment during the CMOR test also first received tactile and visual tests. An extra set of rats (13 GAERS, 13 NEC) received a short delay (5 min *vs.* 1 h) version of the visual test. Additional rats were then used to assess the effects of Z944 (10 mg/kg) on the tactile test (9 GAERS, 9 NEC) and visual test (13 GAERS: 11 treated with drug, 2 treated with vehicle; 13 NEC: 12 treated with Z944, 1 treated with vehicle). Rats that received Z944 treatment during the tactile or visual tests did not receive the other tests.

2.5. Visual, tactile, and crossmodal recognition memory

Recognition memory testing procedures were adapted from previously published protocols (Ballendine et al., 2015; Winters and Reid, 2010). The testing apparatus consisted of a Y-shaped box with one entrance arm and two object arms (10 × 27 cm) (Fig. 1). Object recognition testing included three distinct tests: the tactile, visual, and crossmodal memory tests (Fig. 1A,B,C). Tactile exploration was conducted in red light illumination that prevented the rats from seeing the objects, but allowed recording of the rats' behavior *via* an overhead video camera. During visual exploration, transparent plastic barriers were inserted in front of the objects to prevent tactile exploration. Three habituation sessions (10 min) occurred prior to testing. The first two habituation sessions were paired whereas the last one occurred individually. White and red overhead illuminations were separately presented for half of each habituation session, with the order of illumination counterbalanced.

Testing began one day after the last habituation session in the following order: tactile, then visual, with CMOR testing on the last day. Each test included a 3 min sample phase and 2 min test phase with a 1 h delay between phases with the exception of the short delay visual test where a 5 min delay occurred between the sample and test phases.

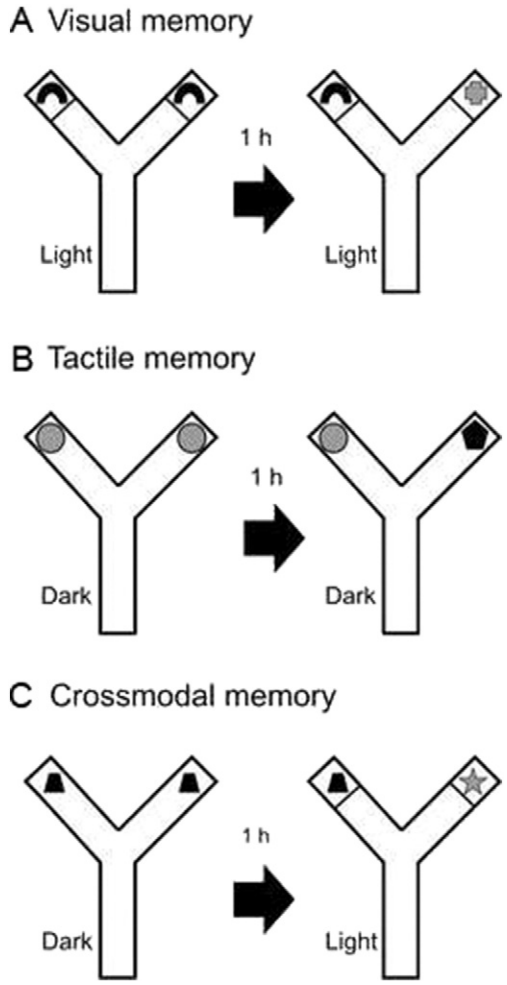

Fig. 1. Schematic overhead view of the three distinct crossmodal object recognition tests, visual test (A), tactile test (B), and crossmodal test (C).

During the sample phase, the maze contained two identical copies of an object at the end of each of the exploration arms. Objects were constructed of glass, plastic, or porcelain and were all similar in size (~10 cm in height and length). During the test phase, the maze contained a third copy of the sample phase object with a novel object in the opposing arm, and presentation of novel and familiar objects was counterbalanced between arms of the maze. Between trials, the maze and objects were wiped with 70% EtOH. Object exploration was scored by a trained experimenter blind to the treatment status of the rat and the designation of the object as either novel or familiar for a given trial. Significantly greater tactile exploration of a novel object when it was paired with a familiar object that had previously been touched (but not seen) during a sample trial is referred to as tactile recognition memory (Fig. 1B). Significantly greater time spent looking at a novel object when it was paired with a familiar object that had only been seen (but not touched) during a sample trial is considered visual recognition memory (Fig. 1A). CMOR memory refers to significantly greater time looking at a novel object that was paired with an object that had been touched, but not seen, in the opposing arm (Fig. 1C).

2.6. Data analysis

The data were analyzed using the Statistical Package for the Social Sciences version 20 for Windows (IBM). Statistical significance for all comparisons was set at $p \leq 0.05$. All results are reported as group means ± standard error of the mean (SEM). Corrections were made for violations of homogeneity of variance (Levene's Test). All analyses were initially run with Sex as a factor. However, the main effect of Sex and all interactions of Sex with Strain or Treatment were non-significant (all $p \geq 0.10$), therefore the sexes were combined for analyses. Univariate analysis of variance (ANOVA) with Strain and Z944 treatment as between-subjects factors were predominantly used for analysis. Where appropriate, independent samples *t*-tests were used with Strain or Treatment as the between-subjects factor. *Post hoc* analyses were performed using Dunnett's test and Bonferroni corrections. One sample *t*-tests comparing to a mean of 0 were used to determine whether novel object exploration was significantly above chance levels. Tactile exploration was scored when a rat actively explored an object with its nose directed within 2 cm of the object and its head or vibrissae moving, but not if the rat was standing on top of the object or not directing attention towards it. Visual exploration was scored when a rat gazed in the direction of an object within 2 cm of the transparent plastic barrier (Ballendine et al., 2015; Winters and Reid, 2010). Analyses of object exploration during the sample phase of each task were run on object exploration over the entire 3 min. Memory was quantified using a discrimination ratio (DR), which was calculated as the time spent exploring (novel − familiar) / (novel + familiar) objects (Ballendine et al., 2015; Cazakoff and Howland, 2011; Howland et al., 2012). Separate analyses were run for min 1 (DR1) and min 1 + 2 (DR1 + 2) of the test phase as most object exploration occurs during the first minute of testing.

3. Results

3.1. Characterization of absence seizures during crossmodal testing

GAERS and NECs were first assessed for SWDs with freely moving EEG similar to previous studies (Marks et al., 2016; Powell et al., 2014). During a 20 min habituation period in the Y-shaped box prior to the crossmodal sample phase, GAERS displayed spontaneous SWDs resembling those described previously (Marescaux et al., 1992; Marks et al., 2016; Powell et al., 2009, 2014). NECs did not display absence seizures or SWD activity (Fig. 2). SWD were observed during 15.2 ± 2.3% of the recording period in GAERS, occurring at 0.9 ± 0.1 seizures per minute, with a mean duration of 10.1 ± 1.1 s. SWDs displayed a spike frequency of 6.6 ± 0.1 Hz. No SWDs were observed in GAERS or NECs during either the sample or test phase of COMR testing.

3.2. Drug naïve trials

Analysis of sample phase exploration times (Table 1) revealed significant differences between NEC and GAERS animals on the tactile ($t(22) = 3.99$, $p = 0.001$) and CMOR ($t(22) = 4.89$, $p < 0.001$) tests. For both of these tests, GAERS had significantly decreased exploration times during tactile phases. A significant strain difference in test phase exploration time (Table 1) was also found for the tactile test ($t(22) = 5.40$, $p < 0.001$) with GAERS exploring objects less than NECs.

Analysis of DR1 and DR1 + 2 of the tactile, visual, and CMOR memory tests (Fig. 3A,B) showed significant strain differences on both tactile recognition memory, DR1 ($t(13.31) = 3.23$, $p = 0.006$), and CMOR memory, DR1 ($t(22) = 2.13$, $p = 0.045$) and DR1 + 2 ($t(22) = 2.51$, $p = 0.020$). Although GAERS showed significantly lower tactile recognition memory, further analyses using one sample *t*-tests comparing DRs to a mean of 0 showed that both GAERS and NEC had significant memory above chance for both DR1 and DR1 + 2 (all $p \leq 0.002$). For CMOR memory, only NECs showed significant novel object exploration for both DR1 and DR1 + 2 in the CMOR test (both $p \leq 0.019$). Although significant strain differences were not found for visual recognition memory, GAERS only showed significant memory above chance levels for DR1 ($t(11) = 2.21$, $p = 0.050$). NECs showed significant visual recognition memory above chance for both DR1 and DR1 + 2 (both $p \leq 0.005$).

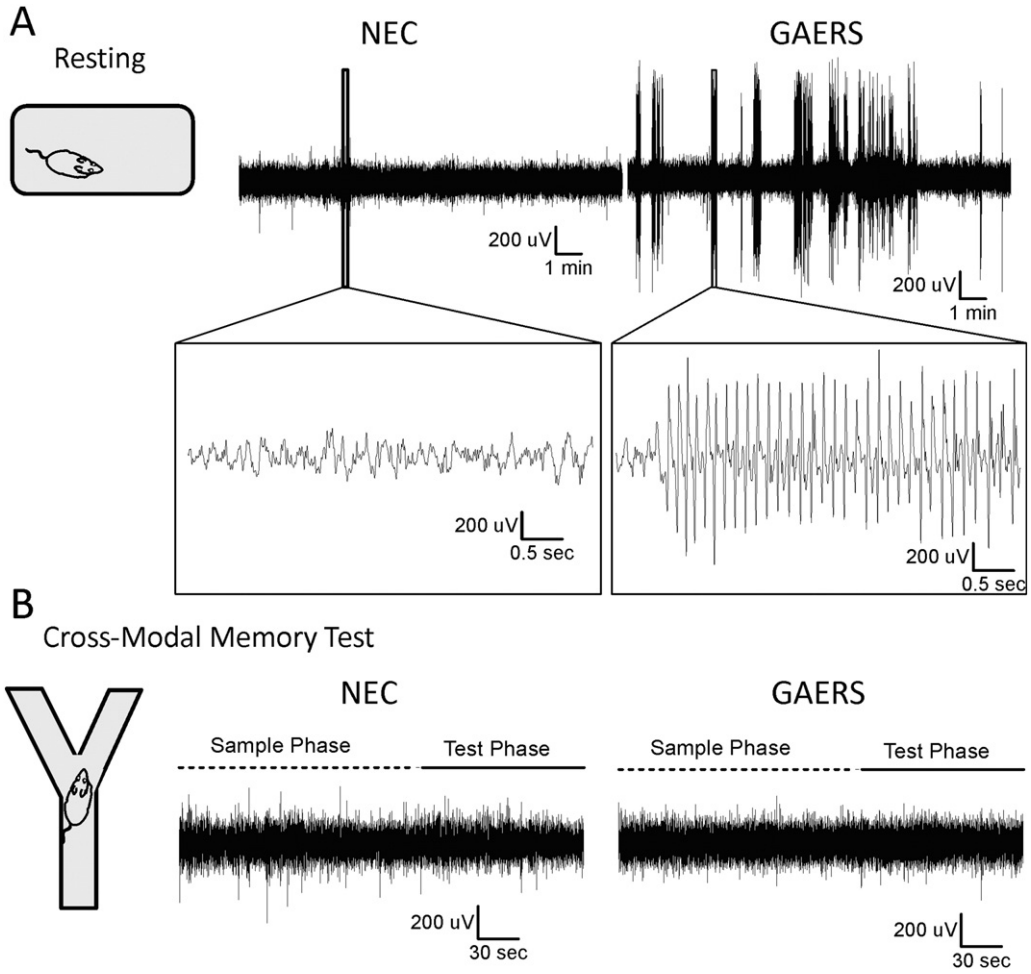

Fig. 2. EEG analysis of seizures during rest and crossmodal memory test. Freely-moving EEG analysis was performed on NEC ($n = 3$) and GAERS ($n = 4$) using wireless telemetry. (A, B) Representative EEG traces are shown for recording sessions performed at rest (A) and also during the sample and test phases of the crossmodal memory test (B). A complete summary of seizure data before and during the trials is presented in the Results section.

3.3. Z944 trials

3.3.1. Crossmodal recognition memory with Z944 treatment

Analysis of sample phase exploration (Table 2) revealed significant main effects of Strain ($F(1,99) = 30.14$, $p < 0.001$) and Treatment ($F(3,99) = 50.07$, $p < 0.001$), as well as a significant Strain by Treatment interaction ($F(3,99) = 4.90$, $p = 0.003$) for the CMOR test. *Post hoc* analysis of the data revealed a significant decrease in exploration time in GAERS relative to NECs during the sample phase, but only for the vehicle ($t(33) = 6.19$, $p < 0.001$) and 10 mg/kg Z944 ($t(22) = 5.50$, $p < 0.001$) treatment conditions. *Post hoc* tests further revealed that, relative to vehicle treatment, all doses of Z944 decreased sample phase exploration in the NEC strain (all $p < 0.001$); whereas, only the 3 mg/kg ($p = 0.003$) and 10 mg/kg ($p < 0.001$) doses significantly decreased sample phase exploration time relative to vehicle treatment in GAERS. When the test phase exploration time was considered (Table 2), a significant main effect of Treatment was observed ($F(3,99) = 7.78$, $p < 0.001$). *Post hoc* analyses revealed that only the 10 mg/kg dose of Z944 produced significantly lower test phase exploration times in the CMOR test in both GAERS and NEC ($p < 0.001$). The Strain by Treatment interaction for test phase object exploration time was not significant (DRs shown in Fig. 4).

Analysis of DR1 and DR1 + 2 of the CMOR test phase exploration with Z944 treatment (Fig. 4A,B) revealed non-significant main effects of Strain and Treatment for both time intervals. However, a significant Strain by Treatment interaction was found for DR1 ($F(3,99) = 3.01$, $p = 0.034$). Planned *a priori post hoc* analyses of the vehicle treatment condition revealed a significant strain difference in CMOR memory for DR1 ($t(33) = 2.40$, $p = 0.022$), where NEC rats explored novel objects significantly more than GAERS. Non-significant strain differences were found for all other treatment conditions. Further *post hoc* analyses revealed that CMOR memory was not significantly affected by the 1 and 3 mg/kg doses of Z944 in both the GAERS and NEC strains. Planned *a priori post hoc* analyses comparing vehicle to 10 mg/kg Z944 treatment

Table 1
Total exploration time of the objects (s ± SEM) during the sample and test phase of the tactile, visual, visual short delay, and crossmodal recognition memory tests. The total time for both the sample and test phases are presented. GAERS showed significantly decreased exploration times for both the sample and test phase of the tactile memory test (*$p < 0.05$). Significantly decreased exploration time was also observed in GAERS during the sample phase of the crossmodal memory test (*$p < 0.05$).

Strain	Test phase	Tactile	Visual	Visual short delay	Crossmodal
NEC	Sample	66.66 ± 2.46	8.25 ± 1.19	6.64 ± 1.04	66.31 ± 4.05
	Test	50.70 ± 3.38	5.51 ± 1.13	7.48 ± 1.97	5.03 ± 0.61
GAERS	Sample	42.93 ± 5.41*	6.54 ± 0.77	5.29 ± 0.73	39.62 ± 3.67*
	Test	27.64 ± 2.61*	3.71 ± 0.37	4.06 ± 0.45	4.56 ± 0.50

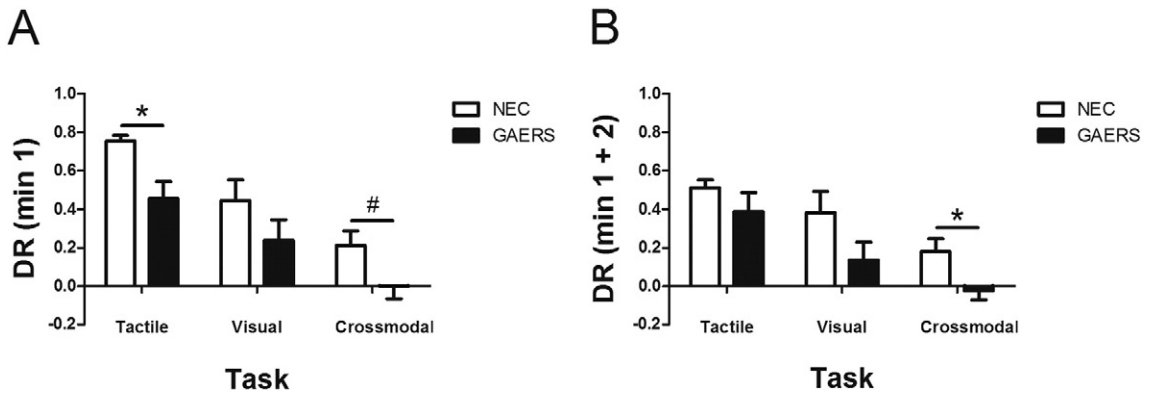

Fig. 3. Tactile, visual, and crossmodal recognition memory discrimination ratios (DR) for minute 1 (DR1; A) and minute 1 plus 2 combined (DR1 + 2; B) for drug naïve GAERS and NECs. GAERS showed significantly reduced tactile (*$p < 0.05$; A) recognition memory for DR1. GAERS also demonstrated significantly reduced crossmodal recognition memory for DR1 (#$p < 0.05$; A) and DR1 + 2 (*$p < 0.05$; B).

within strains revealed a significant treatment effect only in GAERS for both DR1 ($t(28) = -2.71$, $p = 0.011$) and DR1 + 2 ($t(28) = -2.13$, $p = 0.043$). Analyses using one sample *t*-tests comparing DR1 and DR1 + 2 to a mean of 0 found that in the NEC strain, only animals in the vehicle treatment condition showed significant memory compared to chance, DR1 ($t(16) = 2.65$, $p = 0.017$), and DR1 + 2 ($t(16) = 2.98$, $p = 0.009$). In the GAERS strain, rats treated with both 3 mg/kg Z944, DR1 + 2 ($t(11) = 2.28$, $p = 0.043$), and 10 mg/kg Z944, DR1 ($t(11) = 2.50$, $p = 0.030$) and DR1 + 2 ($t(11) = 2.70$, $p = 0.021$), showed significant memory when compared to chance performance.

All animals in the Z944 CMOR received drug naïve tactile and visual tests prior to the crossmodal trials (Fig. 4C,D). Analysis of these data revealed some similar and some unique trends to the initial drug naïve trials. During the tactile sample phase (Table 3), GAERS showed reduced object exploration ($t(106) = 9.31$, $p < 0.001$). In contrast to initial drug naïve trials, analysis of visual sample phase object exploration times (Table 3) revealed a significant difference in exploration between the GAERS and NEC strains ($t(108) = 3.98$, $p < 0.001$) with decreased exploration observed in GAERS. These patterns of exploration continued into the test phase for both the tactile ($t(96.64) = 7.49$, $p < 0.001$) and visual ($t(85.81) = 2.92$, $p = 0.004$) tests with decreased exploration observed in GAERS (Table 3). A strain difference in tactile recognition memory (Fig. 4C,D) was observed for this group, DR1 ($t(106) = 2.04$, $p = 0.044$). GAERS displayed significantly decreased tactile recognition memory relative to NECs. Interestingly, a strain difference in visual recognition memory was also observed, DR1 ($t(108) = 3.74$, $p < 0.001$), DR1 + 2 ($t(108) = 3.32$, $p = 0.001$), whereby GAERS showed a significant decrease in visual recognition memory. One sample *t*-tests revealed significant tactile recognition memory above chance levels in both the GAERS and NEC strains for DR1 and DR1 + 2 (all $p \leq 0.001$). Significant visual recognition memory above chance was only observed in NECs, both DR1 and DR1 + 2 ($p \leq 0.001$).

In light of the significantly decreased memory performance observed in GAERS for visually dependent CMOR and visual test phases, a short delay visual test was run to determine whether poor performance was possibly the result of a visual sensory deficit in GAERS. Analysis of sample and test phase object exploration for the short delay visual test (Table 3) revealed non-significant differences between the GAERS and NEC strains. When DR1 and DR1 + 2 for the short delay visual object recognition test was analyzed, non-significant differences between the GAERS and NEC strains were observed (Fig. 4C,D). When performance was compared to chance levels, both NEC and GAERS showed significant short delay visual recognition memory for both the DR1 and DR1 + 2 time intervals (all $p \leq 0.021$).

3.3.2. Tactile recognition memory with Z944 treatment

As the 10 mg/kg dose of Z944 had the most profound effect for the CMOR test, only this dose was used for further pharmacology experiments. A significant main effect of Strain ($F(1,122) = 23.58$, $p < 0.001$), Treatment ($F(1,122) = 72.39$, $p < 0.001$), and a significant Strain by Treatment interaction ($F(1,122) = 6.74$, $p = 0.011$) was found for tactile sample phase exploration (Table 3). Further analysis of the data revealed significantly lower exploration times in GAERS rats for both no drug ($t(106) = 9.31$, $p < 0.001$) and 10 mg/kg Z944 ($t(16) = 2.73$, $p = 0.015$) treatment conditions. *Post hoc* tests also revealed that within both the NEC and GAERS strains, Z944 treated rats had significantly decreased tactile sample phase exploration times, ($t(60) = 8.87$, $p < 0.001$) and ($t(33.79) = 7.18$, $p < 0.001$) respectively. When test phase exploration was considered, a significant main effect of Strain ($F(1,122) = 18.20$, $p < 0.001$) and Treatment ($F(1,122) = 20.09$, $p \leq 0.001$) was observed (Table 3). GAERS showed significantly reduced object exploration, and 10 mg/kg Z944 also significantly reduced exploration time. The Strain by Treatment interaction for test phase object exploration time was non-significant (Fig. 5A,B).

Exploration of tactile object recognition memory (Fig. 5A,B) revealed a significant main effect of Treatment ($F(1,122) = 7.16$, $p = 0.008$) for the DR1 + 2 time interval. Rats administered 10 mg/kg Z944 showed increased tactile recognition memory relative to non-treated rats. All

Table 2
Total exploration time of the objects (s ± SEM) during the sample and test phases of the crossmodal recognition memory test with Z944 treatment (vehicle, 1 mg/kg, 3 mg/kg, and 10 mg/kg). The total time for both the sample and test phases are presented. A significant decrease in sample phase exploration was found for GAERS, but only for the vehicle and 10 mg/kg dose of Z944 (*$p < 0.05$). All doses of Z944 decreased sample phase exploration in NEC relative to vehicle treated NECs (#$p < 0.05$). However, only GAERS treated with the 3 and 10 mg/kg dose of Z944 showed decreased sample phase exploration relative to vehicle treated GAERS (&$p < 0.05$). In both strains, 10 mg/kg Z944 significantly reduced object exploration during the test phase (**$p < 0.05$).

Strain	Test phase	Vehicle	1 mg/kg Z944	3 mg/kg Z944	10 mg/kg Z944
NEC	Sample	63.72 ± 2.2	41.88 ± 3.29#	38.73 ± 4.10#	28.77 ± 2.06#
	Test	6.43 ± 0.51	5.85 ± 0.70	5.53 ± 0.59	3.69 ± 0.29**
GAERS	Sample	43.33 ± 2.41*	40.75 ± 3.41	31.18 ± 2.27&	14.97 ± 1.43*&
	Test	5.12 ± 0.54	6.62 ± 0.57	5.00 ± 0.46	3.76 ± 0.42**

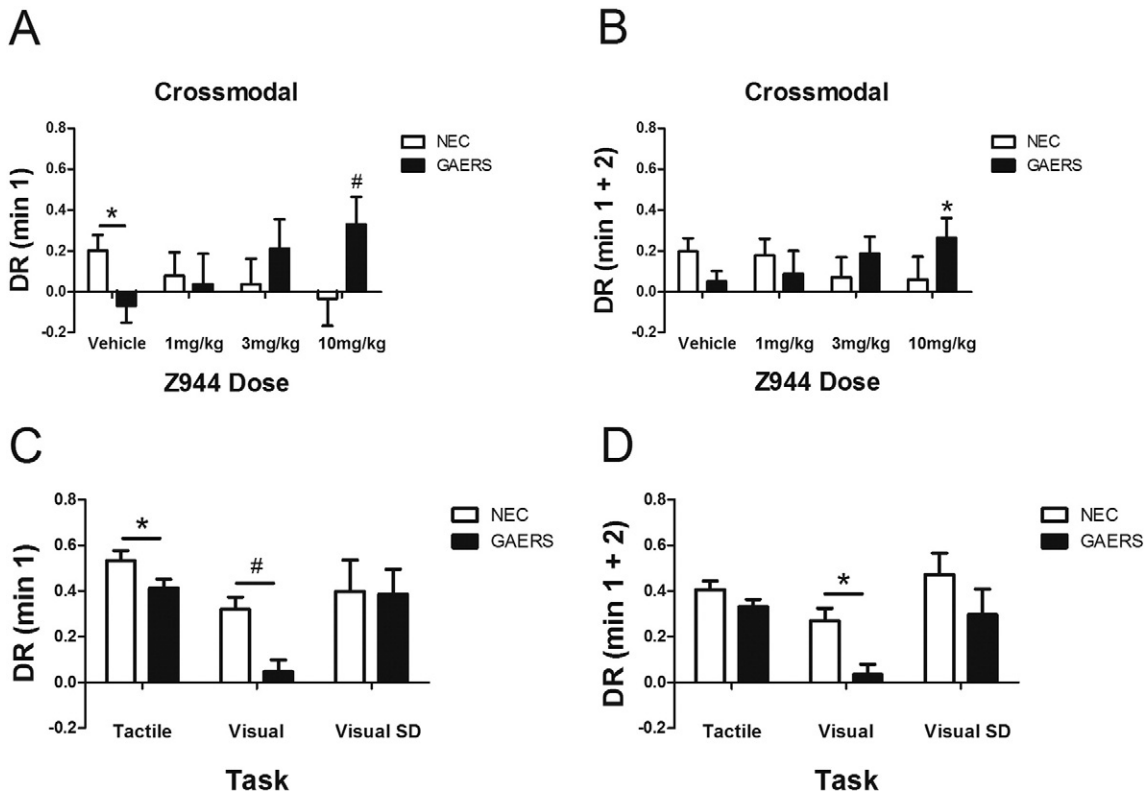

Fig. 4. Crossmodal recognition memory discrimination ratios (DR) for minute 1 (DR1; A) and minute 1 plus 2 combined (DR1 + 2; B) for vehicle and Z944 treated (1, 3, and 10 mg/kg) GAERS and NECs. GAERS show significantly reduced crossmodal recognition memory in vehicle treated rats for DR1 (*$p < 0.05$; A). GAERS treated with 10 mg/kg Z944 show significantly increased crossmodal recognition memory compared to vehicle treated rats for DR1 (#$p < 0.05$; A) and DR1 + 2 (*$p < 0.05$; B). Tactile, visual and visual short delay (SD) DR1 (C) and DR1 + 2 (D) for drug naïve trials prior to crossmodal testing for GAERS and NECs. GAERS showed significantly reduced tactile recognition memory for DR1 (*$p < 0.05$; C). GAERS showed significantly reduced visual recognition memory for both DR1 (#$p < 0.05$; C) and DR1 + 2 (*$p < 0.05$; D).

other main effects and interactions were non-significant. Analyses comparing tactile exploration DR1 and DR1 + 2 to chance levels revealed that all rats showed significant tactile exploration of the objects (all $p \leq 0.001$), with the exception of GAERS treated with 10 mg/kg Z944 for DR1 only.

Table 3
Total exploration time of the objects (s ± SEM) during the sample and test phases of the tactile and visual memory test with 10 mg/kg Z944 treatment. The total time for both the sample and test phases are presented. In the tactile memory test, GAERS showed significantly lower exploration time during the sample phase (*$p < 0.05$). Z944 treatment significantly reduced sample phase exploration time across both the NEC and GAERS strains (#$p < 0.05$). Overall GAERS showed significantly reduced exploration during the tactile test phase relative to NECs (&$p < 0.05$). Z944 treatment significantly reduced tactile test phase exploration in both strains (**$p < 0.05$). For the visual memory test, sample phase exploration was significantly reduced by Z944 treatment in both strains (^$p < 0.05$). In the no drug treatment group, GAERS showed decreased exploration during the sample phase relative to NECs (^^$p < 0.05$). GAERS and NECs treated with Z944 showed decreased exploration during visual test phase exploration ($$p < 0.05$).

Strain	Group	Tactile	Visual
NEC	**No drug**		
	Sample	73.21 ± 1.84	10.13 ± 0.60
	Test	42.85 ± 1.62	6.5 ± 0.52
	10 mg/kg Z944		
	Sample	32.56 ± 2.29#	2.98 ± 0.56^
	Test	27.25 ± 3.08**	4.15 ± 0.57$
GAERS	**No drug**		
	Sample	45.93 ± 2.27*	7.11 ± 0.46^^
	Test	27.80 ± 1.20&	4.77 ± 0.30
	10 mg/kg Z944		
	Sample	24.29 ± 1.99*#	3.53 ± 1.13^
	Test	20.58 ± 0.98&**	4.22 ± 0.82$

3.3.3. Visual recognition memory with Z944 treatment

Analysis of visual sample phase exploration (Table 3) revealed a significant main effect of Treatment ($F(1,129) = 37.20$, $p < 0.001$) and a significant Strain by Treatment interaction ($F(1,129) = 4.13$, $p = 0.044$) on exploration time; whereas the main effect of Strain was non-significant. Similar to the crossmodal and tactile tests, *post hoc* tests revealed visual sample phase object exploration time was significantly decreased by 10 mg/kg Z944 treatment across both strains (all $p \leq 0.012$). *Post hoc* tests also revealed significantly reduced sample phase exploration in drug naïve GAERS relative to drug naïve NEC animals ($t(108) = 3.98$, $p < 0.001$). A significant main effect of Treatment was found for test phase exploration ($F(1,129) = 4.47$, $p = 0.036$) where 10 mg/kg Z944 treatment also produced decreased exploration across both strains. All Strain and Strain by Treatment interactions were non-significant (Fig. 5C,D).

Significant Strain by Treatment interactions were observed for both DR1 ($F(1,129) = 12.25$, $p = 0.001$) and DR1 + 2 ($F(1,129) = 5.58$, $p = 0.020$) of the visual object recognition memory test (Fig. 5C,D). *Post hoc* analyses revealed that GAERS showed significant decreases in object recognition memory only in the no drug treatment condition, DR1 ($t(108) = 3.32$, $p = 0.001$), and DR1 + 2 ($t(108) = 3.98$, $p < 0.001$). *Post hoc* tests also revealed that GAERS treated with 10 mg/kg Z944 showed a significant increase in visual test phase object exploration during the first minute relative to non-treated rats' DR1 ($t(65) = -2.59$, $p = 0.012$). One sample *t*-test analyses revealed that NECs show significant memory over chance performance for the visual test at all DR time intervals (both $p < 0.001$); whereas NECs treated with 10 mg/kg Z944 did not show significant memory over chance. For the GAERS strain, significant object exploration above chance was observed for rats treated with 10 mg/kg Z944, DR1 ($t(10) = 2.63$, $p = 0.025$.

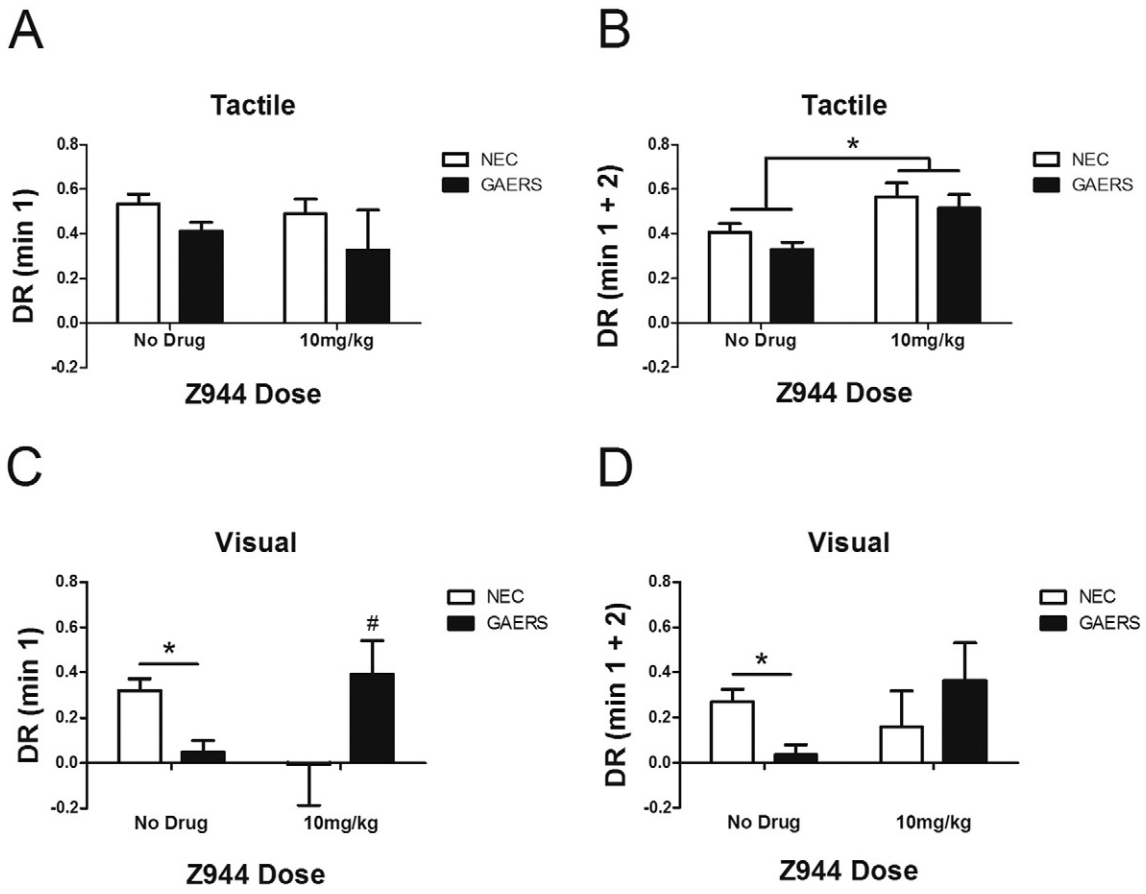

Fig. 5. Tactile (A,B) and visual (C,D) recognition memory discrimination ratios (DR) for minute 1 (DR1) and minute 1 plus 2 combined (DR1 + 2) for treatment naïve (no drug) and 10 mg/kg Z944 treated GAERS and NECs. For tactile recognition memory, rats treated with 10 mg/kg Z944 showed significantly increased memory relative to drug naïve rats for DR1 + 2 (*$p < 0.05$; B). Treatment naïve GAERS show significantly decreased visual recognition memory for both DR1 (*$p < 0.05$; C) and DR1 + 2 (*$p < 0.05$; D). GAERS treated with 10 mg/kg Z944 show significantly increased visual recognition memory relative to treatment naïve GAERS for DR1 (#$p < 0.05$; C).

However, GAERS in the no drug treatment condition did not show visual recognition memory.

4. Discussion

In a series of experiments, we characterized the effects of acute systemic treatment with the T-type calcium channel blocker, Z944, on CMOR memory performance in GAERS and NEC rats. Recognition memory deficits in the tactile, visual and CMOR tests were observed in drug naïve GAERS relative to NECs, although the deficit in tactile recognition memory was less pronounced (Figs. 3, 4). In GAERS, Z944 had a robust dose-dependent effect on crossmodal test performance with a significant reversal of recognition memory deficits at the 10 mg/kg dose (Fig. 4). Of further note, the 10 mg/kg dose of Z944 also completely reversed the visual recognition memory deficits observed in GAERS (Fig. 5). Z944 also decreased both crossmodal and visual recognition memory performance in the NEC strain such that NECs no longer performed above chance levels (Figs. 4, 5).

4.1. CMOR memory deficits in drug naïve GAERS

The present study shows that GAERS are impaired in cognitive tasks that do not involve aversive stimuli. Of particular relevance, interpretation of the results from the present study are less confounded by the enhanced anxiety in GAERS produced by aversive tasks (see Marks et al., 2016; Marques-Carneiro et al., 2016), and therefore provide an important contribution to the characterization of the behavioral phenotype associated with the GAERS model of CAE. Overall, tactile and visual recognition memory deficits were not as consistent and robust as the deficits observed for the CMOR test. The CMOR test is likely the most challenging as the NEC animals showed the lowest DRs for this task, a pattern observed previously in our laboratory when testing rats on these tests (Ballendine et al., 2015). Thus, CMOR may be more sensitive for detecting recognition memory deficits in GAERS. It is also possible that GAERS are more impaired on tasks that depend on the PRh as the crossmodal and visual tests are PRh dependent (Winters and Reid, 2010) and GAERS have altered PRh functioning (Akman et al., 2010). Previous studies have shown that the thalamus may act as a critical relay between the PRh and other areas such as the frontal lobes to control visual object recognition memory (Warburton and Brown, 2015). Indeed, in monkeys, mediodorsal thalamic lesions produced severe deficits in visual object recognition memory (Parker and Gaffan, 1998), thus highlighting the importance of thalamic regions in these tasks. GAERS have documented alterations in thalamic protein expression, as well as heightened excitability and enhanced glucose metabolism in thalamic regions (Danis et al., 2011; Dufour et al., 2003; Toth et al., 2007). Future research should focus on examining the network systems responsible for object recognition deficits in GAERS.

Although not always statistically significant, GAERS displayed a consistent decrease in object exploration during the sample and test phases of all tasks that was especially pronounced during tactile exploration (Tables 1, 2, 3). These results mirror previous findings where GAERS showed significantly decreased distance travelled in an open field (Bouilleret et al., 2009; Dezsi et al., 2013; Jones et al., 2008; Marks et al., 2016; Powell et al., 2014). Overall, these data suggest that GAERS generally display reduced locomotor activity relative to NECs. It is important to note that reduced object exploration does not necessarily translate to a reduction in recognition memory in GAERS. Despite

displaying a significant reduction in object exploration relative to NECs in the tactile sample phase, GAERS still displayed significant memory during the test phase. It is also important to note that the reduction of object exploration observed in GAERS during the sample and test phases are not the result of seizure activity during task performance as EEG recordings revealed that GAERS did not display SWDs while performing the task. A possible explanation for the reduction in activity observed in GAERS is that they were more anxious during task performance and thus displayed less overall movement. Indeed, our lab and others have demonstrated that GAERS display an anxiety-like phenotype in the elevated plus maze (Jones et al., 2008; Marks et al., 2016; Powell et al., 2014) open field (Bouilleret et al., 2009; Dezsi et al., 2013; Jones et al., 2008; Powell et al., 2014), and in response to startling acoustic stimuli (Marks et al., in press; Jones et al., 2010) compared to NEC.

4.2. Z944 rescues visual and CMOR memory deficits in GAERS

To the best of our knowledge, the present report is the first to demonstrate a robust reversal of memory deficits with a T-type calcium channel blocker. This effect was observed for both the visual and crossmodal tests in which GAERS demonstrated the most severe and consistent deficits. Our results extend on previous research demonstrating that the anticonvulsant and T-type calcium channel blocker, ethosuximide (MacDonald and Kelly, 1995), ameliorates behaviors consistent with a psychiatric phenotype in GAERS. It was shown that chronic ethosuximide treatment increased both exploration and the number of entries into the inner area of an open field in GAERS; however, acute treatment was ineffective (Dezsi et al., 2013). It is possible that we observed a behavioral improvement with an acute dose as a result of the significantly increased potency and selectivity of Z944 as a T-type calcium channel blocker (Tringham et al., 2012).

Previous research suggests that the PRh and posterior parietal cortex are critically involved in cross-modal object recognition with the PRh particularly relevant to visual recognition memory (Winters and Reid, 2010). As GAERS express a mutation in Cav3.2 T-type calcium channels (Powell et al., 2009) and the PRh is particularly abundant in T-type calcium channel expression (Talley et al., 1999), cognitive tasks dependent upon the PRh may be selectively impaired in GAERS. Indeed, we observed enhanced in object recognition memory in GAERS in the visual and crossmodal tests following Z944 treatment. Importantly, tactile recognition memory was only mildly increased in both strains during DR1 + 2 indicating that the effects of Z944 are most robust in tasks dependent on the PRh. Interestingly, blockade of glutamatergic activity in the PRh impairs object recognition memory in rodents and primates (Malkova et al., 2015; Winters and Bussey, 2005). Recent evidence suggests that $Ca_V3.2$ channels play a direct role in the regulation of synaptic NMDA receptor transmission. Specifically, expression of a childhood absence epilepsy-linked mutant $Ca_V3.2$ channel, $hCa_V3.2$ (C456S), results in enhanced glutamatergic transmission at synapses (Wang et al., 2015). Thus, Z944 may alter object recognition memory through effects on glutamatergic signalling in the PRh. Alternatively, T-type calcium channel blockade in the thalamus may also account for the memory enhancement observed in GAERS with Z944 treatment. Indeed, increased T-type calcium channel mRNA expression, currents, and expression of a *Cacna1h* mutation-sensitive splice variant have previously been demonstrated in the thalamus of GAERS (Powell et al., 2009; Talley et al., 2000; Tsakiridou et al., 1995).

Acute Z944 treatment significantly reduced object exploration for the GAERS and NEC strains during both the sample and test phases of all the CMOR tests. A potential explanation for this observed effect is that Z944 affects alertness. T-type channels have a well documented role in the generation of electroencephalogram waves observed during sleep (Crunelli et al., 2014). These waves are thought to be produced by a network of activity involving the corticothalamic loop (Crunelli et al., 2014), areas all densely populated with T-type calcium channels (Talley et al., 1999). Another structurally distinct T-type calcium channel blocker, TTA-A2, has been shown to suppress active wakefulness and has been recognized as potential therapeutic targets for sleep disorders (Kraus et al., 2010). Contrastingly, previous research has demonstrated that an acute systemic 10 mg/kg dose of Z944 did not produce the delta brainwaves observed during drowsiness (Tringham et al., 2012). Also, the 10 mg/kg dose of Z944 was not significantly different from vehicle treatment on behavioral measures of sedation (Tringham et al., 2012). An alternative explanation for the decrease in exploratory activity observed is that Z944 affects motor performance in GAERS and NECs. Previous research has demonstrated motor impairments in Cav3.1 KO mice. In elevated beam, rotarod and pole tests, Cav3.1 KO mice took more time to complete the tasks (Ly et al., 2013), a behavioral phenotype consistent with the Z944 treated rats in this study. Thus, behaviors dependent on cerebellar functioning may be similarly affected by T-type calcium channel blockers in both GAERS and NEC animals. Future research should focus on determining whether Z944 does indeed alter cerebellar physiology. Another potential explanation for the observed decrease in object exploration is that Z944 increased anxiety-like behavior in both the GAERS and NEC strains and thus reduced overall levels of locomotion. Although the effect of Z944 on anxiety-like behavior in rodents is not known, Cav3.2 deficient mice have demonstrated an anxiety-like phenotype in the elevated plus maze and light/dark conflict test (Gangarossa et al., 2014).

4.3. T-type calcium channels mediate object recognition memory in NECs

Although blockade of T-type calcium channels in GAERS improved recognition memory, similar treatment in NECs may be deleterious. Z944 produced a pronounced dose-dependent decrease on object recognition memory in NECs for both the visual and crossmodal tests. Interestingly, the recently developed drug, ST101, which activates T-type calcium channels, was shown to improve recognition memory in olfactory bulbectomized and scopolamine treated mice (Moriguchi et al., 2012; Yamamoto et al., 2013). Further, a significant decrease in T-type calcium channels, as observed in Cav3.2 deficient mice, impaired performance on novel object and spatial object recognition tasks (Gangarossa et al., 2014). Thus, previous research and the results from this study suggest that there may be an optimal level of T-type calcium channel activity required for object recognition memory.

T-type calcium channels also mediate cognition in tasks other than recognition memory. Recently, we have shown that Z944 significantly decreased prepulse inhibition (Marks et al., in press), a measure of sensorimotor gating dependent on a network of limbic, cortical, striatal, pallidal, and pontine brain activity (Swerdlow et al., 2000). Cav3.2 KO mice are impaired in the hippocampal dependent context-cued trace fear conditioning and step-down and step-through passive avoidance tasks (Chen et al., 2012). Given the breadth of cognitive networks affected by T-type calcium channel expression, further research into the function of these channels in cognition and behavior is warranted.

5. Conclusion

In a series of experiments, we demonstrated visual and CMOR memory deficits in drug naïve GAERS relative to NECs. Z944 rescued visual and CMOR deficits in GAERS, whereas, NEC animals showed impaired visual and CMOR performance in response to Z944 treatment. Tactile recognition memory was moderately increased by Z944 treatment in both strains. In general, object recognition memory specifically dependent on visual processing was robustly affected by Z944 treatment in both the GAERS and NEC strains. Overall, the current study demonstrates that Z944 does not have a global effect on recognition memory. Rather, its effects depend on both genetic variability between animal strains as well as the specific task considered. These findings are important in that they demonstrate that T-type calcium channel blockers are effective not only as anticonvulsants (Casillas-Espinosa et al., 2015;

Tringham et al., 2012) and for the reduction of pain (LeBlanc et al., 2016; M'Dahoma et al., 2016), but also as cognitive enhancers in the GAERS model of CAE. This highlights the likely critical role T-type calcium channels have in mediating cognitive deficits associated with psychiatric illness and neurological disorders, symptoms that are known to be difficult to treat. Future research should focus on the effects of longer-term Z944 treatment on memory performance and potential side effects specifically related to locomotor activity, alertness, and sleep functioning which may pose a problem for treatment in humans. Continued research into the therapeutic potential of T-type calcium channel regulation may be particularly fruitful for the treatment of neurological disorders and psychiatric illnesses characterized by visual memory deficits, such as CAE and schizophrenia.

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