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Research article

The effect of prenatal and postnatal caffeine exposure on pentylentetrazole induced seizures in the non-epileptic and epileptic offsprings

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ABSTRACT

Caffeine, a central nervous system stimulant, has been reported to modulate seizure activity in various studies. In this study the effects of caffeine exposure on the pentylenetetrazole (PTZ) induced seizure thresholds and seizure stages in the Wistar and genetic absence epilepsy model offsprings were examined. Adult female and male Wistar rats and genetic absence epilepsy rats from Strasbourg (GAERS) consumed caffeine dissolved in water (0.3 g/L) before conception, during the gestational periods and lactation period whereas control groups of each strain received tap water. All offsprings at postnatal day 30 (PN30) subjected to 70 mg/kg of PTZ were evaluated in terms of overall seizure stages, the latency to the first generalized seizure and the c-Fos protein activity in the brain regions of somatosensorial cortex (SSCx), reticular thalamic nucleus (Rt), ventrobasal thalamus (VB), centromedial nucleus (CM) and lateral geniculate nucleus (LGN). The Wistar caffeine group had significantly shorter latency to the first generalized seizure (1.53 ± 0.49 min) comparing to the Wistar control offsprings (3.40 ± 0.68 min). GAERS caffeine group (6.52 ± 2.48 min) showed significantly longer latency comparing to Wistar caffeine group (1.53 ± 0.49 min). Although statistically not significant, GAERS caffeine group showed a longer latency comparing to the GAERS control group (4.71 ± 1.82 min). In all regions of SSCx, Rt, VB, CM and LGN, GAERS caffeine group had lower c-Fos protein expression comparing to the GAERS control group ($p < 0.05$). Wistar caffeine rats had lower expression of c-Fos protein comparing to the Wistar control group only in SSCx. In CM, GAERS rats expressed lower c-Fos protein comparing to the Wistar control ($p < 0.05$). In conclusion differential effects of caffeine in the seizure modulation may involve c-Fos protein activity-dependent protection mechanisms.

1. Introduction

The relationship between caffeine and seizures was shown in many experimental and clinical settings. There is some controversy regarding the effects of caffeine on seizure and seizure threshold. Some reports supported that the long-term application of caffeine may increase the seizure threshold in mice and rat models [1–3]. However caffeine is a central nervous system stimulant, and in isolated hippocampal slices it has been shown to induce epileptiform activities [4,5]. *In vivo* studies in the motor seizure models showed that the caffeine may shorten the latent period of seizure activity [6] and can exacerbate the seizure activity in electrical kindling models [7], as it also did in a chemical model of epilepsy induced by pentylenetetrazole (PTZ) [8]. In humans caffeine was found to lengthen or sustain the seizures evoked by electroconvulsive therapy [9,10]. In the genetic absence epilepsy models, caffeine has been shown to acutely suppress the spike-and-wave discharges (SWDs) in Wistar Albino Glaxo Rats from Rijswijk (WAG/Rij) [11] and Genetic Absence Epilepsy Rats from Strasbourg GAERS [12], while the chronic exposure to caffeine dissolved in drinking water had no effect on SWD expression of GAERS, other adenosine receptor agonists seem to increase the SWD activity in the same model [12]. On the other hand, the age of exposure to caffeine seems to modulate the responses differently. For instance, while exposure to caffeine at earlier stages of such as; postnatal 7–11 (PN7-PN11) or PN13-PN17 can raise

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the threshold for the seizure induction, caffeine can be proconvulsive at the PN25 [13]. In terms of prenatal exposure it has been shown that caffeine alters the hippocampal excitability and increases the percentage of animals that display seizures, although no change of locomotor activity that persists into adulthood was reported [14].

c-Fos protein, the marker of activated neuronal pathways, has been shown to be induced following seizure activity [15–17], and discussed to increase the survival of neurons [18]. Caffeine was reported to increase the c-Fos protein activity in certain brain regions such as striatum or nucleus accumbens [19]. The accumulation of c-Fos protein activity was also found in the hippocampus, cingulate cortex, frontal cortex and the thalamic nuclei in rats with generalized seizures [20]. The brain regions; somatosensorial cortex (SSCx), reticular thalamic nucleus (Rt), ventrobasal thalamus (VB), centromedial nucleus (CM) and lateral geniculate nucleus (LGN) of thalamus were associated with absence epilepsy [21,22]. The SWDs exhibited in the absence epilepsy are especially known to be initiated at cortical perioral sites in the SSCx [23] and synchronized and paced in the thalamic level, including Rt and VB [24–26]. CM has been shown to be degenerated on the course of absence epilepsy in WAG/Rij model [27]. The findings of increased calcium current found in LGN in absence epilepsy models compared to the controls and the spindle like activity produced by LGN was substituted by SWDs upon the application of penicillin, suggest that CM and LGN can be the contributors to the absence epilepsy pathology [28,29].

In this project, we aimed to investigate the effects of prenatal caffeine exposure on the convulsive seizure thresholds and seizure stages in the Wistar and GAERS offsprings at PN30. For this purpose adult female and male Wistar rats and GAERS consumed caffeine dissolved in water before conception, during the gestational periods and lactation period to detect whether caffeine can change the 70 mg/kg PTZ-induced seizure threshold in the offsprings in comparison with the control offsprings exposed to tap water. Further, the c-Fos protein level which was induced following seizure activity [16,30] was evaluated to determine the effect of caffeine on the early post seizure modifications in the molecular level in the SSCx, Rt, VB, CM and LGN in the same offsprings.

2. Material and methods

2.1. Animals

All procedures performed on animals were approved by the Ethical Committee for Experimental Animals of Marmara University, in compliance with the EU Directive 2010/63/EU for animal experiments and all efforts were made to minimize the discomfort that may arise as the result of all experiments (Protocol no: 89.2015.mar). Adult Wistar (n = 4) and GAERS (n = 4) female and male rats (3–4 months old, 300–350 g) were obtained from Experimental Research Animals Unit of Marmara University Faculty of Medicine (DEHAMER) and the GAERS breeding colony of the Department of Pharmacology and Clinical Pharmacology of Marmara University School of Medicine, respectively. The rats were housed in a temperature controlled (21 ± 3 °C) and a 12-h light/dark cycle (lights on at 8 a.m.) room. For animal mating of each group, Wistar and GAERS female and male rats were placed as 1 male and 1 female in one cage. All animals were provided *ad libitum* food. The male and female rats in caffeine group started to receive caffeine dissolved in water and the control groups received tap water before conception. Pregnant rats were exposed to caffeine dissolved in water or tap water until the offsprings were weaned. All offspring groups were injected with PTZ at PN30 to detect the PTZ induced seizure threshold and seizure stages. The offspring groups were as follows: Group I: Wistar control (tap water, n = 6). Group II: GAERS control (tap water, n = 9). Group III: Wistar caffeine (caffeine dissolved in water, n = 6). Group IV: GAERS caffeine (caffeine dissolved in water, n = 7).

2.2. Drugs

To evaluate the effect of prenatal and postnatal caffeine exposure on the PTZ induced seizure thresholds of the offsprings, either caffeine (Doga İlac., Batch Nr: 06788) which was dissolved in tap water (0.3 g/L) or tap water alone were consumed by the adult animals orally before conception, during the gestational periods and lactation period. The offspring groups were weaned at PN28 and injected subcutaneously (s.c.) with 70 mg/kg PTZ dissolved in saline (Sigma Aldrich) on the day of the experiment (PN30). The daily oral intake of caffeine (ml/day) consumed by the adult females was recorded for Wistar or GAERS caffeine groups.

2.3. Surgery

The offsprings of all groups were weaned on the PN28 and anesthetized with ketamine (100 mg/kg, IP) and xylazine (10 mg/kg, IP). Each animal was placed in a stereotaxic frame (Stoelting Model 51600, Stoelting Co., Illinois, USA). A longitudinal incision was cut over the skull and four stainless steel screws with insulated wires of own production were implanted bilaterally over the frontoparietal cortex for cortical electroencephalography (EEG) recordings. The electrodes were connected by insulated wires to a micro connector for the EEG recordings. The electrodes and wires were covered by dental acrylic and fixed to the skull.

2.4. Assessment of the PTZ induced seizures and EEG recordings

Two days after the electrode implantations, 70 mg/kg of PTZ (s.c.) was applied to the animals of all groups on PN30. PTZ was injected into a loose fold of skin of rat in the midline of the neck. The animals were placed in isolated cages to minimize stress and observed for the next 60 min for the presence or absence of a seizure. The EEG was recorded from all animals in the morning (from 9:00 am to 10:30 am), 30 min before and 60 min after the injection of PTZ.

The PTZ induced seizures were evaluated by behavioral seizure intensity stages according to the modified Racine's scale as previously explained (Table 1) [31]. We have evaluated the stage 4–5 seizures as generalized seizure activity. An animal not displaying stage 4–5 seizures was considered to be "not generalized" and displaying stage 4–5 seizures considered as "generalized". The PTZ injection can produce behavioral arrest, staring, muscle twitches, myclonus-like jerks of limbs and/or trunk followed by generalized clonic seizures (Stage 4) with progression to tonic–clonic seizures (Stage 5) and straub tail [31]. This behavioral seizure scoring was cross-checked and accompanied by the seizure activities on the EEG. EEG was amplified through a BioAmp ML 136 amplifier, with band-pass filter settings at 1–40 Hz, recorded and analyzed using Chart v7 program (PowerLab8S ADI Instruments, Oxfordshire, UK). Together with the behavioral seizure intensities, the latency to the first stage 4–5 generalized seizure was evaluated and considered as the seizure threshold.

Table 1
Seizure Intensity Stages Adapted From Modified Racine's Scale.

Stage	Behavior
0	Whisker trembling
1	Sudden behavioral arrest& motionless staring
2	Facial twitching with muzzle
3	Myoclonic jerks in body
4	Clonic seizure in a sitting position
5	Convulsions including clonic/tonic-clonic seizures while lying on belly/side and/or pure tonic seizures

The revised seizure intensity scale, adapted to five stage scoring system in our study [25].

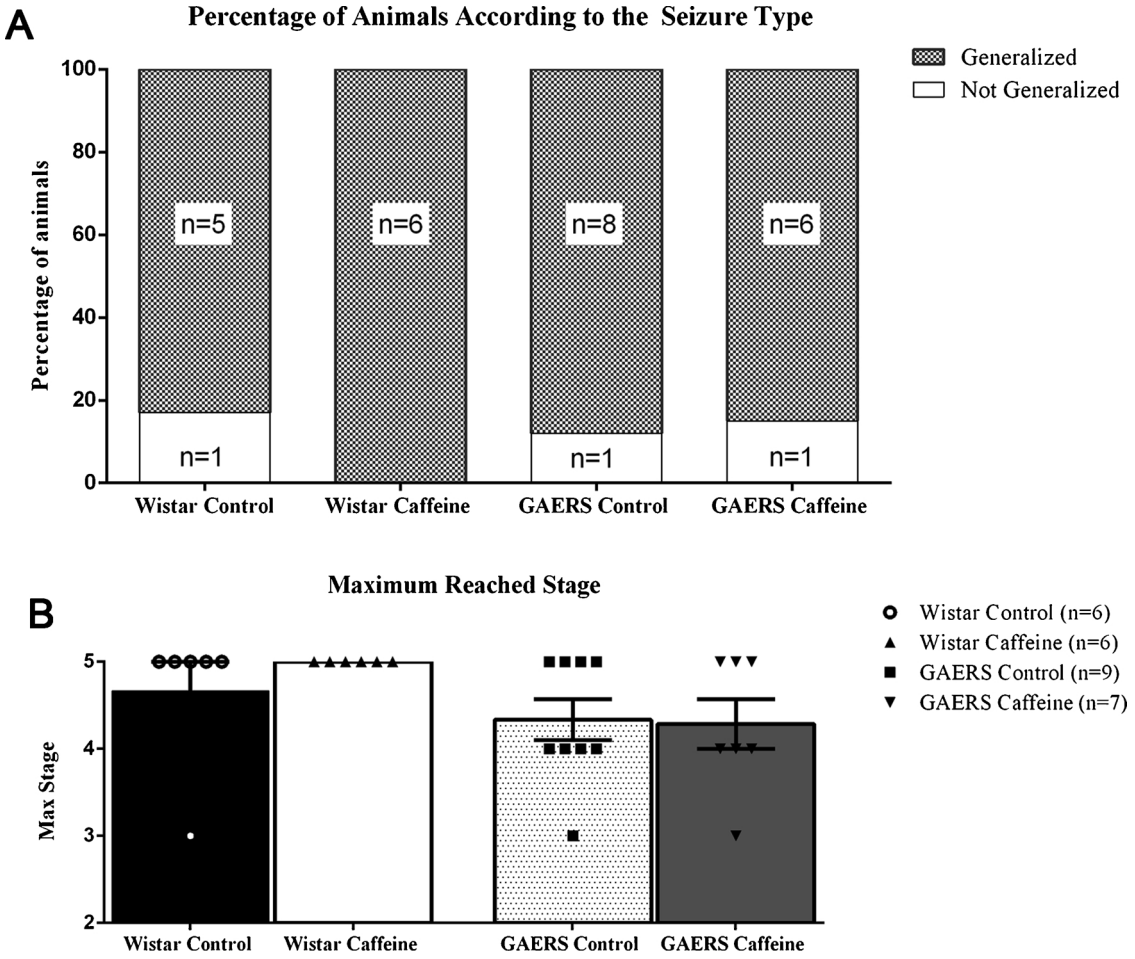

Fig. 1. A) Percentage of offsprings that reached generalized or not generalized seizures following PTZ injection. The generalized seizures are the stage 4–5 seizures according to the modified Racine's scale. B) The maximum reached seizure stages in all groups. Data are expressed as mean ± SEM (n = number of individual rats). Ordinary one-way ANOVA of variance.

2.5. Tissue preparation

Rats were deeply anesthetized with intraperitoneal ketamine (100 mg/kg) and chlorpromazine (1 mg/kg) and perfused transcardially with 6–7 ml of a heparin solution (1000 U/ml of 0.1 M PBS, pH 7.3) and a fixative solution containing 4% paraformaldehyde 30 min after the application of PTZ [32]. Following fixation, the animals were decapitated and the brains were removed. The brains were kept in 4% paraformaldehyde for overnight. After 48 h of incubation in 20% sucrose solution at +4 °C (in 0.1 M PB) for cryoprotection, the samples were kept at +4 °C until c-Fos protein staining. Series of coronal sections, 35 μm thick, were cut using a vibratome (VT 1000S, Leica). Free-floating sections were placed in 6-well plate.

2.6. c-Fos protein immunohistochemistry

For the detection of c-Fos protein expression, the immunoperoxidase procedure was applied as follows. The sections were rehydrated in 0.1% PBS (phosphate-buffered saline solution, 3 × 5 min). This washing procedure was repeated after 10-min incubation in PBS containing 3% H_2O_2 at room temperature. Unspecific binding was blocked with 10 min incubation in 10% bovine serum albumin in PBST (PBS-Tween 20) at room temperature. After washing, the primary antibody (1:1000 polyclonal goat anti-c-Fos, Sigma Aldrich, F7799) was applied for 2 h at 37 ^{0}C. The unbound antibody was removed by washing in PBS and the sections were incubated with the secondary antibody (Universal Kit, Scy Tek Laboratories, ABN125) as instructed. c-Fos immunoreactivity was detected by incubation in diaminobenzidine solution (Vector Laboratories, DAB Peroxidase Substrate Kit SK-4100). The sections were mounted on microscopic glass slides (SuperFrost Plus, Faust, Switzerland).

2.7. Quantification of c-Fos protein expressing neurons

The location of the SSCx, Rt, VB, CM and LGN were identified according to the rat brain atlas by Paxinos and Watson [33]. Four serial sections from each region (SSCx, Rt, VB, CM and LGN) with standard X10 magnification was photographed using AxioCam, Carl Zeiss AG. The sections of SSCx were obtained between bregma -0.60 and -3.2, Rt were between bregma -1.3 and -2.5, VB were between bregma -2.4 and 3.9 mm, CM were between bregma -1.8 and -3.2 and LGN were between bregma -3.3 and -4.3.

The numbers of c-Fos positive neurons were expressed as cell counts/per unit area from the photographs using Image-J program (Wayne Rasband, NIH, USA). Then the average cell counts/per unit from the four sections were calculated. The mean value of the cell counts/per unite area of each animal was used for statistical analysis.

2.8. Statistical analysis

All statistical analyses were performed with GraphPad Prism version 5.00 (GraphPad Software, San Diego, USA). For statistical comparisons of the maximum stage of seizures attained with PTZ and the latencies to the first generalized seizures one-way ANOVA followed by the Tukey's

post-hoc test or Student's *t*-test were used. For the statistical analysis of c-Fos protein levels, two-way ANOVA of variance followed by Tukey's post-hoc test were used.

All data were expressed as the mean ± SEM. Difference of $p < 0,05$ was considered to be statistically significant.

3. Results

The mean daily intake of caffeine dissolved in water (ml/day) during gestational periods and lactation for adult females were as follows; Wistar caffeine: 96.73 ± 5.34 ml and GAERS caffeine: 81.67 ± 5.75 ml.

3.1. PTZ induced seizures and EEG recordings

None of the GAERS pups had matured SWDs that are identified with ≥ 1 s in the duration, a train of SWDs (7–11 Hz) and the amplitude of at least twice the background amplitude of the EEG.

3.1.1. The maximum stage of seizures in animals

PTZ elicited non-generalized clonic seizures of facial, forelimb and trunk muscles and/or subsequently generalized tonic-clonic seizures. In Wistar control group 5 out of 6 animals and in Wistar caffeine group, all of the 6 animals exhibited generalized seizures. In GAERS control group 8 out of 9 rats and in the GAERS caffeine group 6 out of 7 rats exhibited generalized seizures (Fig. 1A).

There was no difference between groups for the maximum reached stage of seizures following PTZ induction. The maximum reached stage of seizures in each group were as follows: Wistar control 4.67 ± 0.33, Wistar caffeine 5 ± 0, GAERS control 4.33 ± 0.24, and GAERS caffeine 4.29 ± 0.29 (Fig. 1B). One animal in each of the Wistar control, GAERS control and GAERS caffeine groups did not reach a generalized seizure stage 4 or 5 (Fig. 1A).

3.1.2. The latency to the first generalized seizure

The latency to the first stage 4–5 generalized seizures in groups were as follows: Wistar control 3.40 ± 0.68 min; Wistar caffeine 1.53 ± 0.49 min; GAERS control 4.71 ± 1.82 min, and GAERS caffeine 6.52 ± 2.48 min. Wistar caffeine group reached to a generalized seizure stage earlier than the Wistar control group ($p < 0.05$) (Fig. 2). There was also a statistical significance between Wistar and GAERS caffeine groups. GAERS caffeine group had a longer latency comparing to the Wistar caffeine group ($p < 0.05$) (Fig. 2). Although GAERS caffeine group showed a slightly longer latency comparing to the GAERS control group there was no statistical significance.

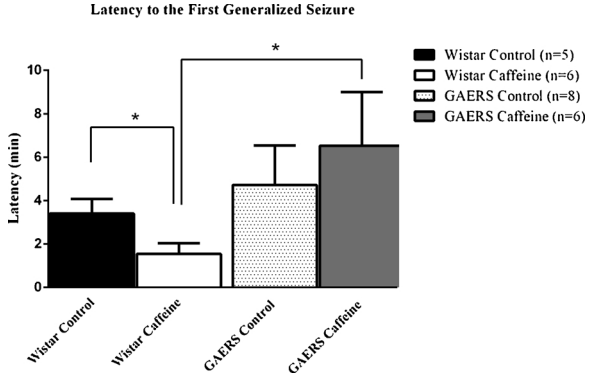

Fig. 2. The latency to the first generalized seizure following PTZ injection. Data are expressed as mean ± SEM (n = number of individual rats). Ordinary one-way ANOVA of variance. *$p < 0.05$, unpaired *t*-test (one-tailed).

3.2. c-Fos protein levels in SSCx, Rt, VB, CM and LGN

The SSCx of Wistar caffeine rats expressed significantly lower c-Fos than the Wistar control group. A similar statistically significant result was also observed between GAERS control and the caffeine groups (Fig. 3). GAERS caffeine group had lower c-Fos protein expression than the GAERS control group. Another statistically significant difference for c-Fos protein expression was between Wistar and GAERS caffeine groups. The SSCx of GAERS caffeine rats expressed lower c-Fos compared to the Wistar caffeine rats ($p < 0.05$) (Fig. 3). The expression of c-Fos in the Rt, VB and LGN regions of GAERS caffeine rats was significantly lower than GAERS control group. The comparison of Wistar and GAERS caffeine groups showed that GAERS caffeine rats expressed significantly lower c-Fos compared to the Wistar caffeine group in these brain regions ($p < 0.05$) (Fig. 3).

A statistically significant difference was found in the c-Fos protein activity in GAERS control group compared to the Wistar control group in the CM thalamic nucleus. c-Fos protein activity of GAERS control group was lower than Wistar control group. Further, GAERS caffeine rats expressed significantly lower c-Fos than the GAERS control group in CM. Comparison of Wistar and GAERS caffeine groups showed that GAERS caffeine group expressed significantly lower c-Fos in the CM compared to Wistar caffeine group ($p < 0.05$) (Fig. 3).

4. Discussion

The main outcomes of this study are as follows: (1) The Wistar caffeine group showed a statistically significant shorter latency to reach a stage 4–5 seizure compared to the Wistar control group; (2) The GAERS caffeine group had a longer latency to reach a stage 4–5 seizure compared to the Wistar caffeine group but not with GAERS control group; (3) c-Fos protein expression was lower in the GAERS caffeine group compared to the GAERS control group in all regions (SSCx, Rt, VB, CM and LGN); (4) c-Fos protein expression was significantly lower in the GAERS caffeine group than the Wistar caffeine group in all regions.; (5) Finally, the only difference for c-Fos protein expression between Wistar control and GAERS control rats was in CM, where the GAERS control rats expressed less c-Fos protein than the Wistar control rats.

The results of the latency to the stage 4–5 generalized seizures, between Wistar control and caffeine rats at PN30 were in line with the studies that the caffeine decreased the seizure threshold in chemical seizure models [34–36]. We have indicated in this study that GAERS offsprings prenatally exposed to caffeine showed a delayed generalized PTZ induced motor seizure compared to the Wistar offsprings at PN30. The adult GAERS rats were shown to have a higher incidence of PTZ induced motor seizures with systemic application of 25 mg/kg or higher doses of PTZ [37]. In this study we evaluated the latency to the first generalized seizure in GAERS at PN30 when they were in the pre-epileptic or non-epileptic phase which they showed a delayed onset to PTZ induced generalized seizures. This may be related with the resistance mechanisms that may be due to the developmental course of absence epilepsy and genetic orientations of GAERS [38]. Another factor to consider is that there is a known increased threshold for seizures in the rats since they require higher doses of PTZ due to their age [39]. The experiments in the rats prenatally exposed to caffeine revealed that this increased seizure thresholds can persist into adulthood, as well [40].

The c-Fos as a marker of neuronal activation, is being widely used in experimental neurological studies, including seizures [41–43]. Although c-Fos has been shown to be induced following PTZ [17], in Glu8 knockout model the thalamic nuclei expressed lower c-Fos upon the application of PTZ [44]. Although following PTZ induced seizures, offsprings of GAERS caffeine group showed significant reductions in the expressions of c-Fos comparing to the control group in all brain regions (SSCx, Rt, VB, CM and LGN), there was a significant reduction in c-Fos protein expression only in the SSCx of the offsprings of Wistar caffeine

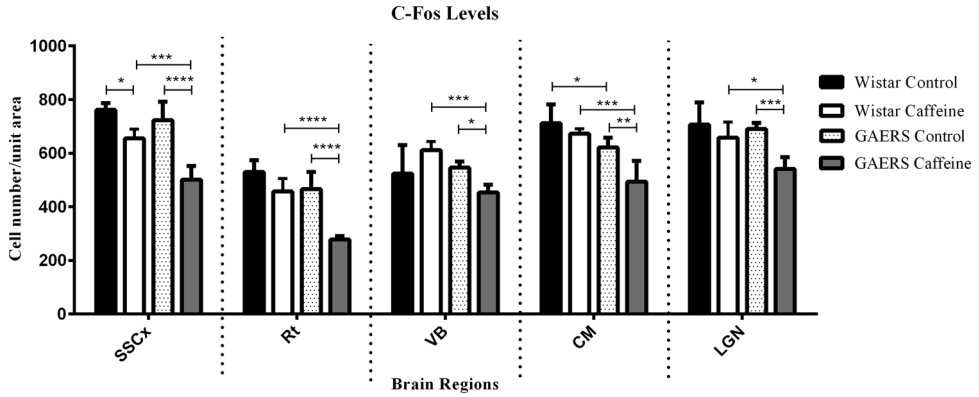

Fig. 3. The cell number per unit of c-Fos protein levels in SSCx, Rt, VB, CM and LGN following PTZ injection. Data are expressed as mean ± SEM (n = number of individual rats). Two-way ANOVA of variance. *p < 0.05, *p < 0,05,**p < 0,01, ***p < 0,001 and ****p < 0,001 Tukey's post-hoc test for multiple comparisons.

group compared to Wistar control group. The relationship between caffeine and c-Fos is controversial in the literature. For instance, Singewald et al., reported that anxiogenic drugs administration showed no significant difference in the c-Fos protein level in the SSCx of Sprague Dawley rats [45]. However, there are studies which showed that caffeine induced the expression of c-Fos mRNA in SSCx of the rat [46,47]. The controversial results can be due to the lack of reflection on the c-Fos protein levels although c-Fos gene expression are induced immediately.

In the present study it is interesting that GAERS control rats showed significant reduction in c-Fos protein levels in the pre-epileptic phase (PN30) compared to the Wistar control in the CM. Further, neuronal death has been reported in the CM of WAG/Rij rat [25].

The EEG evidence in humans suggests that CM participates in the propagation of absence seizure activity [48]. The results of the present study clearly shows involvement of CM in absence epilepsy.

Our results showed that the PTZ induced seizure threshold, the latency to the first stage 4–5 generalized seizure, was higher in GAERS caffeine group compared to Wistar caffeine group, the findings of lower expression of c-Fos protein levels in GAERS caffeine than the Wistar caffeine group can be considered to be consistent. Prenatal consumption of caffeine was reported to induce alpha2 GABA(A) receptor subunit mRNAs in the medulla of the offsprings however it did not induce c-Fos protein expression normally evoked by hypoxia in rats [49]. In our study caffeine exposed offsprings of GAERS had reduced c-Fos expression. In relation with the disruption of GABA receptor activity in GAERS [37], caffeine may be considered to stimulate the GABAergic activity in GAERS. Together with this finding, the Glu8 knockout model study [44] may imply that the caffeine can act through the glutamatergic drive, the PTZ may bring to the scene.

Although in the literature both Wistar and Non-Epileptic Control rats (NECs) strains are recommended to be used as double control to GAERS [50], as a limitation to our study we could not compare our results in NEC animals due to breeding constraints. On the other hand, NEC strain can show metabolic [51], behavioral [50] and GABAergic differences possibly as a result of out casting/excluding other non-absence related genes by selective breeding [52]. Besides there are studies that use Wistar controls as only control to GAERS in epilepsy research [53,54].

5. Conclusion

Further studies are needed for the investigation of why GAERS showed a different progress comparing to the Wistar which may be be partially related with the seizure generating areas in GAERS and possibly through c-Fos mediated pathways. The evaluation of the cognitive, behavioral effects of prenatal caffeine exposure in epilepsy will be valuable as well.

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Declaration of Competing Interest

The authors report no conflicts of interest, they are responsible for the content and writing of this paper.

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References

[1] V. Georgiev, B. Johansson, B.B. Fredholm, Long-term caffeine treatment leads to a decreased susceptibility to NMDA-induced clonic seizures in mice without changes in adenosine A1 receptor number, Brain Res. 612 (1993) 271–277.
[2] B. Johansson, V. Georgiev, T. Kuosmanen, B.B. Fredholm, Long-term treatment with some methylxanthines decreases the susceptibility to bicuculline- and pentylenetetrazol-induced seizures in mice. Relationship to c-fos expression and receptor binding, Eur. J. Neurosci. 8 (1996) 2447–2458.
[3] J.G. Zhi, G. Levy, Effect of chronic caffeine administration on theophylline concentrations required to produce seizures in rats, Proc. Soc. Exp. Biol. Med. 193 (1990) 210–213.
[4] D.G. Margineanu, H. Klitgaard, Caffeine-induced epileptiform field potentials in rat hippocampal slices: a pharmacological characterization, Neuropharmacology 47 (2004) 926–934.
[5] A. Ruangkittisakul, S. Sharopov, C. Kantor, J. Kuribayashi, E. Mildenberger, H.J. Luhmann, W. Kilb, K. Ballanyi, Methylxanthine-evoked perturbation of spontaneous and evoked activities in isolated newborn rat hippocampal networks, Neuroscience 301 (2015) 106–120.
[6] P. Gupta, S.B. Khobragade, V.M. Shingatgeri, Effect of various antiepileptic drugs in zebrafish PTZ-Seizure model, Indian J. Pharm. Sci. 76 (2014) 157–163.
[7] T.E. Albertson, R.M. Joy, L.G. Stark, Caffeine modification of kindled amygdaloid seizures, Pharmacol. Biochem. Behav. 19 (1983) 339–343.
[8] Z. Esmaili, A. Heydari, Effect of acute caffeine administration on PTZ-induced seizure threshold in mice: involvement of adenosine receptors and NO-cGMP signaling pathway, Epilepsy Res. 149 (2019) 1–8.
[9] P.E. Hinkle, C.E. Coffey, R.D. Weiner, M. Cress, C. Christison, Use of caffeine to lengthen seizures in ECT, Am. J. Psychiatry 144 (1987) 1143–1151.
[10] C.E. Coffey, G.S. Figiel, R.D. Weiner, W.B. Saunders, Caffeine augmentation of ECT, Am. J. Psychiatry 147 (1990) 579–585.
[11] F. Dede, S. Karadenizli, O.D. Ozsoy, F.C. Eraldemir, D. Sahin, N. Ates, Antagonism of adenosinergic system decrease SWD occurrence via an increment in thalamic NFkB and IL-6 in absence epilepsy, J. Neuroimmunol. 326 (2019) 1–8.
[12] K. Germe, J.B. Faure, E. Koning, A. Nehlig, Effect of caffeine and adenosine receptor ligands on the expression of spike-and-wave discharges in Genetic Absence Epilepsy Rats from Strasbourg (GAERS), Epilepsy Res. 110 (2015) 105–114.
[13] J. Tchekalarova, H. Kubova, P. Mares, Postnatal period of caffeine treatment and time of testing modulate the effect of acute caffeine on cortical epileptic after discharges in rats, Brain Res. 1356 (2010) 121–129.

[14] C.G. Silva, C. Metin, W. Fazeli, N.J. Machado, S. Darmopil, P.S. Launay, A. Ghestem, M.P. Nesa, E. Bassot, E. Szabo, Y. Baqi, C.E. Muller, A.R. Tome, A. Ivanov, D. Isbrandt, Y. Zilberter, R.A. Cunha, M. Esclapez, C. Bernard, Adenosine receptor antagonists including caffeine alter fetal brain development in mice, Sci. Transl. Med. 5 (2013) 197ra104.
[15] D. Akin, T. Ravizza, M. Maroso, N. Carcak, T. Eryigit, I. Vanzulli, R.G. Aker, A. Vezzani, F.Y. Onat, IL-1β is induced in reactive astrocytes in the somatosensory cortex of rats with genetic absence epilepsy at the onset of spike-and-wave discharges, and contributes to their occurrence, Neurobiol. Dis. 44 (2011) 259–269.
[16] L. Kaczmarek, From c-Fos to MMP-9: in control of synaptic plasticity to produce healthy and diseased mind, a personal view, Postepy Biochem. 64 (2018) 101–109.
[17] E. Samokhina, A. Samokhin, Neuropathological profile of the pentylenetetrazol (PTZ) kindling model, Int. J. Neurosci. (2018) 1–11.
[18] J. Zhang, D. Zhang, J.S. McQuade, M. Behbehani, J.Z. Tsien, M. Xu, C-fos regulates neuronal excitability and survival, Nat. Genet. 30 (2002) 416–420.
[19] P. Svenningsson, B. Johansson, B.B. Fredholm, Caffeine-induced expression of c-fos mRNA and NGFI-A mRNA in caudate putamen and in nucleus accumbens are differentially affected by the N-methyl-D-aspartate receptor antagonist MK-801, Brain Res. Mol. Brain Res. 35 (1996) 183–189.
[20] P. Barone, M. Morelli, G. Cicarelli, A. Cozzolino, G. DeJoanna, G. Campanella, G. DiChiara, Expression of c-fos protein in the experimental epilepsy induced by pilocarpine, Synapse 1 (1993) 1–9.
[21] M. Steriade, F. Amzica, Sleep oscillations developing into seizures in corticothalamic systems, Epilepsia 44 (Suppl. 12) (2003) 9–20.
[22] S.Y. Abbas, S.W. Ying, P.A. Goldstein, Compartmental distribution of hyperpolarization-activated cyclic-nucleotide-gated channel 2 and hyperpolarization-activated cyclic-nucleotide-gated channel 4 in thalamic reticular and thalamocortical relay neurons, Neuroscience 141 (2006) 1811–1825.
[23] E. Sitnikova, G. van Luijtelaar, Cortical control of generalized absence seizures: effect of lidocaine applied to the somatosensory cortex in WAG/Rij rats, Brain Res. 1012 (2004) 127–137.
[24] G. van Luijtelaar, E. Sitnikova, Global and focal aspects of absence epilepsy: the contribution of genetic models, Neurosci. Biobehav. Rev. 30 (2006) 983–1003.
[25] H.K. Meeren, E.L. van Luijtelaar, F.H. Lopes da Silva, R.K. Berdiev, N.E. Chepurnova, S.A. Chepurnov, A.M. Coenen, [The cortico-thalamic theory for generalised spike-wave discharges], Usp. Fiziol. Nauk 35 (2004) 3–19.
[26] T. Seidenbecher, R. Staak, H.C. Pape, Relations between cortical and thalamic cellular activities during absence seizures in rats, Eur. J. Neurosci. 10 (1998) 1103–1112.
[27] M. Jafarian, F. Karimzadeh, F. Alipour, F. Attari, A.A. Lotfinia, E.J. Speckmann, M.R. Zarrindast, A. Gorji, Cognitive impairments and neuronal injury in different brain regions of a genetic rat model of absence epilepsy, Neuroscience 298 (2015) 161–170.
[28] M. Avoli, P. Gloor, Role of the thalamus in generalized penicillin epilepsy: observations on decorticated cats, Exp. Neurol. 77 (1982) 386–402.
[29] T. Kanyshkova, P. Ehling, M. Cerina, P. Meuth, M. Zobeiri, S.G. Meuth, H.C. Pape, T. Budde, Regionally specific expression of high-voltage-activated calcium channels in thalamic nuclei of epileptic and non-epileptic rats, Mol. Cell. Neurosci. 61 (2014) 110–122.
[30] P.F. Fabene, A. Andrioli, M.R. Priel, E.A. Cavalheiro, M. Bentivoglio, Fos induction and persistence, neurodegeneration, and interneuron activation in the hippocampus of epilepsy-resistant versus epilepsy-prone rats after pilocarpine-induced seizures, Hippocampus 14 (2004) 895–907.
[31] A. Luttjohann, P.F. Fabene, G. van Luijtelaar, A revised Racine's scale for PTZ-induced seizures in rats, Physiol. Behav. 98 (2009) 579–586.
[32] V.N. Barros, M. Mundim, L.T. Galindo, S. Bittencourt, M. Porcionatto, L.E. Mello, The pattern of c-Fos expression and its refractory period in the brain of rats and monkeys, Front. Cell. Neurosci. 9 (2015) 72.
[33] G. Paxinos, C. Watson, The Rat Brain in Stereotaxic Coordinates, 3rd, Academic Press, San Diego, 1997.
[34] N.S. Chu, Caffeine- and aminophylline-induced seizures, Epilepsia 22 (1981) 85–94.
[35] C. Cutrufo, L. Bortot, A. Giachetti, S. Manzini, Differential effects of various xanthines on pentylenetetrazole-induced seizures in rats: an EEG and behavioural study, Eur. J. Pharmacol. 222 (1992) 1–6.
[36] K. Wong, A. Stewart, T. Gilder, N. Wu, K. Frank, S. Gaikwad, C. Suciu, J. Dileo, E. Utterback, K. Chang, L. Grossman, J. Cachat, A.V. Kalueff, Modeling seizure-related behavioral and endocrine phenotypes in adult zebrafish, Brain Res. 1348 (2010) 209–215.
[37] M. Vergnes, A. Boehrer, S. Reibel, S. Simler, C. Marescaux, Selective susceptibility to inhibitors of GABA synthesis and antagonists of GABA(A) receptor in rats with genetic absence epilepsy, Exp. Neurol. 161 (2000) 714–723.
[38] M.T. Kendirli, E.H. Bertram, Genetic resistance to kindling associated with alterations in circuit function, Neurobiol. Dis. 105 (2017) 213–220.
[39] R. Guillet, L. Dunham, Neonatal caffeine exposure and seizure susceptibility in adult rats, Epilepsia 36 (1995) 743–749.
[40] R. Guillet, Neonatal caffeine exposure alters seizure susceptibility in rats in an age-related manner, Brain Res. Dev. Brain Res. 89 (1995) 124–128.
[41] H.J. Bennett, K. Semba, Immunohistochemical localization of caffeine-induced c-Fos protein expression in the rat brain, J. Comp. Neurol. 401 (1998) 89–108.
[42] J.E. Marques-Carneiro, A. Nehlig, J.C. Cassel, E.F. Castro-Neto, J.J. Litzahn, A. Pereira de Vasconcelos, M.D.G. Naffah-Mazacoratti, M. Fernandes, Neurochemical Changes and c-Fos Mapping in the Brain after Carisbamate Treatment of Rats Subjected to Lithium-Pilocarpine-Induced Status Epilepticus, Pharmaceuticals (Basel) 10 (2017).
[43] V. Baille-Le Crom, J.M. Collombet, M.F. Burckhart, A. Foquin, I. Pernot-Marino, G. Rondouin, G. Lallement, Time course and regional expression of C-FOS and HSP70 in hippocampus and piriform cortex following soman-induced seizures, J. Neurosci. Res. 45 (1996) 513–524.
[44] A.M. Linden, M. Baez, M. Bergeron, D.D. Schoepp, Increased c-Fos expression in the centromedial nucleus of the thalamus in metabotropic glutamate 8 receptor knockout mice following the elevated plus maze test, Neuroscience 121 (2003) 167–178.
[45] N. Singewald, P. Salchner, T. Sharp, Induction of c-Fos expression in specific areas of the fear circuitry in rat forebrain by anxiogenic drugs, Biol. Psychiatry 53 (2003) 275–283.
[46] B. Johansson, K. Lindstrom, B.B. Fredholm, Differences in the regional and cellular localization of c-fos messenger RNA induced by amphetamine, cocaine and caffeine in the rat, Neuroscience 59 (1994) 837–849.
[47] P. Svenningsson, R. Nergardh, B.B. Fredholm, Regional differences in the ability of caffeine to affect haloperidol-induced striatal c-fos mRNA expression in the rat, Neuropharmacology 37 (1998) 331–337.
[48] L. Tyvaert, S. Chassagnon, A. Sadikot, P. LeVan, F. Dubeau, J. Gotman, Thalamic nuclei activity in idiopathic generalized epilepsy: an EEG-fMRI study, Neurology 73 (2009) 2018–2022.
[49] N. Picard, S. Guénin, N. Larnicol, Y. Perrin. Maternal caffeine ingestion during gestation and lactation influences respiratory adaptation to acute alveolar hypoxia in newborn rats and adenosine A2A and GABA A receptor mRNA transcription, Neuroscience 156 (October (3)) (2008) 630–639.
[50] J.E. Marques-Carneiro, J.B. Faure, B. Cosquer, E. Koning, A. Ferrandon, A.P. de Vasconcelos, J.C. Cassel, A. Nehlig, Anxiety and locomotion in Genetic Absence Epilepsy Rats from Strasbourg (GAERS): inclusion of Wistar rats as a second control, Epilepsia 55 (2014) 1460–1468.
[51] F. Fauvelle, J. Boccard, F. Cavarec, A. Depaulis, C. Deransart, Assessing susceptibility to epilepsy in three rat strains using brain metabolic profiling based on HRMAS NMR spectroscopy and chemometrics, J. Proteome Res. 14 (2015) 2177–2189.
[52] C. Bombardi, M. Venzi, V. Crunelli, G. Di Giovanni, Developmental changes of GABA immunoreactivity in cortico-thalamic networks of an absence seizure model, Neuropharmacology 136 (2018) 56–67.
[53] J.G. Yague, A. Cavaccini, A.C. Errington, V. Crunelli, G. Di Giovanni, Dopaminergic modulation of tonic but not phasic GABAA-receptor-mediated current in the ventrobasal thalamus of Wistar and GAERS rats, Exp. Neurol. 247 (2013) 1–7.
[54] G. Jarre, T. Altwegg-Boussac, M.S. Williams, F. Studer, M. Chipaux, O. David, S. Charpier, A. Depaulis, S. Mahon, I. Guillemain, Building up absence seizures in the somatosensory cortex: from network to cellular epileptogenic processes, Cereb. Cortex 27 (2017) 4607–4623.