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Research Paper

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

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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,

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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

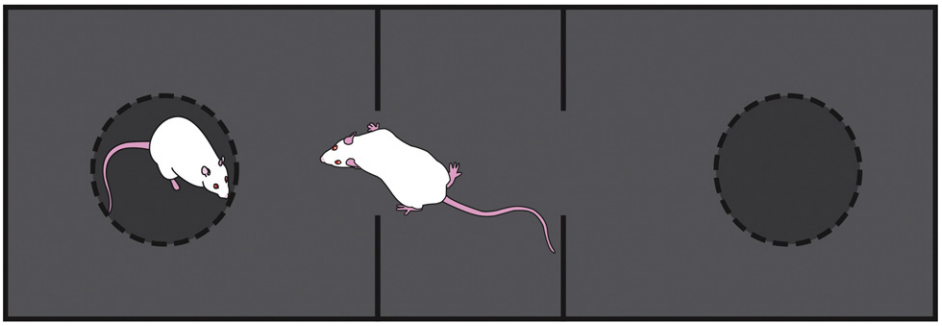

Fig. 1. Schematic of the three-chambered sociability test. See Materials and methods for details.