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THE T-TYPE CALCIUM CHANNEL ANTAGONIST Z944 DISRUPTS PREPULSE INHIBITION IN BOTH EPILEPTIC AND NON-EPILEPTIC RATS

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Abstract—The role of T-type calcium channels in brain diseases such as absence epilepsy and neuropathic pain has been studied extensively. However, less is known regarding the involvement of T-type channels in cognition and behavior. Prepulse inhibition (PPI) is a measure of sensorimotor gating which is a basic process whereby the brain filters incoming stimuli to enable appropriate responding in sensory rich environments. The regulation of PPI involves a network of limbic, cortical, striatal, pallidal and pontine brain areas, many of which show high levels of T-type calcium channel expression. Therefore, we tested the effects of blocking T-type calcium channels on PPI with the potent and selective T-type antagonist Z944 (0.3, 1, 3, 10 mg/kg; i.p.) in adult Wistar rats and two related strains, the Genetic Absence Epilepsy Rats from Strasbourg (GAERS) and Non-Epileptic Control (NEC). PPI was tested using a protocol that varied prepulse intensity (3, 6, and 12 dB above background) and prepulse-pulse interval (30, 50, 80, 140 ms). Z944 decreased startle in the Wistar strain at the highest dose relative to lower doses. Z944 dose-dependently disrupted PPI in the Wistar and GAERS strains with the most potent effect observed with the higher doses. These findings suggest that T-type calcium channels contribute to normal patterns of brain activity that regulate PPI. Given that PPI is disrupted in psychiatric disorders, future experiments that test the specific brain regions involved in the regulation of PPI by T-type calcium channels may help inform therapeutic development for those suffering from sensorimotor gating impairments.

Key words: sensorimotor gating, GAERS, Wistar, startle response.

INTRODUCTION

Low-voltage-activated T-type calcium channels contribute to the functioning of both normal physiological processes such as sleep and heart pacemaker activity, as well as the pathophysiological processes involved in absence epilepsy and neuropathic pain (Nelson et al., 2006; Cain and Snutch, 2013; Cheong and Shin, 2013; Crunelli et al., 2014; Mesirca et al., 2014). T-type calcium channels exhibit unique biophysical properties implicated in the generation of low-threshold spikes that can lead not only to the oscillatory behavior in the brain observed during sleep, but also the burst-firing observed during pathological events such as absence seizures (Cain and Snutch, 2010, 2013).

The term sensorimotor gating describes the phenomenon of a weak sensory event inhibiting a motor response to a subsequent larger sensory event (Swerdlow et al., 2000). PPI is a form of sensorimotor gating that reflects the normal suppression of a startle reflex when an intense stimulus, such as a tone, is preceded by a weaker tone prestimulus (Swerdlow et al., 2000). PPI has high cross species validity between humans and rodents, face and predictive validity, ease of implementation, and reliability (Powell et al., 2012). Interestingly, disrupted PPI is observed in patients with psychiatric and neurological disorders such as schizophrenia, obsessive compulsive disorder, Huntington's disease, temporal lobe epilepsy with psychosis, and Tourette's syndrome (Braff et al., 2001; Castellanos et al., 1996; Geyer et al., 2001; Swerdlow et al., 1993, 1995). Consistent with the neural circuits thought to be involved in these disorders (Swerdlow et al., 1992; Klarner et al., 1998), PPI is regulated by limbic and cortico-striato-pallido-pontine (CSPP) circuits (Koch and Schnitzler, 1997; Swerdlow et al., 2000; Fendt et al., 2001; Yeomans et al., 2006), areas in which T-type calcium channels are expressed (Talley et al., 1999).

Previous research has demonstrated that Cav 3.1 T-type channels in the thalamocortical circuit mediate forward suppression (sensory gating) of auditory cortex neurons, a phenomenon observed when a brief sound subsequently suppresses the neuronal responsiveness to a successive sound of equal magnitude presented within hundreds of milliseconds (Bayazitov et al., 2013). Investigation of the role of T-type calcium channels in mediating PPI is warranted for several reasons. First, there is a wide distribution of T-type calcium channel isoforms in the CSPP circuitry. Second, known disruptions in PPI are observed in disorders such as schizophrenia,

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Abbreviations: ANOVA, analysis of variance; CSPP, cortico-striato-pallido-pontine; GABA, γ-Aminobutyric acid; GAERS, Genetic Absence Epilepsy Rats from Strasbourg; NEC, non-epileptic control; NMDA, N-methyl-D-aspartate; PPF, prepulse facilitation; PPI, prepulse inhibition.

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obsessive compulsive disorder, Huntington's disease, temporal lobe epilepsy with psychosis, and Tourette's syndrome which are all characterized by CSPP abnormalities. Lastly, T-type channels are known to contribute to the suppression of neuronal responses to repetitive auditory stimuli. The recently developed drug, Z944, is a robust pan-T-type calcium channel blocker (Tringham et al., 2012) with demonstrated dose-dependent attenuation of absence seizures and the progression of amygdala kindling in rats, and pain in humans (Tringham et al., 2012; Lee, 2014; Casillas-Espinosa et al., 2015). With this in mind, the objective of the present study was to examine the dose-dependent effect of Z944 on PPI performance in three separate strains of rats (Wistar, GAERS, and NEC). The PPI protocol comprised a range of prepulse-pulse intervals (30, 50, 80, and 140 ms) and prepulse intensities (3, 6, and 12 dB) as it has been demonstrated in both clinical populations and rodents that PPI can fluctuate depending on the interaction of drug treatment with specific PPI protocol parameters (Ballendine et al., 2015; Chandna et al., 2015; Duncan et al., 2001; Howland et al., 2012; Pinnock et al., 2015; Swerdlow et al., 2016, 2008). We expected to observe a dose-dependent alteration in PPI following Z944 treatment in the Wistar and NEC strains. GAERS are a well-described rodent model of childhood absence epilepsy that display a gain-of-function missense mutation in the Cav3.2 T-type calcium channel, R1584P (Powell et al., 2009). Given the observed gain-of-function of $Ca_V3.2$ channels in GAERS, we were uncertain as to whether PPI would be altered in this strain following treatment with Z944. We found that the highest dose of Z944 (10 mg/kg) had a profound effect on PPI in the Wistar and GAERS strains. Significant reductions in PPI in the Wistar and GAERS strains following Z944 treatment at varying prepulse intervals and intensities were observed with a dose-dependent effect on startle in the Wistar strain only.

EXPERIMENTAL PROCEDURES

Animals

Male Wistar rats (Charles River Laboratories, Quebec, Canada), and male and female rats from two related strains, GAERS and NEC (University of Saskatchewan Lab Animal Services Unit, Saskatoon, Canada) (Marks et al., 2016a,b) were used for these experiments (Wistar $N = 40$, 8 per treatment group; NEC $N = 60$, 12 per treatment group; GAERS $N = 60$, 12 per treatment group). Wistar rats weighed 300–500 g throughout the course of testing; whereas NEC and GAERS weighed 170–350 g and 130–250 g, respectively. Body weights for all rats are typical for young adults of each strain. 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. Food (Purina Rat Chow) and water were available *ad libitum*. Experimental procedures were carried out during the light phase (lights on at 07:00 h). All experimental procedures were conducted in accordance with the Canadian Council on Animal Care and were approved by the University of Saskatchewan Animal Research Ethics Board.

Drug preparation

Z944 (Tringham et al., 2012; Marks et al., 2016a) was prepared fresh daily in a 0.06 mg/ml, 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 at a volume of 5 ml/kg to yield doses of 0.3 mg/kg, 1 mg/kg, 3 mg/kg, or 10 mg/kg. The highest dose of Z944 used was based on previous research demonstrating significant blockade of T-type calcium channels without altering the state of alertness (Tringham et al., 2012). Z944 or vehicle was administered 15 min prior to prepulse inhibition testing.

Prepulse inhibition

Rats were handled in small groups for 5 min/day at least 3 times before the first PPI session. The PPI testing procedure was conducted according to a previously published protocol (Howland et al., 2012; Marks et al., 2016b). Two SR-Lab startle boxes (San Diego Instruments, San Diego, CA, USA) were used for testing. Each session began with a 5-min acclimatization period during which a background noise (70 dB) was present for the entire testing period. After acclimatization, 6 pulse-alone trials (120 dB, 40 ms) were presented to obtain a steady level of startle amplitude before presentation of the prepulse + pulse trials. Immediately following the 6 pulse-alone trials, a total of 102 trials of 4 different types were presented in pseudorandom order: pulse-alone (6 trials; 120 dB, 40 ms), prepulse alone (6 trials × 3 prepulse intensities, 20 ms) prepulse + pulse (6 trials × 3 prepulse intensities × 4 prepulse-pulse time intervals – parameters described below), or no stimulus (6 trials). Prepulse + pulse trials began with a 20 ms prepulse of 3, 6, or 12 dB above background noise (70 dB). Four different prepulse – pulse intervals of 30, 50, 80, or 140 ms were used between the onset of the prepulse and the onset of the 120-dB pulse. Each testing session ended with another 6 pulse-alone trials (120 dB, 40 ms). The inter-trial interval varied from 3 to 14 s (average 7.5 s) in random order. After each session, boxes were cleaned with 70% ethanol.

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 $p \leqslant 0.05$. Greenhouse-Geisser corrections were made for violations of sphericity (Mauchley's Test) for all repeated measures analysis of variance (ANOVA). Post hoc analyses were performed using Tukey HSD. Significant three-way interactions were followed up with separate ANOVAs and Tukey post hoc tests. PPI was observed for the 50-, 80-, and 140-ms interval; whereas, the 30-ms interval produced prepulse facilitation. Therefore, data for the 30-ms interval were analyzed separately (Howland et al., 2012; Marks et al., 2016b). Startle data were analyzed with two-way mixed factor ANOVAs (Drug Treatment as the between measures

factor and Pulse Block as the repeated measures factor). Three-way mixed factor ANOVAs (Drug Treatment as the between measures factor and Prepulse-pulse interval and Prepulse Intensity as repeated measures factors) were performed separately for each rat strain.

RESULTS

A four-way mixed factor ANOVA (Strain and Treatment as between measures factors and Prepulse-pulse interval and Prepulse Intensity as repeated measures factors) was initially run for all data. The main effect of Strain ($F(2,145) = 2.20$, $p = 0.115$) and the Strain by Treatment interaction ($F(8,145) = 1.86$, $p = 0.071$) were both non-significant. However, given the gain-of-function mutation of T-type calcium channels in the GAERS strain, and the trend toward a significant Strain by Treatment interaction, the remaining analyses are presented with separate three-way mixed factor ANOVAs.

Wistar

Startle (Fig. 1A, B). A significant within-subject effect of Pulse Block indicates that all Wistar rats displayed habituation of the startle response over the course of testing (Fig. 1A, $F(2,70) = 64.36$, $p < 0.001$). Analyses also revealed a significant between-subject main effect of Treatment (Fig. 1A, $F(4,35) = 3.48$, $p = 0.017$). Post hoc analyses show rats treated with 1 mg/kg Z944 had significantly higher startle than rats treated with 10 mg/kg Z944 ($p < 0.05$). Treatment had no effect on baseline reactivity during trials in which no stimulus or the prepulses (3, 6, 12 dB) were presented alone. However, a significant within-subject effect of Prepulse alone indicates that startle in all rats increased as the prepulse alone intensity increased (Fig. 1B, $F(2.26,79.00) = 5.36$, $p = 0.005$). Post hoc analyses revealed that regardless of treatment, Wistar rats showed a significant increase in startle at the 12 dB prepulse alone stimulus relative to background noise ($p < 0.05$). All interactions were non-significant.

PPI (Fig. 2A–C). A significant within-subject effect of Interval (Fig. 2A, $F(1.42,100.20) = 6.90$, $p = 0.006$), Intensity (Fig. 2B, $F(1.61,100.20) = 57.41$, $p < 0.001$), and an Interval by Intensity interaction ($F(2.86,100.20) = 4.79$, $p = 0.004$) demonstrated that Wistar rats showed different levels of PPI at varying levels of prepulse intensity and interval. A significant between-subject effect of Treatment (Fig. 2C, $F(4,35) = 11.87$, $p < 0.001$) revealed Z944 affected PPI averaged across levels of PPI interval and intensity. Post hoc analyses showed Wistar rats treated with 10 mg/kg Z944 showed significantly lower average PPI compared to vehicle, 0.3-, and 1 mg/kg Z944-treated rats (all $p < 0.05$). Similarly, rats treated with 3 mg/kg also showed significantly lower average PPI relative to 0.3 mg/kg Z944- and vehicle-treated rats ($p < 0.05$). Aside from a three-way Treatment by Interval by Intensity interaction that neared significance ($F(11.45,100.20) = 1.85$, $p = 0.053$), all other main effects and interactions were non-significant. When 30-ms interval trials were examined (Fig. 5A), a significant within-subject effect of Intensity ($F(2,70) = 14.92$, $p < 0.001$) was observed indicating a change from prepulse facilitation (PPF) to PPI as prepulse intensity increased. A significant between-subject effect of Treatment was also found

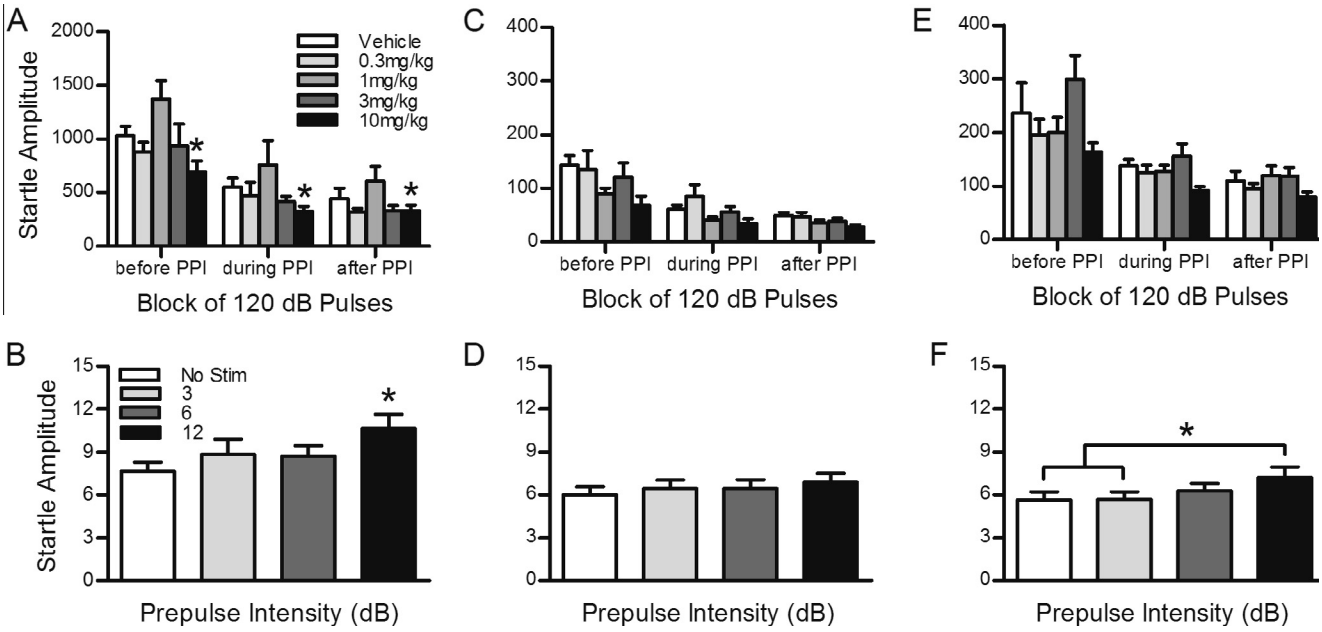

Fig. 1. Acoustic startle response in Wistar (A, B), NEC (C, D), and GAERS (E, F) to the 120 dB pulse in pulse alone trials and to the pre-pulse of varying intensities in pre-pulse alone trials. (A,C,E) All rat strains demonstrated significant habituation of the startle response over the course of testing (significance not depicted on figure). (A) Wistar rats treated with the 10 mg/kg dose showed lower startle response relative to 1 mg/kg-treated rats (A, $^{*}p < 0.05$). (B) An increase in startle was observed in the Wistar strain with a prepulse intensity of 12 dB relative to no stimulus (B, $^{*}p < 0.05$). (D) Startle in the NEC strain was not affected by prepulse alone intensity. (F) An increase in startle was observed in the GAERS strain with a prepulse intensity of 12 dB relative to both the 3 dB prepulse and no stimulus trials (F, $^{*}p < 0.05$).

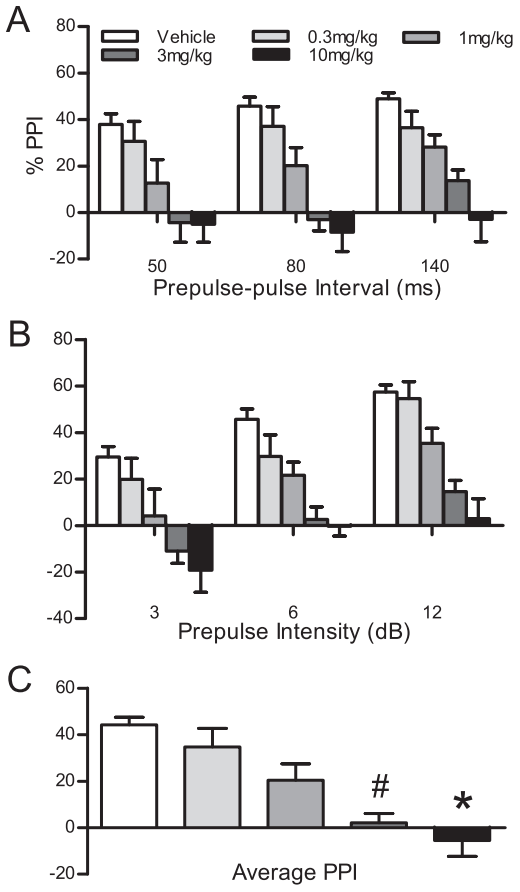

Fig. 2. Prepulse inhibition of the startle response to long prepulse intervals (%PPI) in the Wistar strain. (A) PPI at the 50-, 80-, and 140-ms intervals collapsed across prepulse intensity. (B) PPI at the 3-, 6-, and 12-dB intensities collapsed across prepulse-pulse interval. (A, B) Wistar rats, regardless of treatment group, showed different levels of PPI at varying levels of prepulse intensity and prepulse-pulse interval (significance not depicted on figures). (C) Average PPI collapsed across both prepulse intensity and prepulse-pulse interval. Wistar rats treated with 10 mg/kg Z944 showed significantly lower average PPI compared to vehicle-, 0.3-, and 1 mg/kg-treated rats (C, $^{*}p < 0.05$). Rats treated with 3 mg/kg also showed significantly lower PPI relative to vehicle and 0.3 mg/kg Z944 (C, $^{\#}p < 0.05$).

($F(4,35) = 3.19$, $p = 0.025$). Overall, vehicle-treated rats displayed significant PPI during the 30-ms interval compared to significant PPF observed in rats treated with 10 mg/kg of Z944 ($p < 0.05$). The Treatment by Intensity interaction was not significant at the 30-ms interval.

NEC

Startle (Fig. 1C, D). Statistical analyses revealed a significant within-subject effect of Pulse Block (Fig. 1C, $F(1.26,69.12) = 57.07$, $p < 0.001$) indicating habituation of the startle response in NEC across all treatments. The main effect of Treatment and Treatment by Pulse Block interaction were not significant. Baseline reactivity was not significantly affected by treatment or prepulse alone intensity during no stimulus or prepulse alone trials (Fig. 1D). The interaction term was also non-significant.

PPI (Fig. 3A–C). A significant within-subject effect of Intensity (Fig. 3B, $F(1.63,158.67) = 166.87$, $p < 0.001$)

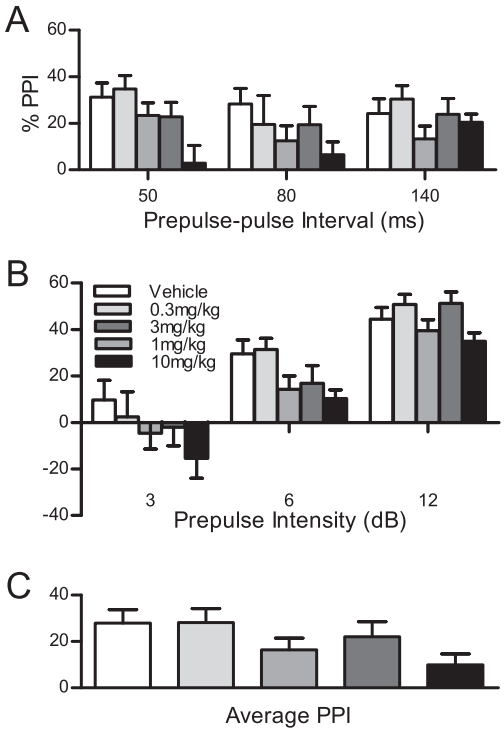

Fig. 3. Prepulse inhibition of the startle response to long prepulse intervals (%PPI) in the NEC strain. (A) PPI at the 50-, 80-, and 140-ms intervals collapsed across prepulse intensity. (B) PPI at the 3-, 6-, and 12-dB intensities collapsed across prepulse-pulse interval. (A, B) NEC rats, regardless of treatment group, showed different levels of PPI at varying levels of prepulse intensity and prepulse-pulse interval (significance not depicted on figures). (C) Average PPI collapsed across both prepulse intensity and prepulse-pulse interval.

and Intensity by Interval interaction (Fig. 3A, B, $F(2.89,158.67) = 8.73$, $p < 0.001$) show that PPI in NEC rats changes depending on the intensity and interval of prepulse presentations. Although a significant three-way Treatment by Interval by Intensity interaction was found ($F(11.54,158.67) = 1.88$, $p = 0.042$), all other main effects and interactions were non-significant. Post hoc analysis of the three-way interaction revealed that for trials with the 12 dB prepulse intensity at each prepulse-pulse interval, animals treated with vehicle, 0.3, and 3 mg/kg of Z944 had significantly higher PPI than animals treated with 3 and 10 mg/kg Z944 during the 3-dB intensity at the 50 and 80 prepulse-pulse intervals ($p < 0.05$; significance not portrayed on the figure). Analysis of the 30-ms interval revealed a significant main effect of Intensity (Fig. 5B, $F(2,110) = 85.22$, $p < 0.001$). PPF was observed at the 3- and 6-dB intensities while PPI was observed at the 12-dB intensity. The main effect of Treatment and Treatment by Intensity interaction were not significant for the 30-ms Interval.

GAERS

Startle (Fig. 1E, F). Habituation of the startle response in GAERS is demonstrated by a significant within-subject effect of Pulse Block (Fig. 1E, $F(1.25,68.87) = 46.11$, $p < 0.001$). A trend toward significance was found for

the between-subject effect of Treatment (Fig. 1E, $F(4,55) = 2.46$, $p = 0.056$). The interaction of Treatment with Pulse Block was non-significant. Analysis of startle reactivity on prepulse alone and no stimulus trials revealed a significant within-subject effect of Prepulse alone (Fig. 1F, $F(1.54,84.67) = 8.74$, $p = 0.001$). Collapsed across treatments, startle reactivity increased at the 12 dB prepulse intensity compared to no stimulus and the 3 dB prepulse intensity ($p < 0.05$). Treatment did not significantly affect startle reactivity during prepulse alone trials, nor was a Treatment by Prepulse alone intensity interaction significant.

PPI (Fig. 4A–C). Analyses revealed a significant within-subject effect of Interval (Fig. 4A, $F(2,110) = 5.25$, $p = 0.007$), and Intensity (Fig. 4B, $F(2,110) = 165.11$, $p < 0.001$) as well as an Interval by Intensity interaction. These results indicated that PPI in GAERS is dependent on the level of prepulse Interval and Intensity. A dose-dependent effect of Z944 on PPI was demonstrated by a significant between-subject effect of Treatment (Fig. 4C, $F(4,55) = 5.50$, $p = 0.001$). Post hoc tests revealed that, averaged across all prepulse intervals and intensities, GAERS treated with 10 mg/kg Z944 showed significantly reduced PPI compared to vehicle- and 0.3 mg/kg-treated rats ($p < 0.05$). A significant interaction between Treatment and Intensity was also found (Fig. 4B, $F(8,110) = 2.85$, $p = 0.006$). Post hoc analyses indicated that at the 3 and 6 dB prepulse intensities, rats treated with 10 mg/kg Z944 had significantly lower PPI than vehicle- and 0.3 mg/kg-treated rats ($p < 0.05$). Post hoc analyses also show that at the 3 dB prepulse intensity, rats treated with 3 mg/kg Z944 had significantly lower PPI than both vehicle- and 0.3 mg/kg Z944-treated rats ($p < 0.05$). At the 30-ms interval, a significant within-subject effect of Intensity was found (Fig. 5C, $F(1.76,96.62) = 82.83$, $p < 0.001$). As prepulse intensity increased from 3 to

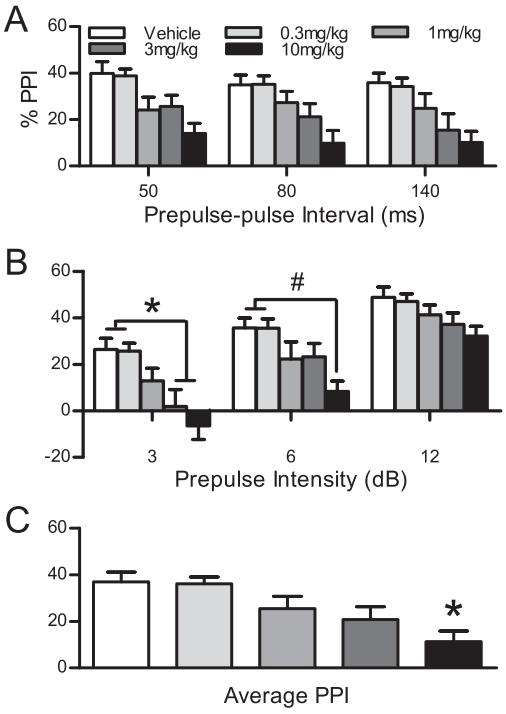

Fig. 4. Prepulse inhibition of the startle response to long prepulse intervals (%PPI) in the GAERS strain. (A) PPI at the 50-, 80-, and 140-ms intervals collapsed across prepulse intensity. (B) PPI at the 3-, 6-, and 12-dB intensities collapsed across prepulse-pulse interval. (A, B) GAERS rats, regardless of treatment group, showed different levels of PPI at varying levels of prepulse intensity and prepulse-pulse interval (significance not depicted on figures). (B) At the 3 dB prepulse intensity, both the 10- and 3 mg/kg-treated GAERS had significantly lower PPI than vehicle- and 0.3 mg/kg-treated rats (B, $^{*}p < 0.05$). At the 6 dB prepulse intensities, GAERS treated with 10 mg/kg Z944 had significantly lower PPI than vehicle and 0.3 mg/kg-treated rats (B, $^{\#}p < 0.05$). (C) Average PPI collapsed across both prepulse intensity and prepulse-pulse interval. GAERS treated with 10 mg/kg Z944 showed significantly reduced PPI compared to vehicle and 0.3 mg/kg-treated rats (C, $^{*}p < 0.05$).

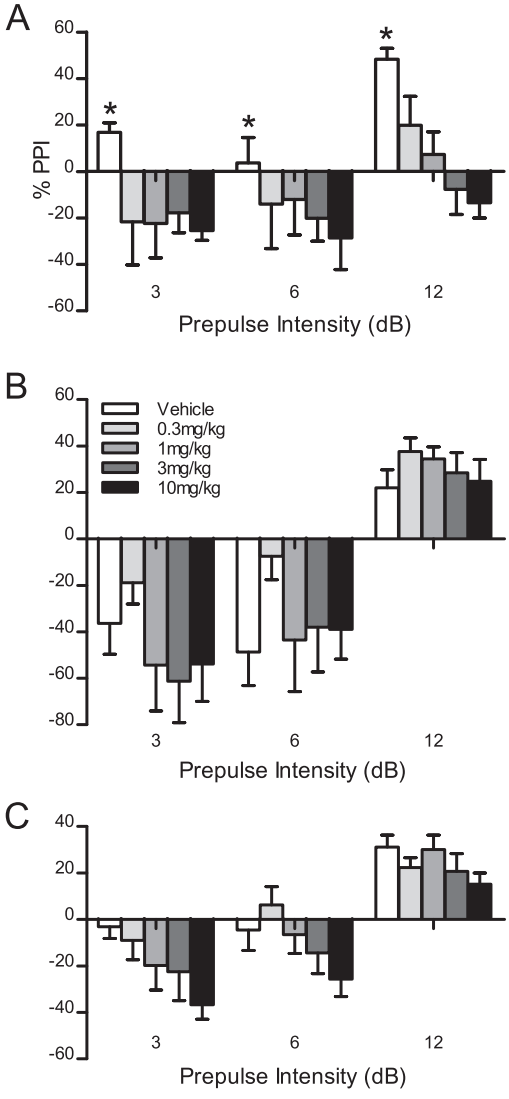

Fig. 5. Prepulse inhibition and facilitation of the startle response to short prepulse intervals (%PPI) in Wistar (A), NEC (B), and GAERS (C). (A–C) A significant change from PPF to PPI was observed in all strains as prepulse intensity increased (significance not depicted on figures). (A) Vehicle-treated Wistar rats displayed significant PPI during the 30-ms interval compared to significant PPF observed in rats treated with 10 mg/kg of Z944 (A, $^{*}p < 0.05$). (B, C) Treatment did not interact with prepulse intensity in the NEC and GAERS strains.

12 dB, a change from PPF to PPI was observed in all treatment groups. All other main effects and interactions were non-significant.

DISCUSSION

We investigated the effects of a recently developed high affinity, selective T-type calcium channel blocker, Z944, on startle and PPI in Wistar, NEC, and GAERS rats. The highest dose of Z944 tested (10 mg/kg) had a profound effect on PPI in the Wistar and GAERS strains. Significant reductions in PPI were observed following Z944 treatment at varying prepulse intervals and intensities with a dose-dependent effect on startle in the Wistar strain only (Figs. 1, 2, 4). Z944 also produced significant prepulse facilitation at the 30 ms prepulse-pulse interval in the Wistar strain only (Fig. 5A).

PPI in three Wistar-derived rat strains

PPI was observed at the 50-, 80-, and 140-ms intervals in Wistar, NEC, and GAERS rats. The degree of PPI observed in the vehicle-treated Wistar rats is slightly lower relative to those observed in other Wistar colonies at the longer prepulse-pulse intervals (Schwabe et al., 2007; Brosda et al., 2011; Goepfrich et al., 2013). We found comparable average PPI to those observed with a 100 ms prepulse-pulse interval at varying intensities in the GAERS strain, albeit slightly lower PPI in the NEC strain (Jones et al., 2010). These trends are similar to those previously reported by our lab for GAERS and NEC (Marks et al., 2016b). The variations in PPI observed in the Wistar and NEC strain could potentially be explained by putative genetic variations between Wistar and NEC colonies (Goepfrich et al., 2013; Powell et al., 2014). Alternatively, variations in PPI could also be explained by differences in startle apparatus used or calibration differences between studies. Interestingly, PPI has been observed in Wistar rats at a shorter 25 ms prepulse-pulse interval (Brosda et al., 2011), which is consistent with our findings. However, prepulse facilitation and PPI have been observed for short prepulse-pulse intervals in the Long-Evans and Sprague–Dawley strains (Mansbach and Geyer, 1991; Howland et al., 2012; Pinnock et al., 2015). Similar to previous findings, prepulse facilitation was observed in NEC rats at the 30-ms interval for low prepulse intensities, whereas GAERS showed no change in startle on these trials (Jones et al., 2010; Marks et al., 2016b). PPI was observed in both strains for trials with 12 dB prepulses (30-ms interval). Taken together, these results suggest that strains of rats with greater PPI for long interval trials have less prepulse facilitation for short interval trials.

Z944 reduces PPI in a dose-dependent manner

Significant dose-dependent decreases in PPI were observed in the Wistar and GAERS strains following acute Z944 treatment. To our knowledge, this is the first study to demonstrate the effects of a specific and potent T-type calcium channel blocking effect on PPI. We used a PPI protocol with a range of intervals as previous research has shown interval-specific effects of some manipulations, including those related to cholinergic and γ-Aminobutyric acid (GABA) ergic receptors, on PPI (Jones and Shannon, 2000; Fendt et al., 2001; Yeomans et al., 2010; Pinnock et al., 2015). These data led to the hypothesis that activation of ionotropic receptors contribute to the inhibition of startle by short-interval prepulses while metabotropic receptor activation contributes to the inhibition observed following longer intervals (100–500 ms). The effects of Z944 on PPI in all strains were quite consistent regardless of the interval used. Thus, it does not appear that the interval-specific effects of some manipulations on PPI extend to T-type calcium channels.

Although sensory gating, i.e., a decrease in the neuronal response elicited by non-startling stimuli, and sensorimotor gating, i.e., a decrease in the motor response elicited by weak stimuli followed by startling stimuli, represent different physiological events, the mechanism underlying the two phenomena may be similar. Z944 attenuates burst firing in thalamic reticular nucleus neurons in both GAERS and NEC rats (Tringham et al., 2012). Recent research has demonstrated that sensory gating in the auditory cortex following repetitive auditory stimuli is attributable to a switch from burst to tonic firing modes within thalamic neurons (Bayazitov et al., 2013). It has also been demonstrated that Cav3.1 T-type calcium channels contribute to the switch in firing modes during sensory gating as Cav3.1 knockdown or inhibition both significantly decreased the suppression of auditory-induced responses in the auditory cortex (Bayazitov et al., 2013). Thus, similar to the decrease in sensory gating produced by knockdown or inhibition of T-type calcium channels, we dose-dependently decreased sensorimotor gating with Z944 treatment. Given that PPI is regulated by the thalamus (Swerdlow et al., 2002), it could be speculated that a decrease in burst-firing in the thalamus produced by Z944 may underlie the decrease in PPI observed. The Z944-medated suppression of sensorimotor gating we observed across the Wistar and GAERS strains indicates that T-type calcium channels contribute to the normal patterns of brain activity that regulate PPI. Although a decrease in PPI was observed in the NEC strain following Z944 treatment, the effect was not significant. Given the trend toward significance, however, an increased sample size in the NEC strain may have resulted in significant effects. Overall, the data suggest that the magnitude of the effect of T-type calcium channel blockade on PPI is complex and likely affected by genetic variations between rat strains.

Regulation of glutamatergic activity and N-methyl-D-aspartate (NMDA) receptor function is well documented to affect PPI. Most notably, blockade of NMDA receptors produces robust disruptions in PPI performance in rats (Klarner et al., 1998; Geyer et al., 2001). The glutamate agonist, 3,5-Dr-L-Phe, acts to both reduce seizures as well as PPI deficits caused by the NMDA antagonist, MK-801 (Cao et al., 2009). Interestingly, high doses of the NMDA agonist, d-cycloserine, reduce PPI in Sprague–Dawley rats (Depoortere et al.,

1999). Thus, numerous lines of evidence suggest common underlying neural mechanisms that mediate both epileptic activity as well as sensorimotor gating, possibly through modulation of glutamatergic activity. Interestingly, both a significant decrease and increase in NMDA receptor signaling results in decreased PPI performance. Of note, recent evidence suggests $Ca_V3.2$ T-type channels play a direct role in the regulation of synaptic NMDA receptor transmission. Specifically, expression of the 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 it could be speculated that Z944 may alter PPI through effects on glutamatergic signaling in the limbic and CSPP circuitry. Given that we observed a decrease in PPI following Z944 administration, it is possible that there is an optimal level T-type calcium channel expression and concomitant glutamatergic activity for optimal PPI behavior.

An alternative explanation for the observed effects of Z944 on PPI is that Z944 significantly affects alertness or sensory processing. T-type channels have a known and 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). Activity in this network of areas is also known to regulate PPI activity (Swerdlow et al., 2000; Li et al., 2009). Other T-type calcium channel blockers, such as TTA-A2, have demonstrated effects on wakefulness and have been recognized as potential therapeutic targets for sleep disorders (Kraus et al., 2010). Despite the known actions of T-type calcium channels on sleep processes, we believe the effects of Z944 are not the result of a decrease in alertness or sensory processing. First, previous research with the agent has demonstrated that the delta brainwaves observed during drowsiness were not produced by an acute 10 mg/kg dose of Z944 (Tringham et al., 2012). Further, the 10 mg/kg dose of Z944 did not differ from vehicle treatment on observable behaviors consistent with sedation (Tringham et al., 2012). Second, in the current study Z944 did not significantly affect startle reactivity to the prepulse alone trials in any of the strains tested. Given that startle was not consistently affected by Z944 treatment, it is unlikely that an altered state of alertness or deficit in sensory processing is the underlying cause of the decrease in PPI observed.

Robust deficits in sensorimotor gating are observed in psychiatric illnesses such as schizophrenia, obsessive compulsive disorder, and Tourette syndrome (Castellanos et al., 1996; Geyer et al., 2001; Swerdlow et al., 1993, 1995). Of note, commonly used typical and atypical antipsychotics exhibit effects on PPI in rodent models. For example, the $D_{2/3}$ receptor antagonist, haloperidol, reduces startle and increases PPI (Schwarzkopf et al., 1993). Further, Wistar rats selectively bred for low PPI demonstrated restored PPI with haloperidol treatment (Hadamitzky et al., 2007). The atypical antipsychotic, clozapine, has been shown to dose dependently decrease both startle amplitude and PPI performance (Wiley, 1994). Interestingly, in combination with drugs that model sensorimotor gating deficits in schizophrenia, both typical and atypical antipsychotics reverse disruptions in PPI (Abekawa et al., 2011; Li et al., 2011; Tournier and Ginovart, 2014). The mechanism of action that underlies the alteration of PPI performance observed with antipsychotic treatment requires further investigation, but of important note, both typical and atypical antipsychotics have demonstrated T-type calcium channel blocking activity (Enyeart et al., 1992; Santi et al., 2002; Choi and Rhim, 2010).

CONCLUSIONS

Z944 had a profound effect on PPI in the Wistar and GAERS strains with significant reductions in PPI observed following Z944 treatment at varying prepulse intervals and intensities. The effects of Z944 in the NEC strain were less robust indicating genetic variation between rat strains may contribute to the effects of T-type calcium channel blockers on PPI. Next generation T-type calcium channel blockers have demonstrated promise in preclinical assays of antipsychotic-like activity. For example, the T-type calcium channel antagonist, TTA-A2, blocked the psychostimulant effects of both amphetamine and MK-801 on psychomotor activity as well as an amphetamine-induced increase in c-fos expression in the nucleus accumbens in rats (Uslaner et al., 2012). Similarly, sensitized hyperlocomotion produced by d-amphetamine or cocaine was blocked by TTA-A2 in C57BL/6J wild-type mice (Gangarossa et al., 2014). Given that PPI is disrupted in human psychiatric disorders, future experiments examining the specific brain regions involved in the regulation of PPI by T-type calcium channels may help inform therapeutic development for those suffering from sensorimotor gating impairments.

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