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The Genetic Absence Epilepsy Rats from Strasbourg model of absence epilepsy exhibits alterations in fear conditioning and latent inhibition consistent with psychiatric comorbidities in humans

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Abstract

Behavioural, neurological, and genetic similarities exist in epilepsies, their psychiatric comorbidities, and various psychiatric illnesses, suggesting common aetiological factors. Rodent models of epilepsy are used to characterize the comorbid symptoms apparent in epilepsy and their neurobiological mechanisms. The present study was designed to assess Pavlovian fear conditioning and latent inhibition in a polygenetic rat model of absence epilepsy, i.e. Genetic Absence Epilepsy Rats from Strasbourg (GAERS) and the non-epileptic control (NEC) strain. Electrophysiological recordings confirmed the presence of spike-wave discharges in young adult GAERS but not NEC rats. A series of behavioural tests designed to assess anxiety-like behaviour (elevated plus maze, open field, acoustic startle response) and cognition (Pavlovian conditioning and latent inhibition) was subsequently conducted on male and female offspring. Results showed that GAERS exhibited significantly higher anxiety-like behaviour, a characteristic reported previously. In addition, using two protocols that differed in shock intensity, we found that both sexes of GAERS displayed exaggerated cued and contextual Pavlovian fear conditioning and impaired fear extinction. Fear reinstatement to the conditioned stimuli following unsignalled footshocks did not differ between the strains. Male GAERS also showed impaired latent inhibition in a paradigm using Pavlovian fear conditioning, suggesting that they may have altered attention, particularly related to previously irrelevant stimuli in the environment. Neither the female GAERS nor NEC rats showed evidence of latent inhibition in our paradigm. Together, the results suggest that GAERS may be a particularly useful model for assessing therapeutics designed to improve the emotional and cognitive disturbances associated with absence epilepsy.

Introduction

The majority of patients diagnosed with epilepsy also experience symptoms of altered cognition and changes in behaviour that may relate to psychiatric illness (Gaitatzis *et al.*, 2004; Hermann *et al.*, 2008; Clancy *et al.*, 2014). For example, children with absence epilepsy experience behavioural (Caplan *et al.*, 2009), cognitive (Mandelbaum & Burack, 1997; Pavone *et al.*, 2001; Henkin *et al.*, 2005; Caplan *et al.*, 2009; Killory *et al.*, 2011; Loughman *et al.*, 2014), and linguistic comorbidities (Caplan *et al.*, 2009) in addition to the bilateral spike-wave discharges (SWDs) characteristic of absence seizures (Tucker *et al.*, 2007). Genetic Absence Epilepsy Rats from Strasbourg (GAERS) is a model of epilepsy closely resembling childhood absence epilepsy (Marescaux *et al.*, 1992). GAERS, but not its non-epileptic control (NEC) strain, show absence seizures that depend on alterations in the thalamocortical circuitry (Danober *et al.*, 1998; Cain & Snutch, 2013). In particular, a mis-sense mutation in Cav3.2 T-type calcium channels is thought to contribute to seizure activity (Powell *et al.*, 2009) and administration of T-type calcium channel blockers attenuates absence seizures in GAERS (Tringham *et al.*, 2012).

Behaviourally, GAERS exhibit anxious and psychotic-like phenotypes, suggesting that they may share some psychiatric comorbidities associated with epilepsy (Jones *et al.*, 2008, 2010; but see also Marques-Carneiro *et al.*, 2014). Anxiety-like behaviour in GAERS is reflected in the elevated plus maze (Jones *et al.*, 2008; Powell

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et al., 2014), open field test (Jones *et al.*, 2008; Bouilleret *et al.*, 2009; Dezsi *et al.*, 2013; Powell *et al.*, 2014), and in response to stimuli that elicit an acoustic startle response (Jones *et al.*, 2010). Enhanced locomotor hyperactivity in response to amphetamine, a common rodent model of acute psychosis, is seen in GAERS both before and after the onset of SWDs, indicating that increased sensitivity is not a secondary effect of seizures (Jones *et al.*, 2010). This hyperactivity may relate to the altered expression of dopamine transporters and receptors in GAERS (Jones *et al.*, 2010), which is relevant to the altered dopaminergic transmission in schizophrenia and other psychoses. As prepulse inhibition (PPI), a measure of sensorimotor gating relevant to psychosis (Braff *et al.*, 2001), was unaltered in GAERS, the strain has been proposed to exhibit some, but not all, endophenotypes of psychosis (Jones *et al.*, 2010).

Given that patients with absence epilepsy exhibit cognitive alterations associated with certain psychiatric disorders, we felt it important that the GAERS absence model also be characterized for cognitive disruptions. The present experiments were designed to assess whether GAERS show deficits in Pavlovian fear conditioning and latent inhibition (LI), two paradigms that depend on interactions between cortical, striatal, limbic, and thalamic brain regions and are commonly used to assess animal models of psychiatric illness (Weiner & Arad, 2009; Milad & Quirk, 2012; Herry & Johansen, 2014). It is of note that we used behavioural paradigms that allow for assessments of learning, memory, extinction, and reinstatement over several testing days. Our findings suggest that increased anxiety-like behaviour is a pervasive trait in the GAERS strain. Further, impaired cued and contextual fear extinction and LI suggest that GAERS exhibit certain psychiatric comorbidities.

Materials and methods

Subjects

Three breeding pairs of the GAERS and NEC strains (total $n = 12$) were supplied as weanlings in December 2013 from the Snutch Laboratory at the University of British Columbia (snutchlab.msl.ubc.ca). This strain was originally established in Canada in 2010 by animals generously provided by Drs Terence J. O'Brien and Kim L. Powell at The University of Melbourne (Powell *et al.*, 2009). Breeding pairs were genotyped to confirm the presence and absence of the R1584P mutation in GAERS and NEC animals, respectively (Powell *et al.*, 2009). Rats were maintained in a temperature-controlled room (21 °C) on a 12 h day/night cycle (lights on at 07:00 h) with *ad libitum* access to standard rat chow and water. Upon arrival, the founder rats were treated with a grain-based fenbendazole diet (Diet S3867; BioServ, Felmington, NJ, USA) for 9 weeks (1 week on diet, 1 week off diet) to eradicate pinworms. Following treatment, the rats were confirmed to be pinworm free and were bred at approximately 5 months of age. Offspring were weaned on postnatal day (P)21–25 and housed in groups of two or three of the same strain and sex. New breeding pairs were obtained from existing litters and bred starting at 2 months of age for a maximum of four litters. 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.

Electrocorticography and local field potential recordings

To confirm the presence and absence of SWDs in adult GAERS and NEC animals, respectively, electroencephalography (EEG) recordings were performed in freely-moving rats. Four-month-old, male NEC rats ($n = 4$ recording periods in $n = 2$ animals) and GAERS ($n = 4$ recording periods in $n = 2$ animals) were implanted with a recording electrode skull screw (bregma, lateral from midline, depth from skull in mm) into the somatosensory cortex (AP + 0.5, ML 4.0, DV − 1.2) and a reference electrode skull screw into the occipital cortex (−6.0, 5.0, −1.2) under isoflurane anaesthesia. The electrocorticography electrodes were used to analyse SWD activity in NEC and GAERS animals. In addition to the EEG electrode, a number of monopolar electrodes (36 G, platinum–iridium wire) were also placed in subcortical structures to confirm or negate the involvement of seizure foci previously associated with GAERS seizures or commonly associated with psychiatric disorders. Platinum–iridium wire local field potential depth electrodes were also placed in the medial prefrontal cortex (+3.0, 0.8, −3.9, $n = 2$ GAERS, $n = 2$ NEC), basolateral amygdala (−2.7, 4.7, −8.8, $n = 1$ GAERS, $n = 1$ NEC), subthalamic nucleus (−3.6, 2.6, −8.4, $n = 1$ GAERS), lateral geniculate nucleus (−4.4, 3.8, −5.2, $n = 1$), and caudate putamen (0.0, 3.5, −5.2, $n = 1$ GAERS, $n = 1$ NEC). Electrodes were connected to a custom EEG interface fitted to the head. Following a surgical recovery period, freely-moving EEG recordings were performed using a wireless headstage and receiver (Multichannel Systems) during 60 min recording sessions. After recordings, DC current (0.30 mA, 15 s) was passed through each electrode to assist in determining placements. Rats were then perfused with saline followed by 10% formalin before the brains were sectioned on a Leica freezing microtome. SWDs were analysed semi-automatically using a custom Matlab script developed by Dr Stuart Cain and Jeff LeDue at the University of British Columbia.

Behavioural testing procedures

Prior to behavioural testing, rats were handled for 5 min/day for 3 days. Males were tested before females to avoid hormonal interference of male behaviour. Testing equipment was cleaned with a 70% ethanol solution between trials. For the elevated plus maze, open field, and PPI experiments, 27 GAERS (15 males, 12 females) and 35 NEC rats (18 males, 17 females) obtained from the first two breedings of the initial founder rats were used for testing. Rats in the elevated plus maze, open field, and PPI experiments were tested at both prepuberty (approximately 5–6 weeks of age) and young adulthood (approximately 8–9 weeks of age). For the low-intensity fear conditioning experiment, 16 GAERS (10 males, six females) and 16 NEC rats (10 males, six females) obtained from the first breeding of the second generation of breeders was used. Rats in this group ranged in age from 14 to 17 weeks. For the high-intensity experiment, 10 male GAERS and nine male NEC rats from the first two breedings of the initial founder rats were used. Rats used for the high-intensity experiment ranged in age from 12 to 20 weeks. For LI, 41 GAERS (20 males, 21 females) and 40 NEC rats (19 males, 21 females) obtained from the first two breedings of the initial founder rats as well as all breedings of the second generation breeders were used for testing. Rats used in the LI task ranged in age from 11 to 19 weeks. The shock reactivity test included eight male GAERS and eight male NEC rats from breedings three and four of the second generation breeders. Animals used for this task ranged in age from 15 to 16 weeks. Animals were tested for measures of anxiety prior to conditioning tasks to reduce the possible effects of repeated footshock on behavioural results.

Elevated plus maze

The base of the maze was constructed from 19-mm-thick plywood (Hannesson *et al.*, 2008), painted white, and the floor and walls

were made of white corrugated plastic. The maze, elevated 45 cm from the ground, consisted of two sets of perpendicular interlocking arms (110 cm in length and 10 cm in width). The interlocking central region bisected the maze into two pairs of arms, one with 45-cm-high walls ('closed arms') and one without walls ('open arms'). Rats were brought into the testing room and tested individually in the apparatus. The trials began by placing a rat in the centre platform of the maze, facing an open arm. Each trial lasted 5 min, after which the rat was removed and returned to its home cage. Using videos of the trials, an experimenter counted the total number of entries into the arms of the maze and recorded the amount of time spent in the open arms. An entry was defined as moving all four paws into the arm. Activity was assessed using the number of entries into all arms. Less time spent in open arms and fewer total arm entries are interpreted as anxiety-like behaviours (Hogg, 1996).

Open field

The open field was made of white industrial plastic, painted black to facilitate tracking with NOLDUS ETHOVISION XT (Version 6) software. The arena was 1.5 m in diameter with 45-cm-high walls. A circle of 1 m diameter in the middle of the arena was called the inner area. Rats were brought into the testing room and placed individually in the arena for 10 min. Distance travelled and time spent in the inner and outer area of the arena were analysed. Less distance travelled and less time spent in the inner area are interpreted as anxiety-like behaviours (Prut & Belzung, 2003).

Prepulse inhibition

Testing was conducted in two standard SR-LAB startle chambers (San Diego Instruments, San Diego, CA, USA) (Howland *et al.*, 2012; Ballendine *et al.*, 2015). Each PPI test session began with a 5 min acclimatization period during which a 70 dB background noise level was presented that remained constant for the entire test session. Following acclimatization, six pulse-alone trials (120 dB, 40 ms) were presented to achieve a relatively stable level of startle amplitude before presentation of the prepulse + pulse trials. The data from these pulse-alone trials were not considered in the analysis of PPI. Immediately following the six initial pulse-alone trials, a total of 102 trials of four different types were presented in a pseudorandom order: pulse alone (six trials, 120 dB, 40 ms), prepulse alone (six trials × three prepulse intensities, 20 ms), prepulse+pulse (six trials × three prepulse intensities × four prepulse–pulse time intervals, discussed below), or no stimulus (six trials). Prepulse+pulse trials consisted of the presentation of a 20 ms prepulse of 3, 6, or 12 dB above background. Prepulse–pulse time intervals were 30, 50, 80, or 140 ms between the onset of the prepulse and onset of the pulse. Each session ended with six pulse-alone trials. The intertrial interval (ITI) varied randomly from 3 to 14 s (average 7.5 s). Two measures were calculated for each rat. The first measure, startle amplitude, represents the mean amplitude of the startle response elicited by each of the six pulse-alone trial blocks. PPI was calculated by averaging the startle amplitudes for each trial type, and the percent PPI for each prepulse intensity was calculated using the formula

$$100 - (100 \times \text{startle amplitude on prepulse + pulse trials}) / (\text{startle amplitude on pulse-alone trials})$$

(Howland *et al.*, 2012; Ballendine *et al.*, 2015). The average startle amplitudes elicited during three blocks of six pulse-alone trials (before, during, and after PPI trials) were compared to measure habituation.

Fear conditioning

Four standard operant conditioning chambers (ENV-008; MedAssociates, St Albans, VT, USA) encased in sound-attenuating cubicles were used. A grid floor wired to a shock generator and scrambler (ENV-414S) delivered footshocks as unconditioned stimuli (USs). The ceiling held a high-frequency speaker (ENV-224BM) that allowed for the presentation of individual tones that were conditioned stimuli (CSs). A video camera located opposite the operant conditioning chambers recorded all behaviour.

The extent of conditioned fear in rats depends on the intensity of the US experienced during training (Cordero *et al.*, 1998). Therefore, two separate experiments using either a high-intensity or low-intensity US were used on separate groups of GAERS and NEC rats. Both protocols contained the following phases: acquisition (day 1), extinction (days 2–5), extended extinction (day 6; required for high-intensity protocol only), and reinstatement (day 6, low-intensity protocol; day 7, high-intensity protocol). All training and testing days were separated by 24 h with the exception of 96 h between days 4 and 5 of extinction.

Both high-intensity and low-intensity protocols consisted of identical training and testing procedures with the exception of the US intensity and extended extinction on day 6. On day 1 (acquisition), rats were administered five CS–US conditioning trials in 20 min. The US was a 1 s footshock (high-intensity US, 0.8 mA; low-intensity US, 0.4 mA). The CS was an auditory tone (4 kHz, 80 dB, 20 s) that coterminated with the US. One CS–US sequence was considered a single fear conditioning trial. The ITI between each trial was 180 s and a stimulus free 180 s period preceded the first trial and followed the last trial. During extinction (days 2–5, low intensity; days 2–6, high intensity), rats were presented with 20 CS-only trials (same parameters as during acquisition) with an ITI of 180 s. On the final day of testing (reinstatement), rats were subjected to a 180 s stimulus-free period followed by two unsignalled footshocks (0.8 or 0.4 mA depending on testing protocol) with an ITI of 120 s. After the second shock, rats were presented with 20 CS-only trials with an ITI of 180 s. Freezing behaviour was defined as the cessation of all movement except for that required for respiration-related movements, and non-awake or rest body postures (Sotres-Bayon *et al.*, 2009).

Latent inhibition

Latent inhibition was conducted in the same chambers as used for fear conditioning (Grecksch *et al.*, 1999; Schauz & Koch, 2000). Rats were split into two groups: one group received pre-exposure to the CS, whereas the other group was only exposed to the operant chambers without CS presentations. LI was conducted in the following sequence: pre-exposure (days 1–3), training (day 4), short-term recall (day 4), and long-term recall (day 5). A 24 h period occurred between all training and testing days with the exception of short-term recall, which occurred immediately after training on day 4. During the first 2 days of pre-exposure, rats were administered 40 CS-only trials (4 kHz, 80 dB, 20 s duration) with a 60 s ITI. On the third day of pre-exposure, rats were administered 20 CS-only

trials with an ITI of 180 s to mimic testing parameters used for fear conditioning and testing. Non-pre-exposed rats were placed into the chambers for the same length of time as pre-exposed rats with no CS presentations on days 1–3. On the training day (day 4), rats were administered 12 CS–US trials (0.475 mA footshock; same tone parameters as used during pre-exposure). Immediately following the 12th CS–US trial, a 180 s stimulus-free period preceded another five CS-only trials (180 s ITI) to test short-term recall. During long-term recall (day 5), rats were exposed to 20 CS-only trials using identical testing parameters as on day 3 of pre-exposure. Similar to fear conditioning, freezing behaviour was defined as the cessation of all movement except for that required for respiration-related movements, and non-awake or rest body postures (Sotres-Bayon *et al.*, 2009).

Shock reactivity test

Shock reactivity occurred in the chambers used for fear conditioning. Briefly, rats were administered footshocks of increasing intensity (0–0.46 mA; increments of 0.02 mA). ITI varied randomly in a range of 40–100 s. The shock intensity producing the first flinch (shoulder twitch or startle) and jump (all four limbs leaving the grid floor) were recorded (Conrad *et al.*, 2001).

Data analysis

All figures summarize means with the error bars representing SEM. SPSS Version 20 for Windows (IBM) was used for statistics. For repeated-measures ANOVA, Greenhouse–Geisser corrections were made for violations of sphericity (Mauchly's test). As strain was not found to significantly interact with age (statistics not reported), P35 and P56 data were analysed separately. Both sexes were analysed together unless otherwise stated. Between-subjects ANOVA interactions were followed up with independent-samples *post hoc t*-tests. Mixed-factor ANOVA interactions were followed with Tukey *post hoc* tests unless otherwise specified. $P < 0.05$ was considered significant for all tests. For the elevated plus maze and open field, data were analysed with two-way (Strain × Sex) ANOVAs. For elevated plus maze data, all analyses were run on the percentage of time spent in the open arm using the formula

$$(\text{time in open arms/total test time}) \times 100$$

Shock reactivity was analysed with a one-way ANOVA with Strain as the between-subjects factor. For PPI, startle and prepulse alone, data were analysed with two-way mixed-factor ANOVAs (Sex and Strain as between-subjects factors; Pulse Block or Prepulse Alone as a repeated-measures factor). Four-way mixed-factor ANOVAs (Prepulse Intensity and Prepulse–Pulse Interval as repeated-measures factors; Strain and Sex as the between-subjects factors) were performed on the PPI data. PPI was calculated by averaging the startle amplitudes for each trial type, and the percent PPI for each prepulse intensity was calculated using the formula

$$100 - (100 \times \text{startle amplitude on prepulse + pulse trials})/(\text{startle amplitude on pulse-alone trials})$$

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 analysed separately from the other intervals (Howland *et al.*, 2012; Ballendine *et al.*, 2015). For low-intensity and high-intensity fear conditioning, freezing during conditioning was binned into blocks of two following the first CS. Freezing to tones during recall and extinction days was binned into blocks of 10. Freezing during conditioning and extinction was analysed with two separate two-way mixed-factor ANOVAs (Strain as the between-subjects factor; CS block as the repeated-measures factor). Rats were considered to have extinguished when levels of freezing within strains to CSs during testing were no longer significantly higher than those observed to the initial CS (CS 1) during conditioning. To determine this, freezing to each tone bin on extinction days were compared with CS 1 on conditioning day with paired-samples *t*-tests using a Bonferroni correction. Freezing to context cues pre-CS 1 and post-CS 1 during conditioning and on each following testing day were also analysed using separate two-way mixed-factor ANOVAs (Strain as the between-subjects factors; Pre-to-post-CS 1 block as the repeated-measures factor). Fear reinstatement was analysed with two separate mixed-factor ANOVAs, one ANOVA analysed fear renewal to context cues following reminder shocks, and the other ANOVA analysed freezing to tone cues (CS in bins of five) following reinstatement of fear. Strain was the between-subjects factor for both ANOVAs, with preshock to postshock context or CS bin serving as the within-subjects factor, respectively. Freezing to tone cues was binned for LI: CS 1 to CS 2–12 on conditioning day, and CS grouped into bins of five for long-term recall. Conditioning and long-term recall of LI were analysed with separate three-way mixed-factor ANOVAs (Strain and Pre-exposure as the between-subjects factors; CS block as the within-subjects factor). Freezing during recall of short-term LI was averaged across all CSs. Short-term LI recall was analysed with a two-way (Strain × Pre-exposure) ANOVA.

Results

Characterization of absence seizures in the Saskatchewan colony

Given that a new colony of GAERS and NEC rats was established at the University of Saskatchewan in Saskatoon, Canada, we first assessed the GAERS and NEC strains for SWDs with freely-moving EEG similar to a recent report (Powell *et al.*, 2014). In their home cages, GAERS displayed spontaneous SWDs of a stereotypical morphology as described previously (Marescaux *et al.*, 1992; Powell *et al.*, 2009, 2014), whereas NEC rats did not display absence seizures or SWD activity (Fig. 1). SWDs were observed during 15.2 ± 2.3% ($n = 4$) of the recording period in GAERS, occurring at 0.9 ± 0.1 seizures/min, with a mean duration of 10.1 ± 1.1 s. SWDs displayed a spike frequency of 8.2 ± 0.11 Hz. It is of note that oscillatory local field potential spike activity was also observed in the medial prefrontal cortex, basolateral amygdala, subthalamic nucleus, lateral geniculate nucleus and caudate putamen that was frequency-locked with cortical SWDs (Fig. 1A and B).

We next performed a series of behavioural analyses on the Saskatchewan colony GAERS and NEC strains using the elevated plus maze, open field, PPI, conditioned fear, and LI.

Increased anxiety-like behaviour of GAERS in the elevated plus maze

Prepuberty

Statistical analyses revealed a significant main effect of Strain on time in open arms (Fig. 2A) ($F_{1,58} = 6.97$, $P = 0.011$) and total open and closed arm entries (Fig. 2B) ($F_{1,58} = 26.21$, $P < 0.001$).

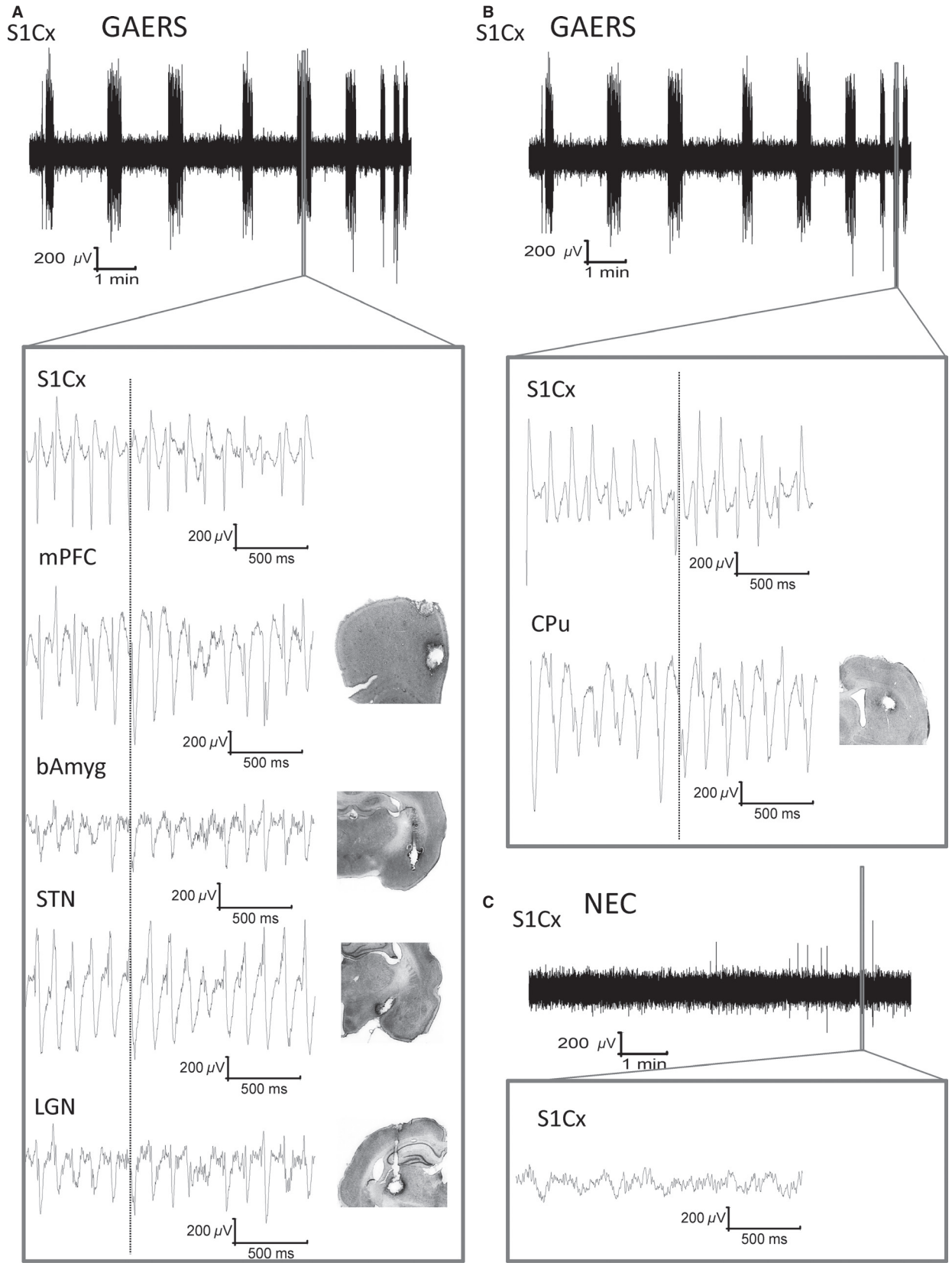

FIG. 1. Absence seizure morphology in GAERS. Electrocorticography and LFP recordings from freely-moving NEC rats and GAERS. Representative recordings from two different GAERS animals (A and B, respectively) are shown at low time resolution (top panel) and high time resolution (lower panels) for a skull screw electrode surgically implanted in the somatosensory cortex (S1Cx) and tungsten wire electrodes surgically implanted in the prelimbic region of the medial prefrontal cortex (mPFC), basolateral amygdala (bAmyg), subthalamic nucleus (STN), lateral geniculate nucleus (LGN) and caudate putamen (CPu). (C) Representative recording from an NEC animal, which do not display absence seizure activity.

Inspection of the data revealed that GAERS spent less time in open arms than NEC rats and had fewer total open and closed arm entries. Males, regardless of strain, also spent significantly less time in the open arms than females (Fig. 2A) ($F_{1,58} = 7.45$, $P = 0.008$) and made fewer total open and closed arm entries (Fig. 2B) ($F_{1,58} = 8.08$, $P = 0.006$). There was no Sex by Strain interaction for time in open arms or total open and closed arm entries ($P \geq 0.56$). Number of open arm entries was also examined and significant main effects of Strain ($F_{1,58} = 19.77$, $P < 0.001$) and Sex ($F_{1,58} = 7.96$, $P = 0.007$) were observed. Overall, NEC rats made more open arm entries than GAERS (12.22 ± 0.62 vs. 7.96 ± 0.78) and females made more open arm entries than males (11.90 ± 0.77 vs. 9.03 ± 0.73).

Young adulthood

Regarding time in open arms (Fig. 2C), significant main effects of Strain ($F_{1,58} = 12.01$, $P < 0.001$), Sex ($F_{1,58} = 8.98$, $P = 0.004$), and a significant Strain by Sex interaction ($F_{1,58} = 5.35$, $P = 0.024$) were found. *Post hoc* analyses indicated that female GAERS spent significantly less time in open arms than the female NEC rats, with no difference between the males. Total arm entries differed (Fig. 2D) by Strain (Fig. 2D) ($F_{1,58} = 40.60$, $P < 0.001$) but not Sex ($F_{1,58} = 1.88$, $P = 0.18$). GAERS had fewer total arm entries than NEC rats. A significant Sex by Strain interaction ($F_{1,58} = 6.15$, $P = 0.016$) was also found. *Post hoc* analyses indicated that NEC male rats had fewer total arm entries than NEC female rats, a pattern that did not exist for GAERS. Analysis of open arm entries revealed a significant main effect of Strain ($F_{1,58} = 17.38$, $P < 0.001$), Sex ($F_{1,58} = 7.48$, $P = 0.008$), and a significant Strain by Sex interaction ($F_{1,58} = 4.22$, $P = 0.044$). *Post hoc* analyses showed that female NEC rats made significantly more open arm entries than female GAERS ($t_{27} = 3.92$, $P = 0.001$) (8.41 ± 0.86 vs. 3.83 ± 0.67). However, a significant strain difference was not observed for males ($P > 0.05$). Regardless of strain, females made significantly more open arm entries than males ($t_{60} = -2.81$, $P = 0.007$) (6.51 ± 0.71 vs. 4.18 ± 0.47).

GAERS displayed altered exploration in the open field

Prepuberty

Statistical analyses revealed a significant main effect of Strain on distance travelled in both the inner (Fig. 3A) ($F_{1,58} = 17.99$, $P < 0.001$) and outer (Fig. 3B) ($F_{1,58} = 31.94$, $P < 0.001$) areas; inspection of the data revealed that GAERS travelled less distance than NEC rats in both the inner and outer areas. A significant main effect of Sex was also observed on distance travelled in both the inner (Fig. 3A) ($F_{1,58} = 7.77$, $P = 0.007$) and outer (Fig. 3B) ($F_{1,58} = 8.30$, $P = 0.006$) areas. Females travelled less distance in the inner area relative to males, but travelled more distance than males in the outer area. No effect of Strain was observed on time spent in the inner area ($F_{1,58} = 0.85$, $P = 0.36$), but a main effect of Sex (Fig. 3C) ($F_{1,58} = 10.28$, $P = 0.002$) revealed that males spent more time in the inner area than females. All Strain by Sex interactions were non-significant.

Young adulthood (P56)

Similar to the prepuberty test, GAERS travelled less distance than NEC rats in both the inner (Fig. 3D) ($F_{1,58} = 23.74$, $P < 0.001$) and outer (Fig. 3E) ($F_{1,58} = 51.33$, $P < 0.001$) areas of the open field. A main effect of Sex on distance travelled was not significant for distance travelled in the inner area ($F_{1,58} = 1.05$, $P = 0.31$), but was significant for distance travelled in the outer area (Fig. 3E) ($F_{1,58} = 6.86$, $P = 0.011$) where males travelled less distance than the females. Sex by Strain interactions for inner and outer distance travelled were not significant, and there were no significant main effects (Strain: $F_{1,58} = 0.56$, $P = 0.46$; Sex: $F_{1,58} = 0.01$, $P = 0.92$) or interactions for time spent in the inner area.

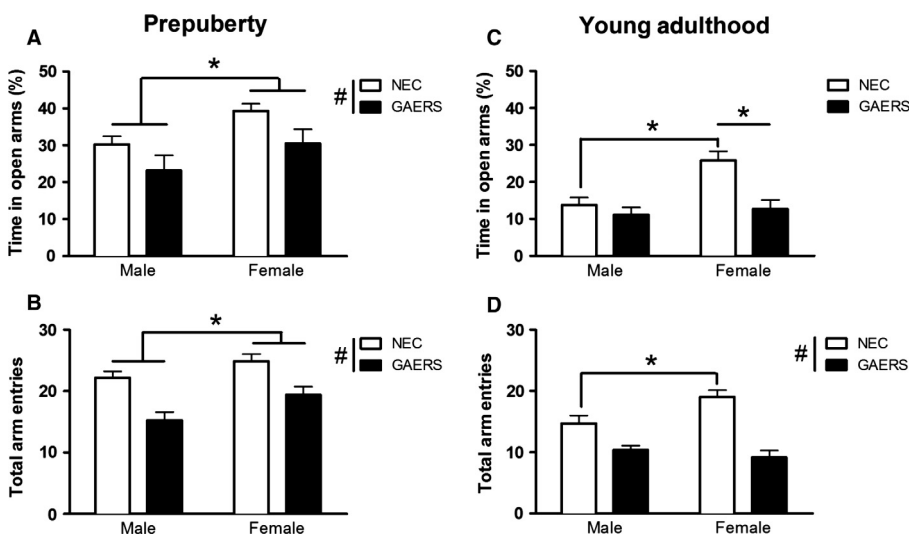

FIG. 2. Elevated plus maze in prepubescent (P35) and young adult (P56) GAERS ($n = 15$ male, $n = 12$ female) and NEC rats ($n = 18$ male, $n = 17$ female). (A) P35 GAERS spend less time in open arms than NEC rats ($^{\#}P < 0.05$). Males spend less time in the open arms than females ($^{*}P < 0.05$). (B) P35 GAERS make fewer total arm entries than NEC rats ($^{\#}P < 0.05$). Males make fewer arm entries than females ($^{*}P < 0.05$). (C) P56 female GAERS spend less time in open arms than female NEC rats ($^{*}P < 0.05$), but the difference is non-significant in the male cohort. NEC males rats spend less time in open arms than NEC female rats ($^{*}P < 0.05$), but there is no significant effect of Sex in the GAERS cohort. (D) P56 GAERS make fewer total arm entries than NEC rats ($^{\#}P < 0.05$), but only the NEC rat cohort shows a significant effect of Sex, with NEC male rats making fewer arm entries than NEC female rats ($^{*}P < 0.05$).

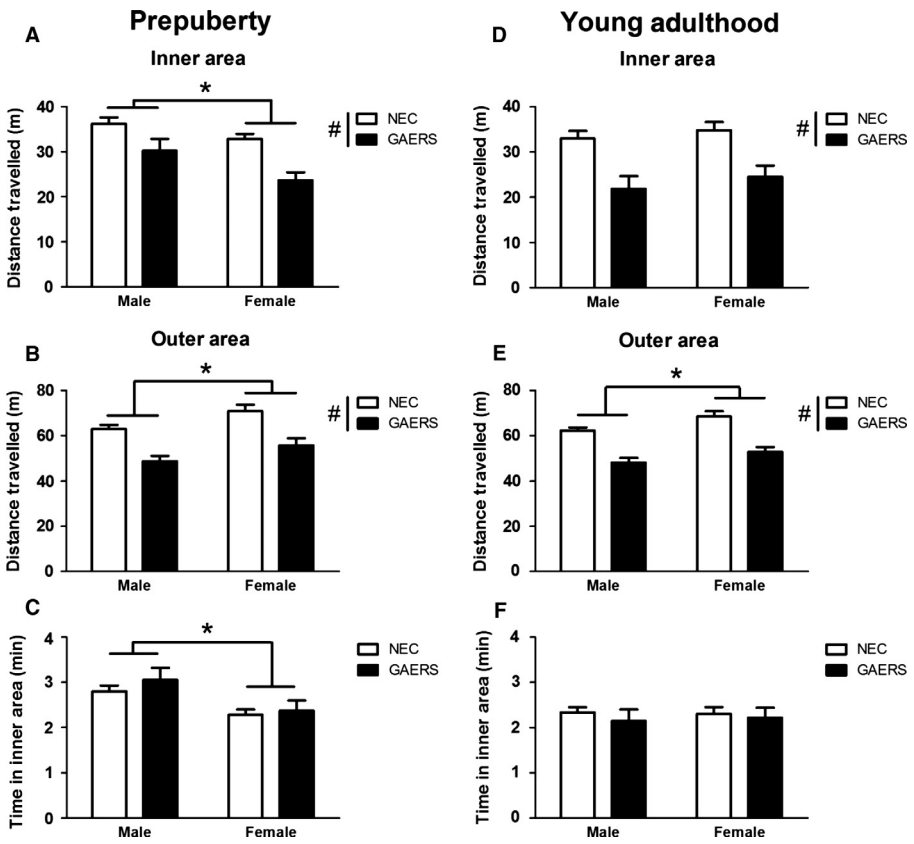

FIG. 3. Open field exploration in prepubescent (P35) and young adult (P56) GAERS (n = 15 male, n = 12 female) and NEC rats (n = 18 male, n = 17 female). GAERS travel less distance in both the inner and outer areas of the arena at P35 and P56 than NEC rats ($^{\#}P < 0.05$) (A, B, D and E). At P35, females travel less distance in the inner area than males (A) ($^{*}P < 0.05$) and greater distance in the outer area (B) ($^{*}P < 0.05$). At P56, females travel greater distance in the outer area than males (E) ($^{*}P < 0.05$), but there is no effect of Sex on distance travelled in the inner area (D). GAERS and NEC rats spend an equal amount of time in the inner area, at both P35 (C) and P56 (F). At P35, females spend less time in the inner area than males ($^{*}P < 0.05$) (C).

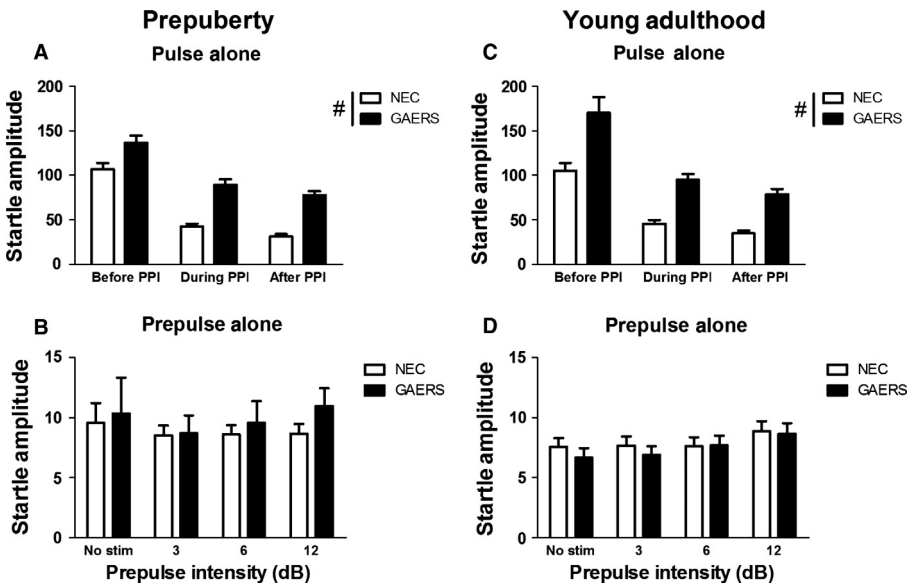

FIG. 4. Acoustic startle response in prepubescent (P35) and young adult (P56) GAERS (n = 27) and NEC rats (n = 35) to the 120 dB pulse in pulse-alone trials and to the prepulse of varying intensities in prepulse-alone trials. GAERS showed an enhanced startle response in the pulse-alone trials at P35 (A) and P56 (C) ($^{\#}P < 0.05$). (B and D) No significant changes in startle to no stimulus or prepulse-alone trials were noted between groups at P35 or P56.

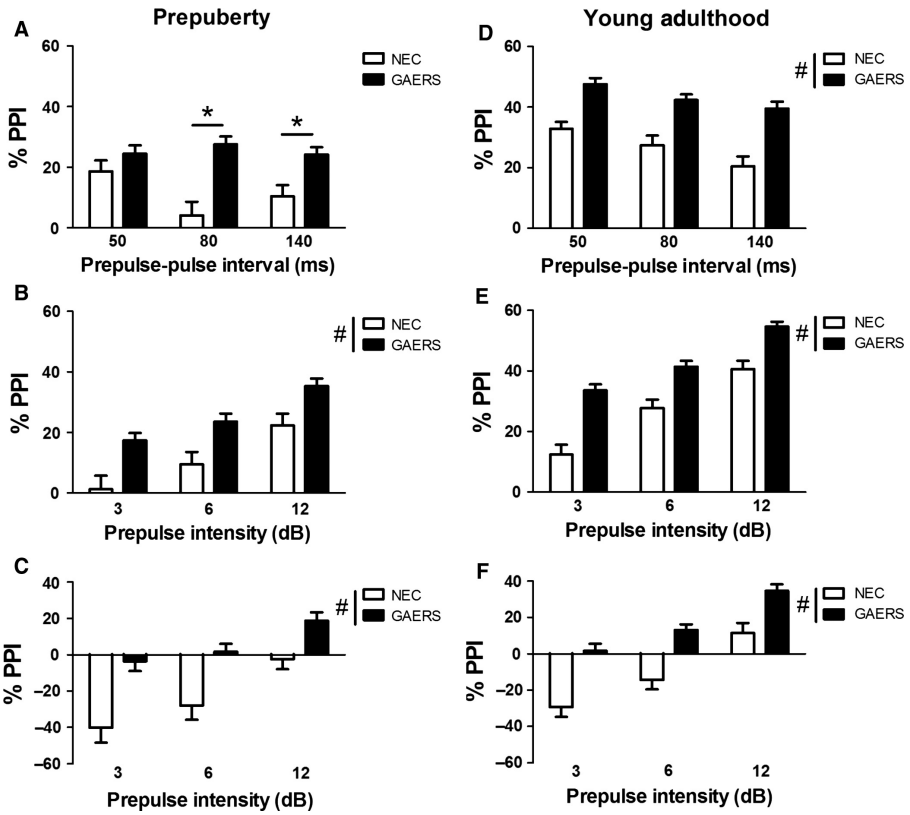

FIG. 5. PPI of the startle response (% PPI) in prepubescent (P35) and young adult (P56) GAERS (n = 27) and NEC rats (n = 35). (A) P35 GAERS showed significantly enhanced PPI at the 80 and 140 ms interval ($^{*}P < 0.05$). (B) At long prepulse–pulse intervals (50, 80 and 140 ms), P35 GAERS show enhanced PPI at all prepulse intensities ($^{\#}P < 0.05$). (C) At the short prepulse–pulse interval (30 ms), P35 GAERS show enhanced PPI ($^{\#}P < 0.05$). Facilitation of the startle response is observed in NEC rats at all prepulse intensities, and in GAERS at the 3 dB intensity. (D) P56 GAERS show enhanced PPI at all prepulse–pulse intervals ($^{\#}P < 0.05$). (E) At long prepulse–pulse intervals, P56 GAERS show enhanced PPI at all prepulse intensities ($^{\#}P < 0.05$). (F) At the short prepulse–pulse interval, P56 GAERS show enhanced PPI ($^{\#}P < 0.05$). Facilitation of the startle response is seen in NEC rats at the 3 and 6 dB intensities.

Acoustic startle and prepulse inhibition were increased in GAERS relative to NEC rats

Prepuberty

Startle. As depicted in Fig. 4A, GAERS exhibited higher startle responses than NEC rats shown by a main effect of Strain ($F_{1,58}$ = 36.72, $P < 0.001$). Sex was not a significant main effect, so males and females are presented together. Both GAERS and NEC rats displayed habituation of startle response to the pulse-alone trials (within-subjects effect of Pulse Block: $F_{1.44,83.41}$ = 191.26, $P < 0.001$). The interaction between Pulse Block and Strain was also significant ($F_{1.44,83.41}$ = 3.70, P = 0.043); however, *post hoc* tests revealed a significant effect of strain at all pulse blocks with GAERS showing increased startle overall. No other significant interactions were found. Strain and Sex had no effect on baseline reactivity during trials in which no stimulus or the prepulses (3, 6, 12 dB) were presented alone (Fig. 4B).

Prepulse inhibition. For PPI trials, males and females were again combined, as there was no main effect of Sex or interaction of Sex with other factors. A main effect of Strain on PPI ($F_{1,58}$ = 10.05, P = 0.002) was observed. The effect of Strain on PPI was qualified by prepulse–pulse interval (Fig. 5A) ($F_{2,116}$ = 13.98, $P < 0.001$), but not intensity (Fig. 5B) ($F_{2,116}$ = 0.31, P = 0.73) interactions. *Post hoc* analyses of the Strain by Interval interaction revealed that the effect of Strain was not observed for the 50 ms interval ($P > 0.05$). Inspection of all other intervals and intensities revealed significantly higher PPI in GAERS relative to NEC rats (all $P < 0.05$). When trials conducted with the 30 ms interval were examined (Fig. 5C), a significant main effect of Strain was observed ($F_{1,58}$ = 13.84, $P < 0.001$). NEC rats showed significant facilitation of PPI at all intensities with facilitation observed in GAERS only at the 3 dB intensity for the 30 ms interval. All other main effects and interactions were non-significant at the 30 ms interval.

Young adulthood

Startle. Higher startle in GAERS was observed during all Pulse Blocks (Fig. 4C) ($F_{1,58}$ = 33.15, $P < 0.001$). A significant main effect of Sex was also observed ($F_{1,58}$ = 5.94, P = 0.018). Males of both strains had higher startle during all pulse blocks. Similar to results observed during prepuberty, both strains showed habituation to the startle response (main effect of the Pulse Block: $F_{1.09,63.26}$ = 84.00, $P < 0.001$). Analysis of startle reactivity on trials with no stimulus, or 3, 6, or 12 dB prepulses alone revealed a main effect of Prepulse Intensity (Fig. 4D) ($F_{3,174}$ = 16.46, $P < 0.001$). All other main effects and interactions were non-significant except for a significant three-way interaction between Prepulse Intensity, Strain, and Sex (statistics not reported).

Prepulse inhibition. For PPI, a significant Sex by Interval interaction was observed ($F_{1.72,100}$ = 3.62, P = 0.037). *Post hoc* analyses revealed that the interaction was not driven by Sex differences within prepulse intervals ($P > 0.05$), but across intervals.

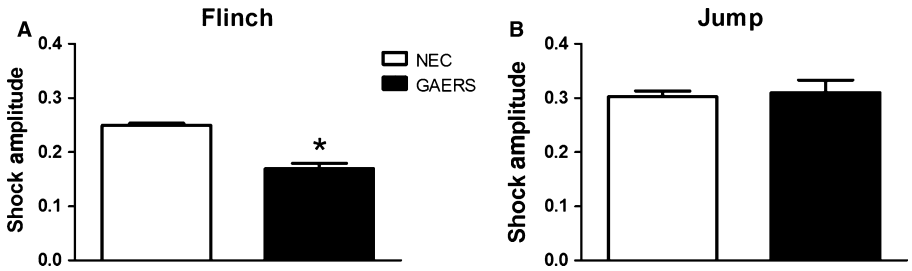

FIG. 6. Sensitivity to footshock in NEC rats (n = 8) and GAERS (n = 8). GAERS show enhanced reactivity to footshock as measured by flinch (A) vs. jump (B). *$P < 0.05$.

Considering that no interactions of Sex with other factors were observed, we combined the sexes for the remaining analyses. A significant main effect of Strain (Fig. 5D and E) ($F_{1,58} = 24.14$, $P < 0.001$) and a Strain by Intensity interaction (Fig. 5E) ($F_{2,116} = 3.53$, $P = 0.032$) were observed for PPI. *Post hoc* analyses revealed that the main effect of strain was still observed within each prepulse intensity (all $P < 0.05$). Thus, at all intensities and intervals, GAERS demonstrated enhanced PPI relative to NEC rats. Examination of trials conducted at the 30 ms prepulse–pulse interval revealed a main effect of Strain (Fig. 5F) ($F_{1,58} = 27.61$, $P < 0.001$). Facilitation of startle (i.e. prepulse facilitation) by the 3 and 6 dB prepulses was observed in NEC rats, whereas PPI was observed at all intensities in GAERS at the 30 ms interval.

GAERS displayed increased freezing during conditioning and impaired extinction of conditioned fear

Shock reactivity was altered in GAERS

Analysis of shock reactivity in NEC rats and GAERS revealed a significant between-group effect for the first flinch (Fig. 6A) ($t_{14} = 7.57$, $P < 0.001$) but not the first jump (Fig. 6B) ($t_{14} = -0.30$, $P = 0.77$). Inspection of the data revealed that GAERS flinched in response to a significantly lower shock amplitude relative to NEC rats.

Low-intensity conditioning

The main effect of Sex and its interactions with all variables during conditioning and extinction were not significant, and thus the sexes were combined for all analyses.

Conditioning. Statistical analyses revealed a significant within-subjects effect of Conditioning (Fig. 7A) ($F_{2,60} = 73.79$, $P < 0.001$) and a significant main effect of Strain (Fig. 7A) ($F_{1,30} = 58.02$, $P < 0.001$) on freezing to tones presented during conditioning. Both GAERS and NEC rats showed increased freezing during conditioning; however, GAERS demonstrated significantly increased freezing during all tones relative to NEC rats. Analysis of contextual freezing during the 180 s prior to and after the first tone presentation (CS 1) also revealed a significant main effect of Strain (Fig. 7B) ($F_{1,30} = 83.83$, $P < 0.001$) and within-subjects effect of Pre-to-Post-CS 1 (Fig. 7B) ($F_{1,30} = 54.77$, $P < 0.001$). A significant Strain by Pre-to-Post-CS 1 interaction was found ($F_{1,30} = 19.85$, $P < 0.001$). *Post hoc* analyses showed that, although GAERS and NEC rats had similar levels of freezing prior to the first tone during conditioning, GAERS froze significantly more than NEC rats after the first tone presentation ($P < 0.05$), demonstrating a heightened initial reactivity to the CS. GAERS also showed a significant increase in freezing from pre-to-post-CS 1 presentation ($P < 0.05$), whereas NEC rats did not ($P > 0.05$).

Extinction. Extinction day 1 was analysed separately as initial recall of conditioned fear is reflected in behaviour on this day. A significant effect of testing day was observed within subjects for extinction day 1 and extinction days 2–4 (Fig. 7A) ($F_{4.61,138.43} = 40.28$, $P < 0.001$). Overall, levels of freezing decreased in both strains from the first 10 CSs of extinction day 1 to the last 10 CSs on extinction day 4. A significant main effect of Strain was also observed for testing days (Fig. 7A) ($F_{1,30} = 178.00$, $P < 0.001$). GAERS showed more freezing during all testing days compared with NEC rats. A Strain by Testing Day interaction was nearly significant ($F_{4.61,138.43} = 395.59$, $P = 0.054$).

A priori planned comparisons investigated freezing during each block of trials to the very first CS presentation (before any shocks were delivered) within each strain (Fig. 7A). NEC rats showed significantly enhanced freezing during CS 1–10 ($t_{15} = -6.81$, $P < 0.001$) and CS 11–20 ($t_{15} = -7.37$, $P < 0.001$) on extinction day 1. NEC rats did not show significantly increased freezing relative to the initial CS presentation during any testing blocks on extinction days 2–4 (all $P > 0.21$), indicating that extinction had occurred. In contrast, GAERS showed enhanced freezing relative to their initial CS levels during CS 1–10 ($t_{15} = -9.32$, $P < 0.001$) and CS 11–20 ($t_{15} = -7.40$, $P < 0.001$) of extinction day 1, as well as CS 1–10 ($t_{15} = -6.51$, $P < 0.001$) of extinction day 2. Freezing levels in GAERS returned to those observed during the initial CS presentation during CS 11–20 of extinction day 2 and extinction day 3 ($P > 0.10$). However, an increase in freezing was observed in GAERS during CS 1–10 of extinction day 4 ($t_{15} = -4.31$, $P = 0.001$). Thus, GAERS took longer to extinguish than NEC rats, with a spontaneous recovery of fear occurring on extinction day 4.

Freezing to contextual cues was also analysed during the 180 s prior to and after the first tone presentation (CS 1) on extinction day 1 and extinction days 2–4 (Fig. 7B). A significant main effect of Strain was found for all testing days (all $P < 0.001$) showing that GAERS froze significantly more overall during pre-CS 1 and post-CS 1 presentations on each test day. During extinction day 1, a significant Strain by Pre-to-Post-CS 1 interaction was found ($F_{1,30} = 5.49$, $P = 0.026$). *Post hoc* analyses revealed that, although NEC rats displayed increased freezing from pre-to-post-CS 1 on extinction day 1, GAERS showed robust freezing to context cues prior to and after the first CS presentation on extinction day 1. Significant within-subjects pre-to-post-CS 1 effects were found for extinction days 2–4 (all $P < 0.001$). These within-subjects effects are confirmed by significant Strain by pre-to-post-CS 1 interactions for extinction days 2 ($F_{1,30} = 21.79$, $P < 0.001$), 3 ($F_{1,30} = 4.19$, $P = 0.050$), and 4 ($F_{1,30} = 10.18$, $P = 0.003$). *Post hoc* analyses revealed that GAERS had significantly enhanced freezing overall

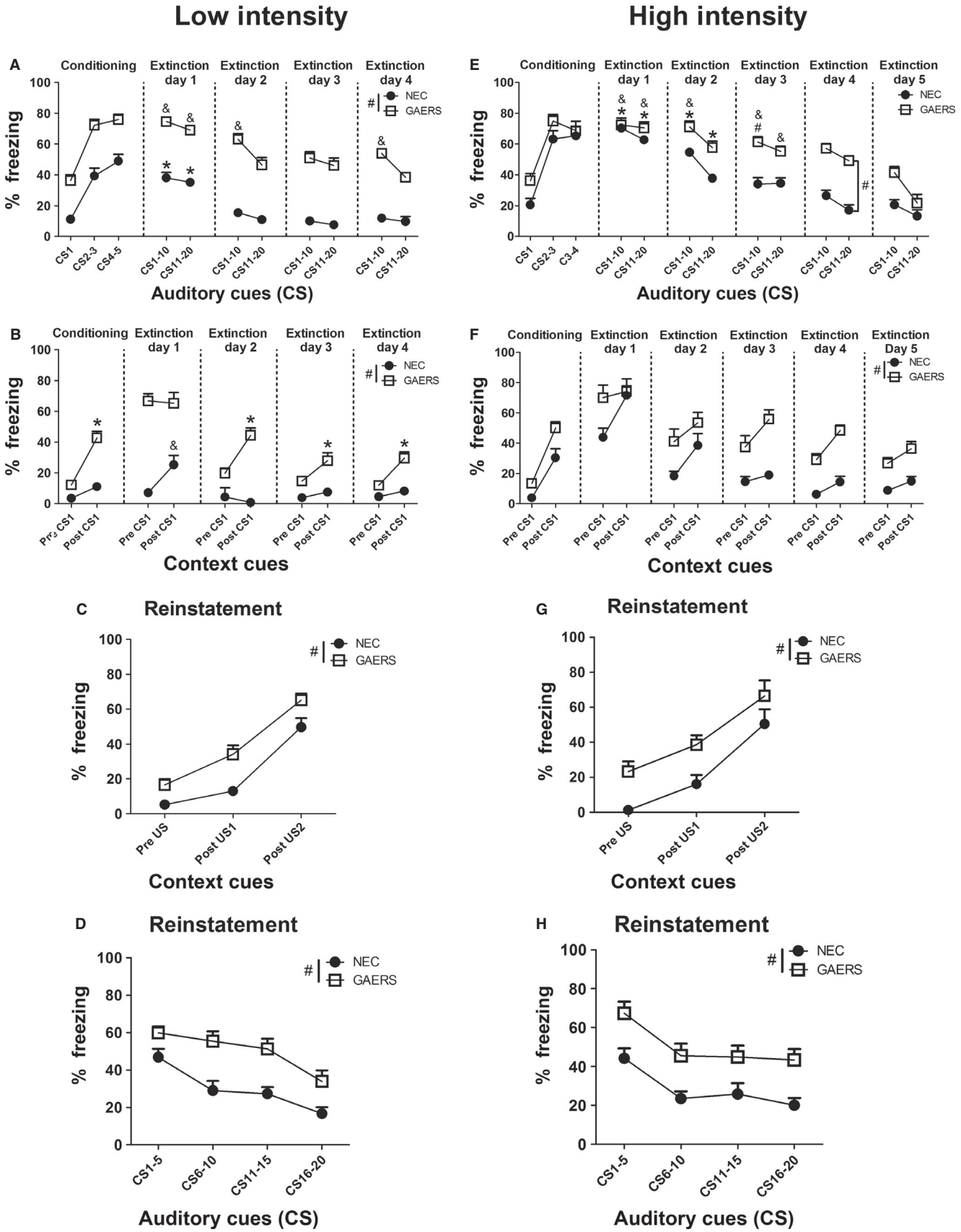

FIG. 7. Conditioned fear, extinction, and reinstatement using a low-intensity and high-intensity protocol. Low-intensity GAERS, $n = 16$; NEC rats, $n = 16$; high-intensity GAERS, $n = 10$; NEC rats, $n = 9$. (A) Low-intensity GAERS show enhanced freezing during conditioning, extinction day 1 and extinction days 2–4 ($^{\#}P < 0.05$). NEC rats show significantly enhanced freezing during extinction day 1 relative to their initial freezing levels during CS 1 of conditioning ($^{*}P < 0.05$). GAERS are slower to extinguish fear than NEC rats as they show significantly enhanced freezing during extinction day 1 and CS 1–10 of extinction day 2 relative to CS 1 of conditioning ($\&P < 0.05$). A recovery of freezing in GAERS occurs again during CS 1–10 of extinction day 4 ($\&P < 0.05$). (B) Overall GAERS show significantly increased freezing to context cues pre-to-post-CS 1 on all testing days ($^{\#}P < 0.05$). Freezing in GAERS increases from pre-to-post-CS 1 during all testing days except for extinction day 1 ($^{*}P < 0.05$). On extinction day 1, NEC rats show a significant increase in freezing from pre-to-post-CS 1 ($\&P < 0.05$). (C and D) A significant strain effect is observed with GAERS showing enhanced freezing to both presentation of the unsignalled footshock and tone cues following unsignalled footshocks ($^{\#}P < 0.05$). (E) High-intensity GAERS and NEC rats show similar levels of freezing until CS 1–10 of extinction day 2 ($^{\#}P < 0.05$), and extinction day 4 ($^{\#}P < 0.05$), where GAERS show significantly enhanced freezing relative to NEC rats. NEC rats show significant increases in freezing relative to their freezing observed during CS 1 during extinction day 1 and extinction day 2 ($^{*}P < 0.05$). GAERS show significantly enhanced freezing relative to their freezing observed during CS 1 during extinction day 1 and CS 1–10 of extinction day 2 ($\&P < 0.05$). Freezing in GAERS spontaneously re-emerges during extinction day 3 ($\&P < 0.05$). (F) Overall, GAERS show significantly increased freezing to context cues relative to NEC rats during pre-to-post-CS 1 presentations on all testing days ($^{\#}P < 0.05$). (G and H) A significant strain effect is observed with GAERS showing enhanced freezing to both presentation of the unsignalled footshock and tone cues following unsignalled footshocks ($^{\#}P < 0.05$).

relative to NEC rats on extinction days 2 and 3 ($P < 0.05$); however, during pre-CS 1 during extinction day 4, GAERS showed similar levels of freezing to NEC rats. Following CS 1 during extinction day 4, GAERS again showed enhanced freezing relative to NEC rats. *Post hoc* analyses also revealed significant increases in freezing in GAERS from pre-to-post-CS 1 on extinction days 2–4 ($P < 0.05$), whereas NEC rats showed similar levels of freezing both pre-CS 1 and post-CS 1 on extinction days 2–4 ($P > 0.05$). These results show that GAERS had a significant recovery of freezing in response to CS 1 during extinction days 2–4, whereas NEC rats did not.

Reinstatement. For reinstatement, contextual freezing was analysed prior to the first shock as well as for the two 120 s periods post-shock (Fig. 7C). A significant main effect of Strain ($F_{1,30} = 19.631$, $P < 0.001$) and a significant within-subjects effect of pre-to-post-shock context ($F_{2,60} = 94.81$, $P < 0.001$) revealed that, whereas both strains showed reinstatement of freezing, GAERS showed enhanced freezing relative to NEC rats both prior to and after unsignalled footshocks. Following two reminder shocks, we assessed reinstated freezing to the CS (Fig. 7D). A significant main effect of Strain ($F_{1,30} = 23.22$, $P < 0.001$) and a significant within-subjects effect of CS block ($F_{3,90} = 14.90$, $P < 0.001$) showed that, whereas both GAERS and NEC rats showed decreased freezing from CS 1–5 to CS 16–20, GAERS continued to show enhanced freezing overall to presentation of the CS. All interaction terms were non-significant.

High-intensity conditioning

Conditioning. Mixed-designs ANOVA revealed a significant within-subjects effect of Conditioning (Fig. 7E) ($F_{2,34} = 36.50$, $P < 0.001$), and a significant main effect of Strain (Fig. 7E) ($F_{1,17} = 7.21$, $P = 0.016$) on freezing to tones presented during conditioning. Both strains demonstrated robust increases in freezing during conditioning; however, GAERS had significantly increased freezing during all tones relative to NEC rats, including elevated freezing before shock presentation. Contextual freezing was analysed for the 180 s prior to and after the first tone presentation (CS 1). Analyses revealed a significant main effect of Strain (Fig. 7F) ($F_{1,17} = 13.61$, $P = 0.002$) and within-subjects effect of pre-to-post-CS 1 (Fig. 7F) ($F_{1,17} = 82.06$, $P < 0.001$). The Strain by pre-to-post-CS 1 interaction was non-significant. These results indicate that, although both GAERS and NEC rats showed significant increases in freezing from pre-to-post-CS 1, GAERS had significantly enhanced freezing relative to NEC rats during both pre-CS 1 and post-CS 1.

Extinction. Similar to low-intensity fear conditioning, extinction day 1 was analysed separately. A significant within-subjects effect of Day was observed for extinction day 1 and extinction days 2–5 (Fig. 7E) ($F_{4.40,74.82} = 50.14$, $P < 0.001$). Freezing decreased in both GAERS and NEC rats from the first 10 CSs during extinction day 1 to the last 10 CSs during day 5 of extinction. A significant main effect of Strain across days (Fig. 7E) ($F_{1,17} = 49.74$, $P < 0.001$) and Strain by Day interaction ($F_{4.40,74.82} = 4.436$, $P = 0.002$) were found. *Post hoc* analyses revealed similar levels of freezing in both strains during extinction day 1 and extinction day 2 ($P > 0.05$). On extinction day 3, NEC rats showed significantly reduced freezing relative to GAERS during CS 1–10 ($P < 0.05$). Significantly reduced freezing was again observed in NEC rats during extinction day 4 ($P < 0.05$), an effect that disappeared by extinction day 5 ($P > 0.05$).

A priori planned comparisons showed that NEC rats had significantly enhanced freezing during CS 1–10 ($t_8 = -16.18$, $P < 0.001$) and CS 11–20 ($t_8 = -22.145$, $P < 0.001$) on extinction day 1, as well as CS 1–10 ($t_8 = -8.60$, $P < 0.001$) and CS 11–20 ($t_8 = -4.33$, $P = 0.003$) on extinction day 2 (Fig. 7E). NEC rats did not show significantly increased freezing relative to their freezing observed during the initial CS presentation on extinction days 3–5 (all $P > 0.45$), suggesting that rats had extinguished to the CS by these days. GAERS also showed enhanced freezing during extinction day 1, CS 1–10 ($t_9 = -6.25$, $P < 0.001$) and CS 11–20 ($t_9 = -6.55$, $P < 0.001$), as well as during CS 1–10 on extinction day 2 ($t_9 = -4.82$, $P = 0.001$). GAERS did not show enhanced freezing relative to their initial CS 1 levels observed during conditioning during CS 11–20 on extinction day 2 ($t_9 = -2.92$, $P = 0.017$). However, their freezing levels were increased significantly during CS 1–10 ($t_9 = -4.63$, $P = 0.001$) and CS 11–20 ($t_9 = -3.63$, $P = 0.004$) on extinction day 3 showing that, overall, GAERS took longer to extinguish to CS than NEC rats. GAERS did not show significantly enhanced freezing during extinction days 4 or 5 (all $P > 0.006$), suggesting that rats had extinguished to CS cues by extinction day 4.

Freezing to contextual cues during the 180 s prior to and after the first tone presentation (CS 1) on extinction day 1 and extinction days 2–5 is presented in Fig. 7F. Although a significant main effect of Strain was not found during extinction day 1 ($F_{1,17} = 2.85$, $P = 0.11$), extinction days 2–5 did show a main effect of Strain (all $P \leq 0.037$). Significant within-subjects effects were also observed for extinction day 1 and extinction days 2–5 (all $P \leq 0.041$). These results show that, although initially both strains displayed similar levels of freezing following conditioning, GAERS froze significantly more to contextual cues on all extinction days. Further, the results indicate that both GAERS and NEC rats displayed increased freezing from pre-to-post-CS 1 on all test days. All Strain by pre-to-post-CS 1 day interactions were non-significant.

Reinstatement. Contextual freezing was analysed prior to the first shock exposure as well as for the two 120 s periods postshock (Fig. 7G). A significant main effect of Strain ($F_{1,17} = 9.03$, $P = 0.008$) and a significant within-subjects effect of pre-to-post-shock context ($F_{2,34} = 40.48$, $P < 0.001$) revealed that, although both GAERS and NEC rats showed reinstatement of freezing, GAERS showed enhanced freezing relative to NEC rats including the period prior to unsignalled footshocks. Assessment of reinstated freezing to the CS (Fig. 7H) revealed a significant main effect of Strain ($F_{1,17} = 13.97$, $P = 0.002$) and a significant within-subjects effect of CS bin ($F_{3,51} = 15.32$, $P < 0.001$). These results show that, although both GAERS and NEC rats showed decreased freezing from CS 1–5 to CS 16–20, GAERS continued to show enhanced freezing overall. The interaction terms for contextual and tone cues were non-significant.

Latent inhibition was disrupted in male GAERS

The sexes were analysed separately due to a significant main effect of Sex on LI long-term recall ($F_{1,73} = 4.39$, $P = 0.040$).

Males

Freezing to the initial CS presented during conditioning (prior to shock delivery) and CS 2–12 was analysed (Fig. 8A). Significant within-subjects effects of Conditioning ($F_{1,35} = 31.29$, $P < 0.001$), Conditioning by Pre-exposure interaction ($F_{1,35} = 12.15$, $P = 0.001$), and a three-way Strain by Conditioning by Pre-exposure interaction ($F_{1,35} = 6.83$, $P = 0.013$) were found. *Post hoc* analyses of these interactions showed that, during CS 1, NEC rats pre-exposed to the CS had significantly lower freezing than NEC rats not pre-exposed to the CS ($P < 0.05$). Also, both pre-exposed NEC rats and GAERS showed a significant increase in freezing from CS 1 to CS 2–12 ($P < 0.05$). All other groups showed non-significant increases in freezing from CS 1 to CS 2–12 ($P > 0.05$).

Analysis of freezing during short-term recall revealed a significant main effect of Strain (Fig. 8A) ($F_{1,35} = 30.92$, $P < 0.001$) where GAERS showed significantly increased freezing. No other main effects or interactions were significant during short-term recall. During long-term recall, a significant within-subjects effect of CS block (Fig. 8A) ($F_{2.43,85.02} = 26.85$, $P < 0.001$) revealed that both strains and exposure groups showed a decrease in freezing from CS 1–5 to CS 16–20. Significant main effects of Strain (Fig. 8A) ($F_{1,35} = 94.74$, $P < 0.001$) and Pre-exposure (Fig. 8A) ($F_{1,35} = 8.88$, $P = 0.005$), as well as a significant Strain by Pre-exposure interaction ($F_{1,35} = 9.07$, $P = 0.005$) were also found. Further analyses of the data showed significantly enhanced freezing in non-pre-exposed NEC rats relative to pre-exposed NEC rats during long-term recall ($t_{17} = 4.37$, $P < 0.001$), although significant differences in freezing were not noted between pre-exposed and non-pre-exposed GAERS. Thus, NEC rats showed LI, whereas GAERS did not. *Post hoc* tests of between-subjects effects also revealed that, overall, GAERS froze significantly more than NEC rats across all CSs presented during long-term recall regardless of pre-exposure group (all $P < 0.001$).

Females

Analyses of freezing to the initial CS and CS 2–12 during conditioning revealed a significant within-subjects effect of Conditioning ($F_{1,38} = 223.35$, $P < 0.001$), Conditioning by Strain interaction ($F_{1,38} = 58.05$, $P < 0.001$), and a significant Conditioning by Pre-exposure interaction ($F_{1,38} = 42.13$, $P < 0.001$) (Fig. 8B). *Post hoc* analyses of these interactions showed a significant strain effect during both CS 1 and CS 2–12 where GAERS froze significantly more than NEC rats overall ($P < 0.05$). *Post hoc* tests also showed that, collapsed across strain, pre-exposure to the CS cue prior to conditioning significantly reduced freezing compared with non-pre-exposed rats ($P < 0.05$). This pattern of behaviour was not observed during CS 2–12 of conditioning ($P > 0.05$).

Analysis of freezing behaviour during short-term recall revealed a significant main effect of Strain (Fig. 8B) ($F_{1,38} = 82.80$, $P < 0.001$), where GAERS showed significantly increased freezing overall during short-term recall. No other main effects or interactions were significant during short-term recall. Analysis of long-term recall revealed a significant within-subjects effect of CS block ($F_{2.45,93.13} = 6.71$, $P < 0.001$) and a significant Strain by CS block interaction ($F_{2.45,93.13} = 5.13$, $P = 0.005$). A significant main effect of Strain was also observed for long-term recall CS presentations ($F_{1,38} = 123.77$, $P < 0.001$). Significant between-subject effects as well as *post hoc* tests confirmed enhanced freezing in GAERS over-

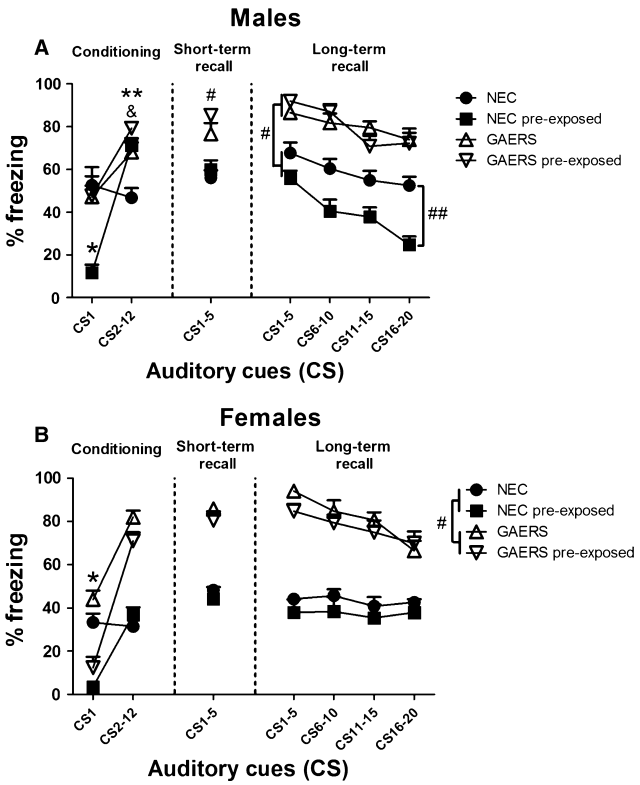

FIG. 8. LI in male and female GAERS and NEC rats. Female pre-exposed GAERS, $n = 11$; NEC rats, $n = 11$; non-pre-exposed GAERS, $n = 10$; NEC rats, $n = 10$; male pre-exposed GAERS, $n = 10$; NEC rats, $n = 10$; non-pre-exposed GAERS, $n = 10$; NEC rats, $n = 9$. (A) Male NEC rats pre-exposed to the CS show significantly reduced freezing to the first cue presented during conditioning ($^{*}P < 0.05$). Both NEC rats (**) and GAERS (&) pre-exposed to the CS show a significant increase in freezing from CS 1 to CS 2–12 during conditioning ($P < 0.05$). During short-term and long-term recall, GAERS froze significantly more than NEC rats regardless of pre-exposure group ($^{\#}P < 0.05$). NEC rats pre-exposed to the CS show significantly decreased freezing during long-term recall compared with non-pre-exposed NEC rats ($^{\#\#}P < 0.05$). (B) Both GAERS and NEC female rats pre-exposed to the CS show decreased freezing to the first cue during conditioning compared with non-pre-exposed rats ($^{*}P < 0.05$). GAERS females show enhanced freezing during conditioning, short-term and long-term recall ($^{\#}P < 0.05$).

all during long-term recall compared with NEC rats ($P < 0.05$). All other main effects and interactions were non-significant.

Discussion

We characterized the behavioural phenotype of a new colony of GAERS and NEC rats established in 2013. EEG recordings from both strains confirmed the presence of SWDs in GAERS but not NEC rats. Deep electrode local field potential recordings revealed that the medial prefrontal cortex, basolateral amygdala, subthalamic nucleus, lateral geniculate nucleus and caudate putamen brain regions all display oscillatory activity that follows the SWD. These data are largely in agreement with previous studies designed to specifically map GAERS seizures with the exception that, in our hands, the basolateral amygdala electrode displayed oscillatory activity (Vergnes *et al.*, 1987, 1990; Marescaux *et al.*, 1992; Danober *et al.*, 1998; Zheng *et al.*, 2012). However, low-amplitude local field potential data should be interpreted with caution given that the electrodes used were monopolar and therefore may display oscillations as a result of SWD activity at the reference electrode over the occipital cortex. Dramatic behavioural differences existed between the strains in a battery of tests related to anxiety, sensorimotor gating, and cognition. Decreased time spent in the open arms of the elevated plus maze before puberty (Fig. 2A) and elevated startle at both age groups (Fig. 4A and C) indicate an anxiety-like phenotype in GAERS. Our novel findings of increased footshock reactivity (Fig. 6) as well as enhanced freezing to conditioned fear-associated cues in GAERS provide additional evidence of an exaggerated anxiety-like response (Fig. 7A and E). Further, we report significant deficits in extinction of conditioned fear in GAERS (Fig. 7A, B, E and F) and the long-term recall of LI in male GAERS (Fig. 8A).

GAERS show enhanced anxiety-like behaviours compared with NEC rats

Characterization of GAERS from the Melbourne colony revealed increased anxiety-like behaviour in the elevated plus maze (Jones *et al.*, 2008; Powell *et al.*, 2014), open field (Jones *et al.*, 2008; Bouilleret *et al.*, 2009; Dezsi *et al.*, 2013; Powell *et al.*, 2014), and in response to startling acoustic stimuli (Jones *et al.*, 2010) when compared with NEC rats. Our results are consistent with these reports except that GAERS did not spend less time in the inner area of the open field than NEC rats and thus did not display increased anxiety-like behaviour in this specific task (Jones *et al.*, 2008; Bouilleret *et al.*, 2009). Interestingly, differences in anxiety-like behaviour in the open field were noted when rats were tested at P91–98 in previous studies, but only trended toward significance at P49. As open field tests were conducted at P35 and P56 in the present study, testing of older rats from our colony may reveal increased anxiety-like behaviour for GAERS in the open field test that was not observed for the age group that we examined. In addition, rats of both strains travelled roughly 10 times further in the 10 min open field test in the present experiments than was previously reported (Bouilleret *et al.*, 2009), suggesting that the rats responded to the test differently between the experiments. However, significantly reduced locomotor activity of GAERS was consistent in both the open field and elevated plus maze tests. In general, males and females of both strains displayed similar behaviour in the tests of anxiety, with some differences noted for the test at P35. Male NEC rats showed a surprising reduction in open arm time at P56 (Fig. 2C), which is inconsistent with their behaviour in the open field or startle chambers. It should be noted that increased anxiety-like behaviour is not a phenotype shared by other rat strains with absence seizures (van Luijtelaar, 2011) and the behaviour of the NEC strain as a 'control' has been drawn into question (Marques-Carneiro *et al.*, 2014). In the present experiments, NEC rats generally performed similarly to Long Evans rats in these tests (Hannesson *et al.*, 2008; Chandna *et al.*, 2015). An exception is the relatively short time spent in the open arms of the elevated plus maze by the NEC male rats on P56 compared with the Long Evans male rats tested in Chandna *et al.* (2015). Alternatively, variability (14–32%) in the percent time that male Wistar rats spend in the open arms of the elevated plus maze has been reported previously (Goepfrich *et al.*, 2013; Keeley *et al.*, 2015). Therefore, a possible alternative explanation for the current findings in the elevated plus maze is that NEC rats demonstrate decreased anxiety. Unfortunately, we have not routinely used the Wistar strain for behavioural experiments, which is the founder strain for the GAERS and NEC rats, making direct comparisons of the current data with the Wistar strain difficult.

Prepulse inhibition is enhanced in GAERS relative to NEC rats

GAERS showed robust enhancement of PPI for all prepulse intensities and most prepulse–pulse intervals. Previously, Jones *et al.* (2010) reported no significant differences in PPI for trials with a 100 ms prepulse–pulse interval but a significant increase in PPI for P42 GAERS on trials with a 30 ms prepulse–pulse interval. Thus, GAERS from our colony exhibit a more pronounced change in PPI. In both studies, startle responses were consistently increased by two-fold or threefold in the GAERS, which may influence PPI (Swerdlow *et al.*, 2000). It is worth noting that, during prepuberty, GAERS display consistent PPI across all intervals, whereas NEC rats show lower PPI at the 80 and 140 ms intervals. For these specific factors, an alternative interpretation of the data is that the NEC rats have decreased PPI. PPI is typically thought to model the alterations in sensorimotor gating observed in psychiatric disorders such as schizophrenia (Braff *et al.*, 2001). As patients with schizophrenia show impaired PPI, the effects in the GAERS are inconsistent with this aspect of the disorder, although similarities between other aspects of the GAERS phenotype and schizophrenia (or psychosis) have been suggested (Jones *et al.*, 2010). Interestingly, the NEC group showed decreased PPI for the longer prepulse–pulse intervals that could be interpreted as an impairment in PPI. These results are lower than those typically obtained from the outbred Wistar strain (Brosda *et al.*, 2011; Goepfrich *et al.*, 2013). These variations in PPI and anxiety could potentially be explained by genetic drift that has been shown to impact not only GAERS, but also the NEC rat behavioural phenotype (Powell *et al.*, 2014).

GAERS show enhanced conditioned fear and extinction deficits

To the best of our knowledge, aspects of Pavlovian fear conditioning have not been examined previously in rodent models of absence epilepsy. GAERS froze significantly more during the conditioning day in both low-intensity and high-intensity protocols. It is of particular interest that freezing was significantly enhanced to the first CS presentation (CS 1) in GAERS, indicating increased initial reactivity to the novel tone cue before the USs (footshocks) were presented. Enhanced freezing during CS 1 in GAERS is consistent with the increased anxiety-like behaviour observed in the other tests. Although similar increases in freezing to the CSs were observed in

GAERS and NEC rats relative to initial freezing prior to footshock, GAERS did display increased freezing to contextual cues in the low-intensity condition during conditioning (Fig. 7B). Thus, these data are interpretable as either enhanced contextual memory in GAERS or as a deficit in contextual memory in the NEC rats. However, the high-intensity conditioning data suggest that NEC rats show intact contextual memory when the UCS is more salient (Fig. 7F). GAERS froze significantly more during extinction day 1 following low-intensity conditioning, but not high-intensity conditioning, a further reflection of heightened anxiety-like behaviour in this strain. Delayed extinction to the CS and context was also found in GAERS for both low-intensity and high-intensity fear conditioning protocols. A similar fear conditioning protocol used on male Sprague-Dawley rats demonstrated significant and near-complete extinction of fear at 2 days after training, suggesting that GAERS exhibit increased freezing relative to other rat strains (Sotres-Bayon *et al.*, 2007).

It is difficult to determine whether the observed increased conditioning to contextual cues and extinction delays were the result of enhanced conditioning, impaired inhibitory learning, or increased anxiety as increases in freezing could be interpreted in any of these three ways. Future research should consider investigating learning and memory processes using appetitive operant tasks that are less anxiety provoking to simplify interpretation of the data. Although GAERS displayed higher levels of freezing overall during reinstatement, both GAERS and NEC rats displayed similar responses to the unsignalled USs and CSs, suggesting that reinstatement of fear was not specifically affected by strain.

Studies using other rodent models of epilepsy have shown dissociable alterations in fear conditioning and tests of anxiety. For example, amygdala kindling, a rodent model of temporal lobe seizures, caused decreased freezing during both fear conditioning and recall (Botterill *et al.*, 2014) and increased anxiety in the elevated plus maze and open field tests (Kalynchuk, 2000; Hannesson *et al.*, 2008). Thus, the mechanisms mediating the changes in emotionality and cognition probably differ between these models. The reduction in extinction learning may be caused by impaired inhibitory learning mechanisms in the medial prefrontal cortex and amygdala relevant to human anxiety disorders such as post-traumatic stress disorder (Milad & Quirk, 2012; Herry & Johansen, 2014).

Impaired long-term recall of latent inhibition in male GAERS

Results of the LI experiment show similarities with the fear conditioning experiment. The reduction in freezing to the first CS in male NEC pre-exposed rats confirms the effectiveness of the pre-exposure phase in habituating the NEC rats to the cue. GAERS males from the pre-exposed group showed no such difference. In addition, the non-pre-exposed male NEC rats showed similar levels of freezing to CS 1 as GAERS, which is distinct from the low level of freezing observed in NEC rats during CS 1 in the fear conditioning studies. We believe that this relative increase in freezing reflects that the NEC male rats had come to expect a chamber with no stimuli during pre-exposure (consistent with what they had experienced) and presentation of the first CS caused an increase in freezing as it was unexpected in the pre-exposure context. Following conditioning, GAERS males of both groups showed increased freezing relative to NEC rats, an effect that we observed in the low-intensity fear conditioning experiment. During long-term recall 24 h later, the NEC male rat groups showed strong evidence of LI, whereas the GAERS males did not. The females of both strains performed in a manner similar to the fear conditioning experiment with both groups of GAERS showing significantly more freezing than NEC rats during conditioning, short-term recall, and long-term recall. Surprisingly, female NEC rats did not show evidence of LI although previous studies have shown LI in male and female Wistar rats (Arad & Weiner, 2010).

Latent inhibition is typically believed to require selective attention, and deficits in LI are measurable in psychiatric disorders including schizophrenia (Weiner & Arad, 2009). Selective attention, as measured by the continuous performance test, is also impaired in children with absence epilepsy (Glauser *et al.*, 2010; Killory *et al.*, 2011). Therefore, the impairment in LI noted for the male GAERS is consistent with this cognitive phenotype of patients. Further characterization of the attentional deficits in GAERS could be achieved with the continuous performance test that has been adapted for use in rodents with touchscreen-equipped operant conditioning chambers (Hvoslef-Eide *et al.*, 2015).

GAERS as a model of the comorbid psychiatric symptoms associated with absence epilepsy

GAERS have been most commonly used to examine the mechanisms underlying absence seizures (Marescaux *et al.*, 1992; Danober *et al.*, 1998; Cain & Snutch, 2013). In addition, the model has been used to study the emotional and psychosis-related phenotypes associated with epilepsy. The present findings extend this characterization to include cognition with the fear conditioning and LI paradigms. Given the association between absence epilepsy and psychiatric comorbidities in humans (Mandelbaum & Burack, 1997; Pavone *et al.*, 2001; Henkin *et al.*, 2005; Caplan *et al.*, 2009), use of the GAERS strain to further understand the aetiology of psychiatric illness is warranted. The mechanism underlying the behavioural changes observed in GAERS is probably complex, given the neural circuitry and pharmacology involved in the tests used. Differences between the GAERS and NEC rats have been reported in several brain areas including the thalamus, cortex, amygdala, and striatum that could contribute to the behavioural changes observed (Danober *et al.*, 1998; Bouilleret *et al.*, 2009; Powell *et al.*, 2009; Jones *et al.*, 2010; Tringham *et al.*, 2012; Cain & Snutch, 2013). Assessing the contribution of absence seizures towards the behavioural changes observed will be an important next step. Absence seizures increase in frequency and length as the GAERS age (Jones *et al.*, 2008) and extended administration of ethosuximide to GAERS has been shown to reduce the anxiety-like profile of GAERS in the open field test (Dezsi *et al.*, 2013). As the electrophysiological recordings were conducted on the rats when they were in their home cage, additional recordings simultaneously with behavior would clarify whether seizures during the tasks contributed to the effects observed. Finally, pharmacological experiments with compounds such as ethosuximide or the T-type calcium channel blocker Z944, known to reduce seizures (Tringham *et al.*, 2012; Dezsi *et al.*, 2013), would provide insight into the possible role of seizures for the behavioral effects observed.

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Abbreviations

CS, conditioned stimulus; EEG, electroencephalography; GAERS, Genetic Absence Epilepsy Rats from Strasbourg; ITI, intertrial interval; LI, latent inhibition; NEC, non-epileptic control; P, postnatal day; PPI, prepulse inhibition; SWD, spike-wave discharge; US, unconditioned stimulus.

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