Experimental Neurology 296 (2017) 16–22

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Research Paper

Sociability impairments in Genetic Absence Epilepsy Rats from Strasbourg: Reversal by the T-type calcium channel antagonist Z944

Mark T. Henbid [a,1], Wendie N. Marks [a,1], Madeline J. Collins [a], Stuart M. Cain [b], Terrance P. Snutch [b], John G. Howland [a,*]

[a] Department of Physiology, University of Saskatchewan, Saskatoon, SK S7N 5E5, Canada
[b] Michael Smith Laboratories, Djavad Mowafaghian Centre for Brain Health, University of British Columbia, Vancouver, BC V6T 1Z4, Canada

ARTICLE INFO

Article history:
Received 6 February 2017
Received in revised form 16 June 2017
Accepted 24 June 2017
Available online 26 June 2017

Keywords:
Absence epilepsy
Sociability task
GAERS
T-type calcium channels

ABSTRACT

Childhood absence epilepsy (CAE) is associated with interictal co-morbid symptoms including abnormalities in social behaviour. Genetic Absence Epilepsy Rats from Strasbourg (GAERS) is a model of CAE that exhibits physiological and behavioural alterations characteristic of the human disorder. However, it is unknown if GAERS display the social deficits often observed in CAE. Sociability in rodents is thought to be mediated by neural circuits densely populated with T-type calcium channels and GAERS contain a missense mutation in the Cav3.2 T-type calcium channel gene. Thus, the objective of this study was to examine the effects of the clinical stage pan-T-type calcium channel blocker, Z944, on sociability behaviour in male and female GAERS and non-epileptic control (NEC) animals. Female GAERS showed reduced sociability in a three-chamber sociability task whereas male GAERS, male NECs, and female NECs all showed a preference for the chamber containing a stranger rat. In drug trials, pre-treatment with 5 mg/kg of Z944 normalized sociability in female GAERS. In contrast, female NECs showed impaired sociability following Z944 treatment. Dose-dependent decreases in locomotor activity were noted following Z944 treatment in both strains. Treatment with 10 mg/kg of Z944 altered exploration such that only 8 of the 16 rats tested explored both sides of the testing chamber. In those that explored the chamber, significant preference for the stranger rat was observed in GAERS but not NECs. Overall, the data suggest that T-type calcium channels are critical in regulating sociability in both GAERS and NEC animals. Future research should focus on T-type calcium channels in the treatment of sociability deficits observed in disorders such as CAE.

1. Introduction

Childhood absence epilepsy (CAE) is characterized by losses in consciousness that can co-occur with mild clonic movements and automatisms during bilateral spike and wave discharges (SWD's) concomitant with seizure activity (Pavone et al., 2001). Similar to other epilepsies, CAE is associated with interictal co-morbid impairments including cognitive (Caplan et al., 2009; Henkin et al., 2005; Killory et al., 2011; MacEachern et al., 2017; Mandelbaum and Burack, 1997; Pavone et al., 2001) and language deficits (Caplan et al., 2009), as well as social behaviour abnormalities (Caplan et al., 2009). The GAERS model of absence epilepsy, but not its related NEC strain, exhibits similar SWD characteristics of CAE (Marescaux et al., 1992; Tringham et al., 2012). Previous research has shown that GAERS also display the cognitive and psychiatric-like phenotypes associated with epilepsy (Bouilleret et al., 2009; Dezsi et al., 2013; Jones et al., 2008; Jones et al., 2010; Marks et al., 2016a; Marks et al., 2016b; Powell et al., 2014, but see also Marques-Carneiro et al., 2014); however, it is unknown whether they demonstrate the social deficits observed in CAE. Social deficits occur in approximately 23% of CAE patients (Caplan et al., 2008). In adulthood, individuals with absence epilepsy are significantly more socially isolated (Olsson and Campenhausen, 1993). These social deficits occur regardless of seizure control (Nickels, 2015). Therefore, the first objective of the present study was to assess sociability in the GAERS model.

T-type calcium channels are abundantly expressed in the circuits implicated in mediating rodent social behaviour including the amygdala, olfactory bulb, piriform cortex, and lateral septum (Ferguson et al., 2001; Talley et al., 1999). Morphometric abnormalities have been observed in the amygdala of GAERS further suggesting the possibility of disrupted social behaviour in these animals (Bouilleret et al., 2009). Seizures in GAERS are produced by abnormalities in thalamocortical circuity at least in part due to gain-of-function missense mutation in the Cav3.2 T-type calcium channel gene (Cain et al., 2015; Powell et al., 2009). In support, the pan-T-type calcium channel blocker, Z944, suppresses seizure activity in GAERS (Tringham et al., 2012). Further,

* Corresponding author at: University of Saskatchewan, Department of Physiology, GB33 Health Sciences, 107 Wiggins Rd, Saskatoon, SK, Canada.
E-mail address: john.howland@usask.ca (J.G. Howland).
[1] Co-first authors.

http://dx.doi.org/10.1016/j.expneurol.2017.06.022
0014-4886/

Z944 administration not only suppresses seizure activity, but also improves cognitive deficits observed in GAERS, specifically, visual and crossmodal memory deficits (Marks et al., 2016a). Given the role of T-type calcium channels in GAERS neuropathology and the cognitive improvements observed in GAERS with Z944 treatment, examining the effect of Z944 on social behaviour in GAERS is warranted. The objective of this study was to examine dose-dependent effects of Z944 on sociability in GAERS and NEC rats using a three chambered sociability task (Millan and Bales, 2013; Nadler et al., 2004). Social interaction in this task is measured by preference to explore a cage containing a stranger rat as opposed to an empty cage. This sociability task is ideal in that social interaction is initiated by the test animal and placement of the stranger animal in a wire cage ensures aggressive behaviours between animals is limited (Crawley, 2007). The work demonstrated sociability deficits in GAERS animals. Further, sociability deficits in GAERS were reversed by Z944 administration.

2. Materials and methods

2.1. Animals

For all experiments, GAERS and NEC rats (12–20 weeks of age) were used (University of Saskatchewan Lab Animal Services Unit, Saskatoon, Canada) (Marks et al., 2016b). Rats were maintained in a temperature controlled room (21 °C) on a 12 hour day-night cycle (lights on at 7 am) with ad libitum access to standard rat chow and water. All rats were housed in groups of 2 or 3 in standard polypropylene cages. Experimental procedures were conducted in accordance with the Canadian Council on Animal Care guidelines for humane animal use and were approved by the University of Saskatchewan Animal Research Ethics Board.

2.2. Drug preparation

The characterization and synthesis of Z944 is described in Tringham et al. (2012). Z944 was prepared daily in a solution of 10% dimethyl sulfoxide (DMSO; Sigma Aldrich, St. Louis, MO) and 90% sodium carboxymethyl cellulose (0.5% in saline, Sigma Aldrich). Injections were administered intraperitoneally at 5 ml/kg in doses of either 5 mg/kg or 10 mg/kg. The highest dose of Z944 was chosen based on previous research demonstrating significant blockade of GAERS seizure activity at the 10 mg/kg dose without altering the state of alertness (Tringham et al., 2012). Injection of Z944 or vehicle was performed 15 min prior to the habituation phase of sociability testing.

2.3. Three chambered social interaction apparatus

The apparatus (150 cm by 40 cm by 40 cm tall) was constructed from black corrugated plastic (Fig. 1). The two side chambers were each 60 cm by 40 cm, while the middle chamber was 30 cm by 40 cm. The middle chamber's walls were made of Plexiglas sheets 12 cm long. The rat cages were constructed of ¾″ plywood painted black, wire mesh, and metal rods. The cages had a diameter of 18 cm and a height of 20 cm. The cage height was extended to 40 cm through the use of vertically placed metal rods to discourage climbing.

2.4. Sociability task procedure

Prior to behavioural testing, rats were handled for 5 min/day for 3 days. The testing apparatus was cleaned with 70% ethanol between trials. For the drug naive sociability trials, 23 GAERS (12 male, 11 female) and 22 NEC (12 male, 10 female) animals were used. For the Z944 trials, 28 female GAERS (11 control, 9 5 mg Z944 treatment, 8 10 mg Z944 treatment) and 37 female NEC (12 control, 12 5 mg Z944 treatment, 13 10 mg Z944 treatment) animals were used. Animals that did not complete the sociability task, defined as not visiting both the stranger and empty cage, were not included in the results. In total, 4 GAERS treated with the 5 mg/kg dose and 8 GAERS treated with 10 mg/kg of Z944 were removed (total N's listed above account for these removals).

The sociability task used was adapted from previously published protocols (Cutuli et al., 2015; Nadler et al., 2004). Briefly, animals were given a habituation session to the apparatus (10 min) immediately before the sociability task. Each sociability trial began by placing the test rat into the middle chamber of the interaction apparatus. Dividers were in place to prevent entry into either of the side chambers. A stranger rat of the same strain and sex was placed into one of the two rat cages in the side chambers. The side placement of the stranger rat and empty cage were randomized and counterbalanced. Test animals had no previous interaction with stranger animals for this task. After 3 min, the dividers were removed and the test rat was given 10 min to freely explore the entire apparatus. The trial was tracked using Noldus Ethovision XT 11.5 to determine: time spent in direct social contact with either the stranger rat containing cage or the empty cage, number of entries into the direct social interaction zones, total distance moved, and latency to first entry into the direct social interaction zones. Direct interaction with either the stranger or empty cage was considered to have occurred when the test rat's nose entered a 2 cm circle around either of the two cages.

2.5. Data analysis

All figures summarize means with the error bars representing standard error of the mean (SEM). SPSS Version 20 for Windows (IBM) was used for statistics. A discrimination ratio (DR) was used to calculate the frequency and duration ratios. The DR formula: (stranger rat cage − empty cage) / (stranger rat cage + empty rat cage) is adapted from DRs used to quantify preferential exploration of novel objects (Ballendine et al., 2015; Cazakoff and Howland, 2011; Howland et al., 2012). Positive DR values indicate a preference for the stranger cage whereas negative DR values indicate a preference for the empty cage. Repeated measures analysis of variance (ANOVA) with 2 min Time

Fig. 1. Schematic of the three-chambered sociability test. See Materials and methods for details.

bins as a within-subjects factor were initially run for all analyses to explore whether Strain, Sex, or Treatment interacted with performance over time. Given that Time did not interact with the other factors, univariate ANOVAs were run for distance travelled, stranger cage exploration, empty cage exploration, frequency DR, and duration DRs with Strain, Sex, or Treatment as the between-subjects factors where appropriate. Analysis of latency to exploration of the stranger and empty cages were performed using univariate ANOVAs with Strain, Sex, or Treatment as the independent variables where appropriate. Post hoc analyses were performed using *t*-tests with a Bonferroni correction (significance set to 0.0125). One sample *t*-tests comparing to a mean of 0 were used to determine whether frequency or duration DR was significantly above chance levels (Ho et al., 2015; Winters and Reid, 2010), thus directly assessing whether a specific group demonstrated evidence of sociability. Effect size was calculated with partial η^2 which represents the total variability in each dependent variable that can be attributed to the independent variables. Partial η^2 values of 0.01, 0.06, and 0.14 are considered small, medium and large effect sizes. Unless stated otherwise, statistical significance was set at $p \leq 0.05$.

3. Results

3.1. Drug naïve trials

Distance travelled within the testing arena was analyzed (Fig. 2A). A significant between-subjects main effects of both Strain ($F(1,41) = 5.42$, $p = 0.025$, partial $\eta^2 = 0.117$) and Sex ($F(1,41) = 14.22$, $p = 0.001$, partial $\eta^2 = 0.258$) revealed that GAERS travelled significantly less distance than NECs and males travelled significantly less distance than females overall.

Latency to first exploration of both the stranger and empty cage were analyzed. Although the effects of Strain and Sex on latency to explore the stranger cage were non-significant (all $p \geq 0.211$; Fig. 2B), it took significantly longer for GAERS ($F(1,41) = 11.04$, $p = 0.002$, partial $\eta^2 = 0.212$) and males ($F(1,41) = 5.29$, $p = 0.027$, partial $\eta^2 = 0.114$) to explore the empty cage compared to NEC and female animals, respectively (Fig. 2C).

The total time spent exploring the stranger or empty cage was analyzed (Fig. 2D, E respectively). All main effects and interactions for total

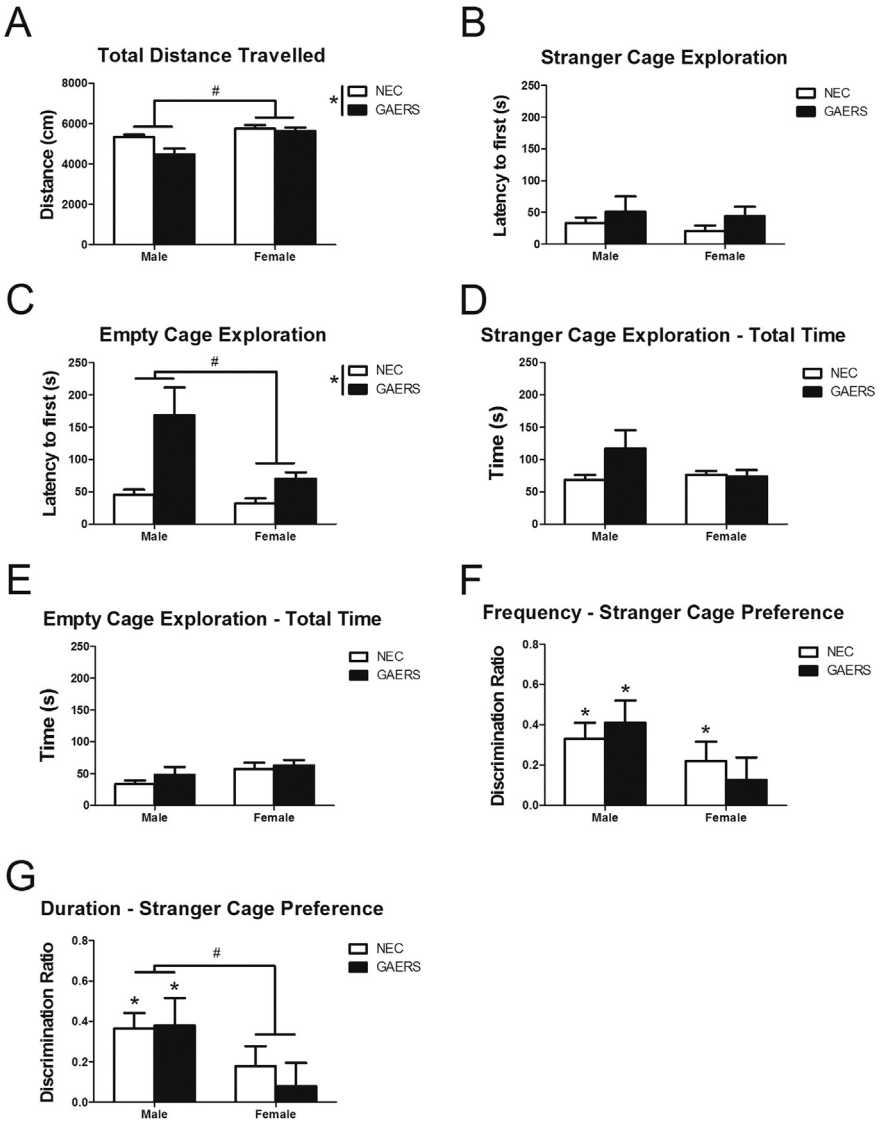

Fig. 2. Distance travelled, latency to stranger and empty cage exploration, total stranger and empty cage exploration, frequency and duration discrimination ratios (DR) for drug naïve male and female GAERS and NECs. Overall GAERS travelled significantly less distance than NECs (*$p < 0.05$; A) and males travelled significantly less distance than female animals (#$p < 0.05$; A). GAERS took significantly longer to explore the empty cage compared to NECs (*$p < 0.05$; C). Males took significantly longer to explore the empty cage relative to female animals (#$p < 0.05$; C). Frequency DR was significantly above chance levels for male and female NECs (*$p < 0.05$; F) and female GAERS (*$p < 0.05$; F). Duration DR was significantly increased in male compared to female animals (#$p < 0.05$; G). Male GAERS and NECs showed significantly increased duration DRs relative to chance levels of exploration (*$p < 0.05$; G).

time spent exploring either the stranger or empty cage were non-significant (all $p \geq 0.053$). Analysis of the DR for the frequency for which the stranger cage was explored in relation to the empty cage (Fig. 2F) revealed nonsignificant differences in Strain, Sex, and the Strain by Sex interaction (all $p \geq 0.058$). A significant between-subjects main effect of Sex ($F(1,41) = 4.84$, $p = 0.033$, partial $\eta^2 = 0.106$) was found with analysis of the DR for the duration of time spent exploring the stranger cage in relation to the empty cage (Fig. 2G). Overall, males spent significantly more time exploring the stranger cage relative to the empty cage compared to females. Further analyses using one sample *t*-tests comparing frequency and duration DRs to a mean of 0 (Fig. 2F, G) showed significant frequency of exploration of the stranger cage above chance in males of both the NEC ($t(11) = 4.10$, $p = 0.002$) and GAERS ($t(11) = 3.69$, $p = 0.004$) strains. Duration of exploration of the stranger cage was also significantly above chance in males of both strains: NEC ($t(11) = 4.75$, $p = 0.001$), GAERS ($t(11) = 2.77$; $p = 0.018$). However, significant exploration of the stranger cage was only found in NEC females for the frequency DR ($t(9) = 2.32$, $p = 0.045$). Thus, GAERS females showed no evidence of preferentially exploring the cage containing the stranger rat.

3.2. Z944 trials

Although female GAERS did not show significantly impaired performance relative to female NECS, female GAERS did display a deficit in sociability relative to chance performance. Thus, Z944 trials were performed in female animals. The 10 mg/kg dose of Z944 impaired the ability of approximately half the animals to perform the sociability task. In light of this we analyzed the data using only the vehicle and 5 mg/kg Z944 groups. The means and standard errors of the means of the distance travelled, stranger and empty cage latencies to explore, total stranger and empty cage exploration times, and frequency and duration DR for the 10 mg/kg group are summarized in Table 1.

Analysis of the distance travelled within the testing arena revealed significant between-subjects main effects of Strain ($F(1,40) = 10.93$, $p = 0.002$, partial $\eta^2 = 0.215$) and Treatment ($F(1,40) = 36.11$, $p < 0.001$, partial $\eta^2 = 0.474$; Fig. 3A). Over the entire testing session, NEC animals travelled significantly more distance than GAERS. Also, both NEC and GAERS animals treated with the 5 mg/kg dose of Z944 travelled less distance overall relative to vehicle treated animals. Although latency to first exploration of the stranger cage was not significantly affected by either Strain or Treatment (all $p \geq 0.110$; Fig. 3B), a significant Strain by Treatment interaction was found for the latency to first exploration of the empty cage ($F(1,40) = 5.33$, $p = 0.026$, partial $\eta^2 = 0.118$; Fig. 3C). However, post hoc analyses failed to reach significance for individual between group comparisons. A significant main effect of Treatment ($F(1,40) = 5.99$, $p = 0.019$, partial $\eta^2 = 0.130$) and a significant Strain by Treatment interaction ($F(1,40) = 10.74$, $p = 0.002$, partial $\eta^2 = 0.212$) was found for total stranger cage exploration (Fig. 3D). Post hoc analyses revealed GAERS treated with 5 mg/kg Z944 showed significantly increased total stranger cage exploration relative to vehicle treated GAERS ($t(18) = -2.99$, $p = 0.008$). A significant Strain by Treatment interaction was also found for total time spent exploring the empty cage ($F(1,40) = 7.73$, $p = 0.008$, partial $\eta^2 = 0.162$; Fig. 3E). Post hoc tests showed vehicle treated GAERS spent significantly more time exploring the empty cage relative to vehicle treated NECs ($t(21) = -3.53$, $p = 0.002$). A significant between-subjects interaction of Strain and Treatment was found for the frequency DR ($F(1,40) = 5.56$, $p = 0.023$, partial $\eta^2 = 0.122$; Fig. 3F). However, post hoc comparisons between the groups did not reach significance. A similar pattern of results was found for duration DR with a significant between-subjects interaction of Strain and Treatment ($F(1,40) = 7.13$, $p = 0.011$, partial $\eta^2 = 0.151$; Fig. 3G). In contrast to the post hoc results for the frequency DR, post hoc analyses for the duration DR revealed that GAERS treated with vehicle show significantly reduced duration DR compared to vehicle treated NECs ($t(21) = 4.06$, $p = 0.001$). One sample *t*-test analyses showed that although only vehicle treated NECs showed significant preference for the stranger cage as demonstrated by significant frequency DR ($t(11) = 4.79$, $p = 0.001$) and duration DR ($t(11) = 6.11$, $p < 0.001$) compared to chance, only GAERS treated with 5 mg/kg Z944 showed significant stranger cage preference, duration DR ($t(8) = 2.44$, $p = 0.041$).

4. Discussion

We first characterized the sociability of drug naïve male and female GAERS and NEC animals. A significant decrease in total distance travelled was found for both male and female GAERS (Fig. 2A). However, an impairment in sociability was only found in female GAERS (Fig. 2F). We then examined the effects of acute treatment with the clinical stage pan-T-type calcium channel blocker Z944 (either 5 mg/kg or 10 mg/kg) on sociability in female GAERS and NECs. A significant decrease in distance travelled following Z944 treatment was noted in both strains (Fig. 3A). The 5 mg/kg dose of Z944 rescued sociability impairments in GAERS and decreased performance in NECs (Fig. 3D, F, G).

4.1. Sociability deficits in drug naïve female GAERS

Sociability deficits were found in female GAERS as demonstrated through a non-significant frequency of preference for the stranger cage relative to chance levels of exploration. Indeed, it has been documented that social abnormalities occur in rodent models of epilepsy. For example, interictal spikes in male juvenile rats, resulting from repeated injections with bicuculline methiodine into the prefrontal cortex, are associated with a failure to show preference for a stranger rat as opposed to a novel object in an opposing cage using a similar sociability task as the one used for the present study (Hernan et al., 2014). Similarly, seizure-prone male and female El mice displayed significantly decreased investigation of a familiar conspecific in a multi-day sociability task (Lim et al., 2007). Further, relative to controls, both male and female neonatal Wistar rats submitted to pilocarpine induced seizures showed significantly reduced preference for the stranger chamber relative to the empty chamber in the sociability task when tested as adults (Castelhano et al., 2013; Castelhano et al., 2015).

The sociability deficit noted in female GAERS is consistent with social abnormalities found in epileptic patient populations. A review of 45 studies on social competence in children with epilepsy confirmed deficits in social adjustment, social performance and social skills in both male and female children (Rantanen et al., 2012). Within the studies reviewed, some demonstrate that certain impairments may be worse in female children. For example, not only were children with epilepsy significantly more likely to display decreased social competence relative to a healthy control group, but females were more likely to display more disrupted social competence than males with epilepsy (Jakovljevic and

Table 1
The means and standard errors of the means (SEM) for total distance travelled, latency to stranger and empty cage exploration, total stranger and empty cage exploration, and total frequency and duration discrimination ratios (DR) for 10 mg/kg Z944 injected GAERS and NEC females.

Strain		Total distance (cm)	Stranger latency (s)	Empty latency (s)	Stranger exploration (s)	Empty exploration (s)	Frequency DR	Duration DR
NEC	Mean	3235.22	27.57	60.28	139.61	107.16	0.13	0.17
	SEM	103.74	6.52	30.74	26.48	33.31	0.20	0.20
GAERS	Mean	2315.58	103.23	325.15	200.16	68.77	0.50	0.46
	SEM	195.39	39.22	80.83	37.16	31.06	0.24	0.25

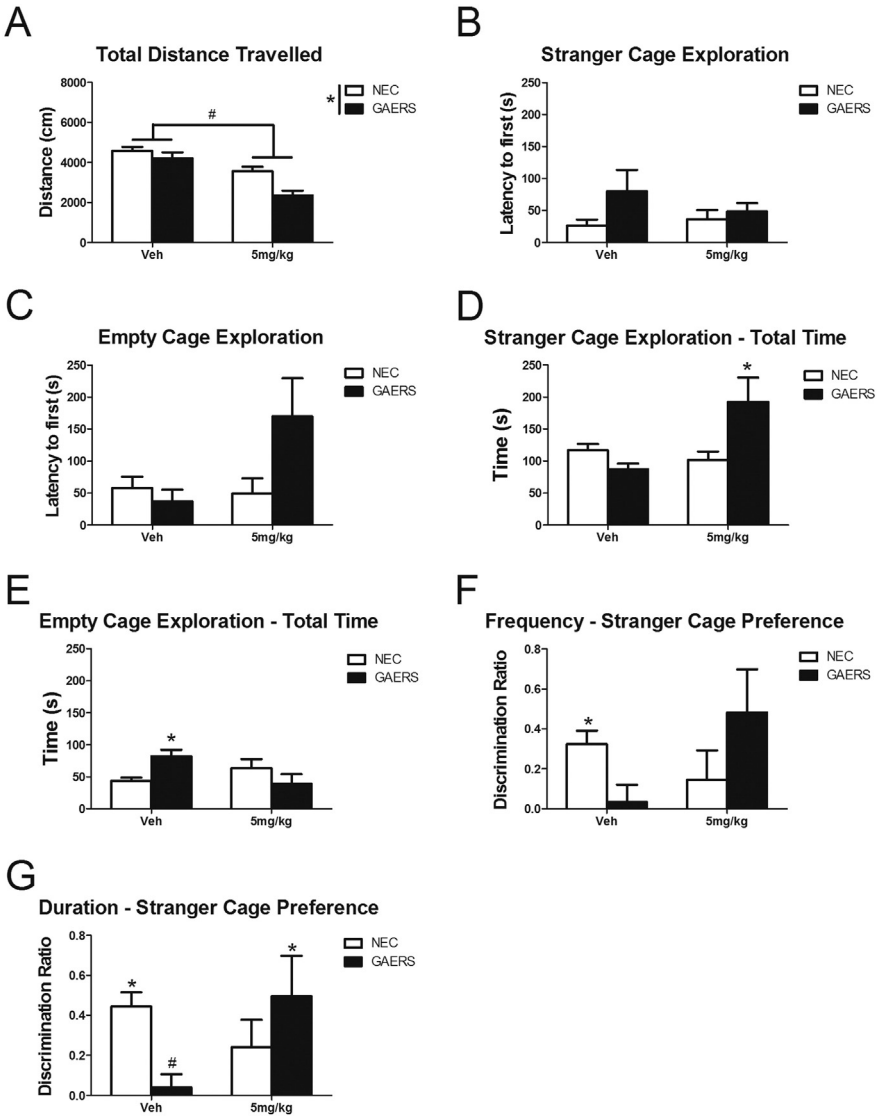

Fig. 3. Distance travelled, latency to stranger and empty cage exploration, total stranger and empty cage exploration, frequency and duration discrimination ratios (DR) for female GAERS and NECs treated with vehicle (Veh) or 5 mg/kg Z944. GAERS travelled significantly less distance than NECs ($^{*}p < 0.05$; A) and animals treated with 5 mg/kg Z944 travelled significantly less distance than vehicle treated animals ($^{\#}p < 0.05$; A). GAERS treated with 5 mg/kg Z944 spent significantly more time exploring the stranger cage relative to vehicle treated GAERS ($^{*}p < 0.05$; D). Vehicle treated GAERS spent significantly more time exploring the empty cage relative to vehicle treated NECs ($^{*}p < 0.05$; E). Only NECs treated with vehicle showed significant frequency DR relative to chance performance ($^{*}p < 0.05$; F). Vehicle treated GAERS displayed significantly reduced duration DR compared to vehicle treated NECs ($^{\#}p < 0.05$; G). Both vehicle treated NECs and 5 mg/kg Z944 treated GAERS demonstrated significant duration DR exploration above chance levels ($^{*}p < 0.05$; G).

Martinovic, 2006). Although we did not correlate female GAERS behaviour with changes in estrous cycle, future research should consider investigating whether alterations in sociability behaviour are dependent on cycling hormones in female animals.

We replicated previous findings that show significant decreases in locomotor behaviour in GAERS relative to NEC animals. Both male and female GAERS travel less distance in an open field task and elevated plus maze (Bouilleret et al., 2009; Dezsi et al., 2013; Jones et al., 2008; Marks et al., 2016b; Powell et al., 2014). Further, male and female GAERS also spend significantly less time exploring objects in a crossmodal object recognition task (Marks et al., 2016a). It should be noted that a decrease in locomotor behaviour alone cannot explain the decrease in sociability found in female GAERS as male GAERS also showed decreased locomotor behaviour yet performed the sociability task similarly to NECs. Decreased locomotor behaviour in GAERS could be indicative of enhanced anxiety during task performance. Indeed, GAERS have increased anxiety-like behaviour in the elevated plus maze (Dezsi et al., 2016; Jones et al., 2008; Marks et al., 2016b; Powell et al., 2014), open field (Bouilleret et al., 2009; Dezsi et al., 2013; Dezsi et al., 2016; Jones et al., 2008; Marks et al., 2016b; Powell et al., 2014), and in response to startling acoustic stimuli (Jones et al., 2010; Marks et al., 2016b, c). Consistent with these data, the present study found latency to first exploration of the empty cage was significantly higher in GAERS, which could be interpreted as increased neophobic behaviour. Although increased anxiety in female GAERS relative to male GAERS could explain the reduced approach behaviour towards a novel conspecific, we have previously reported similar levels of anxiety in both male and female GAERS (Marks et al., 2016b). Further, both male and female GAERS showed similar latencies to exploration of the stranger cage that were non-significant from NEC performance. These results suggest that impaired sociability behaviour in female GAERS is likely not the result of increased anxiety in these animals.

4.2. Sociability deficits in female GAERS are reversed by Z944

The T-type calcium channel blocker, Z944, significantly reversed sociability deficits in female GAERS as demonstrated through not only a significant increase in stranger cage exploration relative to vehicle

treated GAERS, but also a significant increase in duration DR relative to chance levels of exploration. To our knowledge, this is the first report of a reversal of sociability deficits with a T-type calcium channel blocker. We have previously demonstrated that 10 mg/kg of Z944 significantly reversed unimodal and crossmodal object recognition memory deficits in GAERS, an effect that was not attributable to changes in seizures during task performance (Marks et al., 2016a). Chronic treatment with the anticonvulsant and T-type calcium channel blocker, ethosuximide (MacDonald and Kelly, 1995), has also been shown to reverse anxiety-like behaviours in GAERS (Dezsi et al., 2013). In contrast, Z944 impaired sociability performance in NEC animals. This pattern of results mirrors our previously reported effects of Z944 on NEC animals where we observed impaired crossmodal object recognition memory (Marks et al., 2016a). The culmination of these effects suggests T-type calcium channel blockers may be effective at reversing behaviours consistent with a psychiatric phenotype in GAERS animals. However, there may be an optimal level of T-type calcium channel activity required for non-impaired functioning in the NEC strain. Future research should determine whether alterations in sociability in GAERS are related to a reduction in seizures produced by Z944 treatment, or through an effect of T-type calcium channel regulation on behaviour directly.

Social behaviour in rodents is complex, and is believed to involve a circuit including the amygdala, olfactory bulb, piriform cortex, and lateral septum (Ferguson et al., 2001), all areas where T-type calcium channels are expressed (Talley et al., 1999). There is evidence to suggest that Cav3.2 T-type channels contribute to the regulation of *N*-methyl-D-aspartate receptor (NMDAR) transmission as enhanced glutamatergic transmission occurs with expression of the childhood absence epilepsy-linked mutant Cav3.2 channel, hCav3.2 (C456S) (Wang et al., 2015). NMDAR dysfunction may underlie sociability deficits observed in psychiatric disorders such as schizophrenia (Lee and Green, 2016). For example, genetically modified mice with NMDAR hypofunction and wild mice treated with the NMDAR antagonist, MK801, showed decreased preference for the stranger mouse in the sociability task (Halene et al., 2009; Moy et al., 2013). Importantly, reductions in sociability were not attributable to increased anxiety (Halene et al., 2009). This finding suggests there may be an optimal level of NMDA functioning required for sociability. Given that GAERS display a mutation in the Cav3.2 T-type calcium channel (Powell et al., 2009), the reversal of sociability behaviour in GAERS by Z944 may be mediated through altered NMDAR functioning. Future research should determine whether a pathological enhancement in glutamatergic function also results in sociability deficits.

Treatment with Z944 significantly reduced locomotor behaviour in both GAERS and NEC animals as demonstrated by decreased distance travelled during the task. Although the 10 mg/kg dose did not significantly decrease locomotor activity relative to the 5 mg/kg dose (statistics not shown), the 10 mg/kg dose did disrupt the ability of GAERS to successfully perform the sociability task. A possible explanation for this effect is that Z944 dose-dependently increases drowsiness in both strains since T-type channels are known to contribute to sleep oscillations (Crunelli et al., 2014). Other T-type calcium channel blockers, such as TTA-A2, suppress wakefulness and are considered as potential therapeutics for sleep disorders (Kraus et al., 2010). However, the effects of Z944 on drowsiness have been previously explored and at a 10 mg/kg acute systemic dose the delta brainwaves observed during drowsiness were not observed, nor did it significantly increase measures of sedation in rats (Tringham et al., 2012). Alternatively, there is some evidence to suggest that decreased T-type calcium channel functioning may affect motor performance. For example, motor impairments have been observed in Cav3.1 KO mice (Ly et al., 2013). An increase in anxiety-like behaviour could also explain reduced locomotion in GAERS and NEC animals; however, latency to explore the stranger cage was not significantly increased which would be consistent with an anxiety-like phenotype. Future research should examine the effects of Z944 directly on measures of anxiety, and locomotor behaviours such as the rotarod test (Ly et al., 2013). Further, investigating the effects of a lower dose of Z944 may circumvent alterations in locomotor behaviour while still increasing sociability in GAERS.

5. Conclusion

In the present series of experiments, we demonstrated sociability deficits in female GAERS that were reversed by an acute dose of 5 mg/kg of Z944. In contrast, we observed decreased sociability in NECs treated with Z944. Decreased locomotor activity was noted in GAERS as well as in animals treated with Z944. Overall, this study suggests T-type calcium channels play a critical role in the regulation of social behaviour, and may be a fruitful target for therapeutics aiming to treat neurological and psychiatric illnesses characterized by deficits in social behaviour such as CAE, autism, and schizophrenia.

Funding and disclosure

This research was supported by a Brain Canada Multi-Investigator Research Initiative Grant with matching support from Genome British Columbia, the Michael Smith Foundation for Health Research, and the Koerner Foundation (PI: TPS). Work in the laboratory of TPS is supported by an operating grant from the Canadian Institutes for Health Research (CIHR; #10677) and the Canada Research Chair in Biotechnology and Genomics-Neurobiology. Work in the laboratory of JGH is supported by an operating grant from CIHR (#125984) and matching funds for these experiments from the Saskatchewan Health Research Foundation and the University of Saskatchewan. WNM is supported by funding from the Saskatchewan Health Research Foundation. SMC is supported by the CURE Taking Flight Award and by a research grant from the B.C. Epilepsy Society. MJC received salary support from the College of Medicine, University of Saskatchewan.

References

Ballendine, S.A., Greba, Q., Dawicki, W., Zhang, X., Gordon, J.R., Howland, J.G., 2015. Behavioral alterations in rat offspring following maternal immune activation and ELR-CXC chemokine receptor antagonism during pregnancy: implications for neurodevelopmental psychiatric disorders. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 57:155–165. http://dx.doi.org/10.1016/j.pnpbp.2014.11.002 (S0278-5846(14)00218-8 [pii], retrieved from PM:25445065).

Bouilleret, V., Hogan, R.E., Velakoulis, D., Salzberg, M.R., Wang, L., Egan, G.F., et al., 2009. Morphometric abnormalities and hyperanxiety in genetically epileptic rats: a model of psychiatric comorbidity? NeuroImage 45 (2):267–274. http://dx.doi.org/10.1016/j.neuroimage.2008.12.019 (S1053-8119(08)01273-1 [pii], retrieved from PM:19167503).

Cain, S.M., Tyson, J.R., Jones, K.L., Snutch, T.P., 2015. Thalamocortical neurons display suppressed burst-firing due to an enhanced Ih current in a genetic model of absence epilepsy. Pflugers Arch. 467 (6):1367–1382. http://dx.doi.org/10.1007/s00424-014-1549-4 (retrieved from PM:24953239).

Caplan, R., Siddarth, P., Stahl, L., Lanphier, E., Vona, P., Gurbani, S., et al., 2008. Childhood absence epilepsy: behavioral, cognitive, and linguistic comorbidities. Epilepsia 49 (11):1838–1846. http://dx.doi.org/10.1111/j.1528-1167.2008.01680.x (EPI1680 [pii], retrieved from PM:18557780).

Caplan, R., Levitt, J., Siddarth, P., Wu, K.N., Gurbani, S., Sankar, R., et al., 2009. Frontal and temporal volumes in Childhood Absence Epilepsy. Epilepsia 50 (11):2466–2472. http://dx.doi.org/10.1111/j.1528-1167.2009.02198.x (EPI2198 [pii], retrieved from PM:19624714).

Castelhano, A.S., Cassane, G.S., Scorza, F.A., Cysneiros, R.M., 2013. Altered anxiety-related and abnormal social behaviors in rats exposed to early life seizures. Front. Behav. Neurosci. 7:36. http://dx.doi.org/10.3389/fnbeh.2013.00036 (retrieved from PM:23675329).

Castelhano, A.S., Ramos, F.O., Scorza, F.A., Cysneiros, R.M., 2015. Early life seizures in female rats lead to anxiety-related behavior and abnormal social behavior characterized by reduced motivation to novelty and deficit in social discrimination. J. Neural Transm. (Vienna) 122 (3):349–355. http://dx.doi.org/10.1007/s00702-014-1291-2 (retrieved from PM:25139483).

Cazakoff, B.N., Howland, J.G., 2011. AMPA receptor endocytosis in rat perirhinal cortex underlies retrieval of object memory. Learn. Mem. 18 (11):688–692. http://dx.doi.org/10.1101/lm.2312711 (18/11/688 [pii], retrieved from PM:22005749).

Crawley, J.N., 2007. Mouse behavioral assays relevant to the symptoms of autism. Brain Pathol. 17 (4):448–459. http://dx.doi.org/10.1111/j.1750-3639.2007.00096.x (BPA096 [pii], Retrieved from PM:17919130).

Crunelli, V., David, F., Leresche, N., Lambert, R.C., 2014. Role for T-type Ca2+ channels in sleep waves. Pflugers Arch. 466 (4):735–745. http://dx.doi.org/10.1007/s00424-014-1477-3 (retrieved from PM:24578015).

Cutuli, D., Caporali, P., Gelfo, F., Angelucci, F., Laricchiuta, D., Foti, F., et al., 2015. Pre-reproductive maternal enrichment influences rat maternal care and offspring developmental trajectories: behavioral performances and neuroplasticity correlates. Front. Behav. Neurosci. 9:66. http://dx.doi.org/10.3389/fnbeh.2015.00066 (retrieved from PM:25814946).

Dezsi, G., Ozturk, E., Stanic, D., Powell, K.L., Blumenfeld, H., O'Brien, T.J., et al., 2013. Ethosuximide reduces epileptogenesis and behavioral comorbidity in the GAERS model of genetic generalized epilepsy. Epilepsia 54 (4):635–643. http://dx.doi.org/10.1111/epi.12118 (retrieved from PM:23464801).

Dezsi, G., Ozturk, E., Salzberg, M.R., Morris, M., O'Brien, T.J., Jones, N.C., 2016. Environmental enrichment imparts disease-modifying and transgenerational effects on genetically-determined epilepsy and anxiety. Neurobiol. Dis. 93:129–136. http://dx.doi.org/10.1016/j.nbd.2016.05.005 (S0969-9961(16)30096-1 [pii], retrieved from PM:27185593).

Ferguson, J.N., Aldag, J.M., Insel, T.R., Young, L.J., 2001. Oxytocin in the medial amygdala is essential for social recognition in the mouse. J. Neurosci. 21 (20), 8278–8285 (21/20/8278 [pii], retrieved from PM:11588199).

Halene, T.B., Ehrlichman, R.S., Liang, Y., Christian, E.P., Jonak, G.J., Gur, T.L., et al., 2009. Assessment of NMDA receptor NR1 subunit hypofunction in mice as a model for schizophrenia. Genes Brain Behav. 8 (7):661–675. http://dx.doi.org/10.1111/j.1601-183X.2009.00504.x (GBB504 [pii], retrieved from PM:19563516).

Henkin, Y., Sadeh, M., Kivity, S., Shabtai, E., Kishon-Rabin, L., Gadoth, N., 2005. Cognitive function in idiopathic generalized epilepsy of childhood. Dev. Med. Child Neurol. 47 (2), 126–132 (retrieved from PM:15707236).

Hernan, A.E., Alexander, A., Jenks, K.R., Barry, J., Lenck-Santini, P.P., Isaeva, E., et al., 2014. Focal epileptiform activity in the prefrontal cortex is associated with long-term attention and sociability deficits. Neurobiol. Dis. 63:25–34. http://dx.doi.org/10.1016/j.nbd.2013.11.012 (S0969-9961(13)00324-0 [pii], retrieved from PM:24269731).

Ho, J.W., Poeta, D.L., Jacobson, T.K., Zolnik, T.A., Neske, G.T., Connors, B.W., et al., 2015. Bidirectional modulation of recognition memory. J. Neurosci. 35 (39):13323–13335. http://dx.doi.org/10.1523/JNEUROSCI.2278-15.2015 (35/39/13323 [pii], retrieved from PM:26424881).

Howland, J.G., Cazakoff, B.N., Zhang, Y., 2012. Altered object-in-place recognition memory, prepulse inhibition, and locomotor activity in the offspring of rats exposed to a viral mimetic during pregnancy. Neuroscience 201:184–198. http://dx.doi.org/10.1016/j.neuroscience.2011.11.011 (S0306-4522(11)01278-4 [pii], Retrieved from PM:22119062).

Jakovljevic, V., Martinovic, Z., 2006. Social competence of children and adolescents with epilepsy. Seizure 15 (7):528–532. http://dx.doi.org/10.1016/j.seizure.2006.06.005 (S1059-1311(06)00123-3 [pii], retrieved from PM:16860578).

Jones, N.C., Salzberg, M.R., Kumar, G., Couper, A., Morris, M.J., O'Brien, T.J., 2008. Elevated anxiety and depressive-like behavior in a rat model of genetic generalized epilepsy suggesting common causation. Exp. Neurol. 209 (1):254–260. http://dx.doi.org/10.1016/j.expneurol.2007.09.026 (S0014-4886(07)00371-8 [pii], retrieved from PM:18022621).

Jones, N.C., Martin, S., Megatia, I., Hakami, T., Salzberg, M.R., Pinault, D., et al., 2010. A genetic epilepsy rat model displays endophenotypes of psychosis. Neurobiol. Dis. 39 (1):116–125. http://dx.doi.org/10.1016/j.nbd.2010.02.001 (S0969-9961(10)00039-2 [pii], retrieved from PM:20153428).

Killory, B.D., Bai, X., Negishi, M., Vega, C., Spann, M.N., Vestal, M., et al., 2011. Impaired attention and network connectivity in childhood absence epilepsy. NeuroImage 56 (4):2209–2217. http://dx.doi.org/10.1016/j.neuroimage.2011.03.036 (S1053-8119(11)00314-4 [pii], retrieved from PM:21421063).

Kraus, R.L., Li, Y., Gregan, Y., Gotter, A.L., Uebele, V.N., Fox, S.V., et al., 2010. In vitro characterization of T-type calcium channel antagonist TTA-A2 and in vivo effects on arousal in mice. J. Pharmacol. Exp. Ther. 335 (2):409–417. http://dx.doi.org/10.1124/jpet.110.171058 (jpet.110.171058 [pii], retrieved from PM:20682849).

Lee, J., Green, M.F., 2016. Social preference and glutamatergic dysfunction: underappreciated prerequisites for social dysfunction in schizophrenia. Trends Neurosci. 39 (9):587–596. http://dx.doi.org/10.1016/j.tins.2016.06.005 (S0166-2236(16)30056-X [pii], retrieved from PM:27477199).

Lim, C.E., Turner, L.H., Heinrichs, S.C., 2007. Short-term social recognition memory deficit and atypical social and physiological stressor reactivity in seizure-susceptible El mice. Seizure 16 (1):59–68. http://dx.doi.org/10.1016/j.seizure.2006.10.006 (S1059-1311(06)00199-3 [pii], retrieved from PM:17116413).

Ly, R., Bouvier, G., Schonewille, M., Arabo, A., Rondi-Reig, L., Lena, C., et al., 2013. T-type channel blockade impairs long-term potentiation at the parallel fiber-Purkinje cell synapse and cerebellar learning. Proc. Natl. Acad. Sci. U. S. A. 110 (50):20302–20307. http://dx.doi.org/10.1073/pnas.1311686110 (1311686110 [pii], retrieved from PM:24277825).

MacDonald, R.L., Kelly, K.M., 1995. Antiepileptic drug mechanisms of action. Epilepsia 36 (Suppl. 2), S2–12 (retrieved from PM:8784210).

MacEachern, S.J., D'Alfonso, S., McDonald, R.J., Thornton, N., Forkert, N.D., Buchhalter, J.R., 2017. Most children with epilepsy experience postictal phenomena, often preventing a return to normal activities of childhood. Pediatr. Neurol. http://dx.doi.org/10.1016/j.pediatrneurol.2017.03.002 (S0887-8994(16)31041-4 [pii], retrieved from PM:28483399).

Mandelbaum, D.E., Burack, G.D., 1997. The effect of seizure type and medication on cognitive and behavioral functioning in children with idiopathic epilepsy. Dev. Med. Child Neurol. 39 (11), 731–735 (retrieved from PM:9393886).

Marescaux, C., Vergnes, M., Depaulis, A., 1992. Genetic absence epilepsy in rats from Strasbourg—a review. J. Neural Transm. Suppl. 35, 37–69 (retrieved from PM:1512594).

Marks, W.N., Cain, S.M., Snutch, T.P., Howland, J.G., 2016a. The T-type calcium channel antagonist Z944 rescues impairments in crossmodal and visual recognition memory in Genetic Absence Epilepsy Rats from Strasbourg. Neurobiol. Dis. 94, 106–115.

Marks, W.N., Cavanagh, M.E., Greba, Q., Cain, S.M., Snutch, T.P., Howland, J.G., 2016b. The GAERS model of absence epilepsy exhibits alterations in fear conditioning and latent inhibition consistent with psychiatric comorbidities in humans. Eur. J. Neurosci. 43 (1):25–40. http://dx.doi.org/10.1111/ejn.13110 (retrieved from PM:26490879).

Marks, W.N., Greba, Q., Cain, S.M., Snutch, T.P., Howland, J.G., 2016c. The T-type calcium channel antagonist Z944 disrupts prepulse inhibition in both epileptic and non-epileptic rats. Neuroscience 332:121–129. http://dx.doi.org/10.1016/j.neuroscience.2016.06.035 (S0306-4522(16)30274-3 [pii], retrieved from PM:27365170).

Marques-Carneiro, J.E., Faure, J.B., Cosquer, B., Koning, E., Ferrandon, A., de Vasconcelos, A.P., et al., 2014. Anxiety and locomotion in Genetic Absence Epilepsy Rats from Strasbourg (GAERS): inclusion of Wistar rats as a second control. Epilepsia 55 (9):1460–1468. http://dx.doi.org/10.1111/epi.12738 (retrieved from PM:25059093).

Millan, M.J., Bales, K.L., 2013. Towards improved animal models for evaluating social cognition and its disruption in schizophrenia: the CNTRICS initiative. Neurosci. Biobehav. Rev. 37 (9 Pt B):2166–2180. http://dx.doi.org/10.1016/j.neubiorev.2013.09.012 (S0149-7634(13)00221-2 [pii], retrieved from PM:24090822).

Moy, S.S., Nonneman, R.J., Shafer, G.O., Nikolova, V.D., Riddick, N.V., Agster, K.L., et al., 2013. Disruption of social approach by MK-801, amphetamine, and fluoxetine in adolescent C57BL/6J mice. Neurotoxicol. Teratol. 36:36–46. http://dx.doi.org/10.1016/j.ntt.2012.07.007 (S0892-0362(12)00142-0 [pii], retrieved from PM:22898204).

Nadler, J.J., Moy, S.S., Dold, G., Trang, D., Simmons, N., Perez, A., et al., 2004. Automated apparatus for quantitation of social approach behaviors in mice. Genes Brain Behav. 3 (5):303–314. http://dx.doi.org/10.1111/j.1601-183X.2004.00071.x (GBB071 [pii], retrieved from PM:15344923).

Nickels, K., 2015. Seizure and psychosocial outcomes of childhood and juvenile onset generalized epilepsies: wolf in sheep's clothing, or well-dressed wolf? Epilepsy Curr. 15 (3):114–117. http://dx.doi.org/10.5698/1535-7597-15.3.114 (retrieved from PM:26316843).

Olsson, I., Campenhausen, G., 1993. Social adjustment in young adults with absence epilepsies. Epilepsia 34 (5), 846–851 (retrieved from PM:8404736).

Pavone, P., Bianchini, R., Trifiletti, R.R., Incorpora, G., Pavone, A., Parano, E., 2001. Neuropsychological assessment in children with absence epilepsy. Neurology 56 (8), 1047–1051 (retrieved from PM:11320177).

Powell, K.L., Cain, S.M., Ng, C., Sirdesai, S., David, L.S., Kyi, M., et al., 2009. A Cav3.2 T-type calcium channel point mutation has splice-variant-specific effects on function and segregates with seizure expression in a polygenic rat model of absence epilepsy. J. Neurosci. 29 (2):371–380. http://dx.doi.org/10.1523/JNEUROSCI.5295-08.2009 (29/2/371 [pii], retrieved from PM:19144837).

Powell, K.L., Tang, H., Ng, C., Guillemain, I., Dieuset, G., Dezsi, G., et al., 2014. Seizure expression, behavior, and brain morphology differences in colonies of Genetic Absence Epilepsy Rats from Strasbourg. Epilepsia 55 (12):1959–1968. http://dx.doi.org/10.1111/epi.12840 (retrieved from PM:25377760).

Rantanen, K., Eriksson, K., Nieminen, P., 2012. Social competence in children with epilepsy—a review. Epilepsy Behav. 24 (3):295–303. http://dx.doi.org/10.1016/j.yebeh.2012.04.117 (S1525-5050(12)00300-9 [pii], retrieved from PM:22595143).

Talley, E.M., Cribbs, L.L., Lee, J.H., Daud, A., Perez-Reyes, E., Bayliss, D.A., 1999. Differential distribution of three members of a gene family encoding low voltage-activated (T-type) calcium channels. J. Neurosci. 19 (6), 1895–1911 (retrieved from PM:10066243).

Tringham, E., Powell, K.L., Cain, S.M., Kuplast, K., Mezeyova, J., Weerapura, M., et al., 2012. T-type calcium channel blockers that attenuate thalamic burst firing and suppress absence seizures. Sci. Transl. Med. 4 (121):121ra19. http://dx.doi.org/10.1126/scitranslmed.3003120 (4/121/121ra19 [pii], retrieved from PM:22344687).

Wang, G., Bochorishvili, G., Chen, Y., Salvati, K.A., Zhang, P., Dubel, S.J., et al., 2015. CaV3.2 calcium channels control NMDA receptor-mediated transmission: a new mechanism for absence epilepsy. Genes Dev. 29 (14):1535–1551. http://dx.doi.org/10.1101/gad.260869.115 (29/14/1535 [pii], retrieved from PM:26220996).

Winters, B.D., Reid, J.M., 2010. A distributed cortical representation underlies crossmodal object recognition in rats. J. Neurosci. 30 (18):6253–6261. http://dx.doi.org/10.1523/JNEUROSCI.6073-09.2010 (30/18/6253 [pii], retrieved from PM:20445051).