Journal of Research in Pharmacy

Research Article

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Unaltered trace element levels following the absence status induction by an alpha 2A receptor agonist in the cortex and hippocampus of genetic absence epilepsy rats

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Received: 2 February 2024 / Revised: 4 June 2024 / Accepted: 6 June 2024

ABSTRACT: This study builds upon our prior investigation proposing a potential animal model for absence status epilepticus induced by specific alpha-2a adrenergic receptor (α2AAR) activation through intracerebroventricular injection of dexmedetomidine (DEX). Our objective was to explore trace element levels within the cortex of genetic absence epilepsy rats from Strasbourg (GAERS) during absence status induction through α2AAR activation. Stereotaxic surgery was performed on adult GAERS to implant recording electrodes in the frontoparietal cortices under anesthesia. Following intracerebroventricular injection of the α2AAR agonist, DEX, the electroencephalography (EEG) was recorded. After inducing the second period of absence statuses, the rats were euthanized. Trace elements were analyzed using inductively coupled plasma mass spectrometry (ICP/MS) among the groups: GAERS-NAÏVE, GAERS injected with saline (GAERS-SAL), and GAERS injected with DEX (GAERS-DEX). No significant differences of the levels of trace elements were observed in the GAERS-DEX group compared to GAERS-SAL following absence status induction. Conversely, significant differences in trace element levels were identified between the GAERS-NAÏVE and GAERS-SAL or GAERS-DEX groups. Cortical levels of 25Mg, 55Mn, 57Fe, 88Sr, 65Cu, 42Mo, 80Hg, 15P, 52Cr, 59Co, 66Zn, 82Se, 85Rb, 133Cs, and 205Tl were higher in the GAERS-NAÏVE group compared to GAERS-SAL ($p < 0.05$). Similarly, hippocampal levels of 25Mg, 43Ca, 55Mn, 57Fe, 88Sr, 65Cu, 42Mo, 80Hg, 15P, 52Cr, 59Co, 66Zn, 82Se, 85Rb, 133Cs, and 205Tl were higher in the GAERS-NAÏVE group compared to the GAERS-SAL group ($p < 0.05$). Our findings suggest that DEX-induced absence status does not alter trace element levels in the cortex and hippocampus, unlike convulsive forms of epilepsies. However, the influence of trace element modulations on the development of absence status remains open to discussion. Intriguingly, cannula placement appeared to affect trace element levels, prompting inquiries into the current methodology of intracerebroventricular cannula implementation.

KEYWORDS: GAERS; absence status epilepticus; trace elements; dexmedetomidine; alpha 2AR

1. INTRODUCTION

Epilepsy is a neurological disorder with profound negative pathological, social and cognitive consequences [1]. The most critical issue in the progression of epilepsy is that the incidence of a life-threatening phenomenon known as status epilepticus contributes to a 20% increase in mortality rates. [2]. There are two types of status epilepticus. One is the convulsive status, usually manifested with symptoms similar to tonic and clonic movements, as seen in temporal lobe epilepsy [3]. The other, the non-convulsive status epilepticus (NCSE), has recently attracted the attention of clinicians, and some studies have indicated that it may be present in 8% of unconscious patients in intensive care units without clinical signs of seizures [2, 4-6].

Previously, we proposed a model for absence status epilepticus, NCSE, by activating alpha 2a adrenergic receptors (α2AAR) with the agonist dexmedetomidine (DEX) in a genetic model of absence

How to cite this article: Yavuz M, Dolu G, Azevedo R, Almeida A, Onat F. Unaltered trace element levels following the absence status induction by an alpha 2A receptor agonist in the cortex and hippocampus of genetic absence epilepsy rats. J Res Pharm. 2025; 29(2): 590-598.

ISSN: 2630-6344

https://doi.org/10.12991/jrespharm.1662084

epilepsy [7]. The involvement of α2AAR, a specific subtype of α2AAR, has been a focal point in understanding the generation and sustainability of spike-and-wave discharges (SWDs) [8, 9]. Therefore to study absence status epilepticus, our model was a promising candidate.

Other studies in animal models of convulsive status epilepticus showed altered levels of macro and trace elements. For instance, Se has been shown to hinder the development of epileptogenesis in different models of status epilepticus [10]. On the other hand, Co triggered seizures in homocysteine induced status epilepticus in C57BL/6J mouse model [11, 12]. Additionally, NCSE has been reported with tetrathiomolybdate treatment [13]. Besides the upregulation of the calcium channels modulated by Zn has been found to be inversely proportional to absence seizure generations [14]. Zn was also reported to decrease during seizure in a mouse model of epilepsy [15].

The involvement of Fe in post-hemorrhagic stroke epilepsy and post-traumatic epilepsy takes center stage, with significant amounts of Fe from red blood cells hemoglobin being released within the brain, potentially contributing to ferroptosis, as suggested by Mori et al. (2004) and Liu et al. (2023) [16, 17]. Cu, known to increase nerve excitability and susceptibility to epilepsy, may also contribute to neuronal death following seizures [17]. Serum Mg levels exhibit dichotomous behavior, with increased levels in certain seizures, such as maximal electroshock seizures and pentylenetetrazol kindling in normal mice model that is subjected to GPR39 agonist, whileshowing decreased levels in Gpr39-KO mice subjected to pentylenetetrazol kindling [18].

Studies in dogs with idiopathic epilepsy also revealed elevated serum concentrations of several macro and trace elements, including Mn, Fe, Cu, Mo, Zn and Se, with different changes in subgroups, providing crucial insights about the possible association between these elements and epilepsy pathophysiology and response to treatment [19]. In humans with genetic generalized epilepsy, low levels of Mg, Ca, and Zn, and high levels of Cu have been reported [20].

Our present study sought to comprehensively evaluate changes in macro and trace element levels in the absence status epilepticus model, exactly in the 15th minute after absence status induction with DEX in a genetic absence epilepsy model. We aimed to identify whether there is any difference between macro and trace element levels in the cortex and hippocampus of epileptic GAERS following DEX administration to understand whether absence status epilepticus immediately changes the ionic content. The elucidation resulting from these findings promises to provide valuable information about the pathophysiology of absence status and will possibly contribute to defining distinctions between convulsive status epilepticus.

2. RESULTS

2.1 Macro and trace element levels in the cortex

Macro and trace element levels were compared between GAERS-NAIVE and GAERS-SAL groups and between GAERS-SAL and GAERS-DEX groups of. According to the comparison GAERS-NAIVE vs. GAERS-SAL, the levels of Mg, Mn, Fe, Sr, Cu, Mo, P, Hg, Cr, Co, Zn, Se, Rb, Cs, and Tl were statistically higher in the GAERS-NAÏVE group ($p < 0.05$; See Figure 1, Table 1). The differences in Ca, As, and Ni did not reach statistical significance. On the other hand, no significant differences were observed between the GAERS-SAL and GAERS-DEX groups.

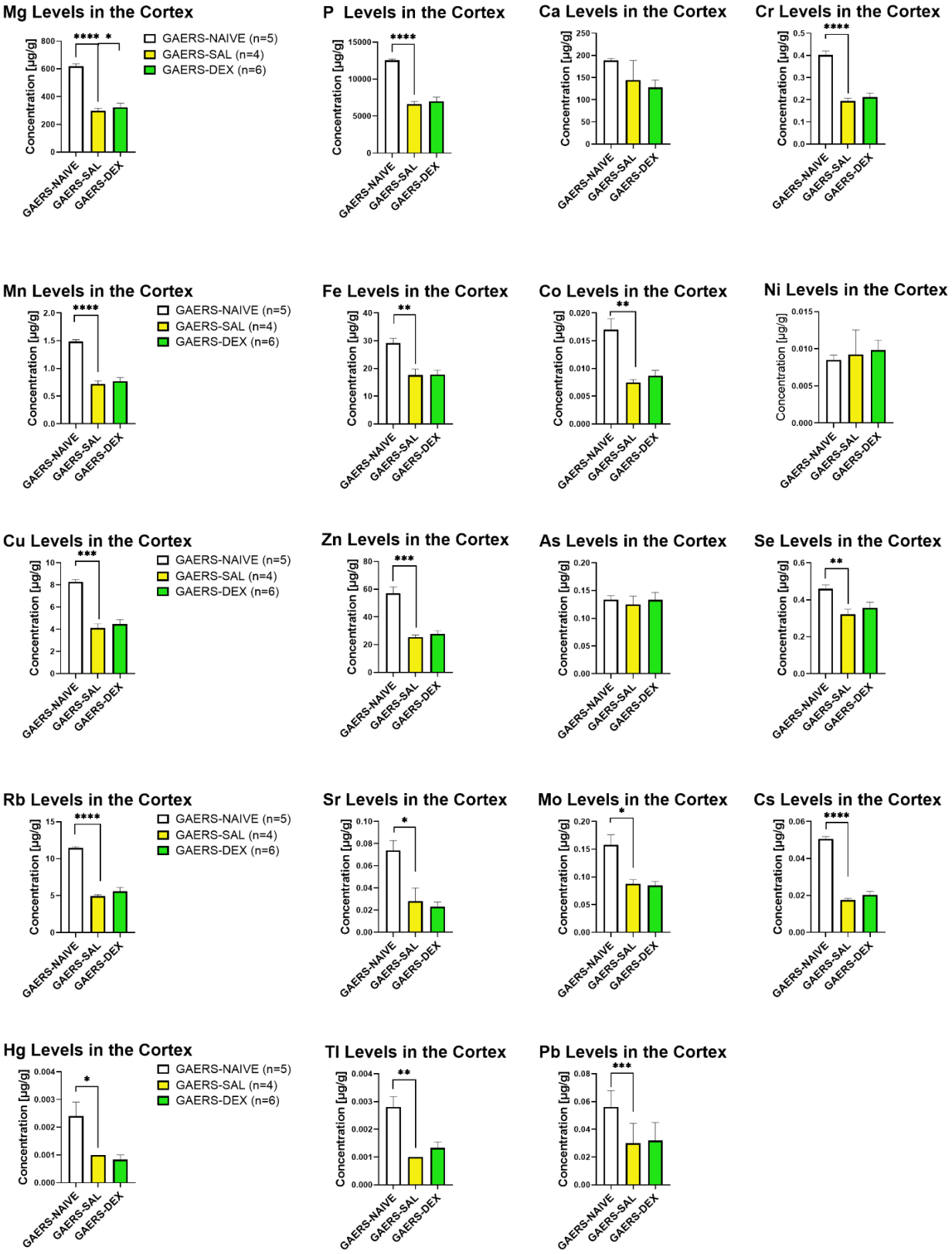

Figure 1. Macro and trace elements in the cortex [µg/g]. All data are expressed as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001 denote significant differences. Significant higher levels of Mg, Mn, Fe, Sr, Cu, Mo, P, Hg, Cr, Co, Zn, Se, Rb, Cs and Tl were observed in the GAERS-NAÏVE group compared to the GAERS-SAL group.

2.2 Macro and trace element levels in the hippocampus

Journal of Research in Pharmacy
Research Article

The concentrations of Mg, Ca, Mn, Fe, Sr, Cu, Mo, P, Hg, Cr, Co, Zn, Se, Rb, Cs and Tl were significantly higher in the GAERS-NAIVE group compared to the GAERS-SAL group ($p < 0.05$), with no statistically significant difference in the levels of Pb, As and Ni. In turn, no statistically significant difference was observed in macro and trace element levels between the GAERS-SAL group and GAERS-DEX ($p < 0.05$; See Figure 2, Table 2).

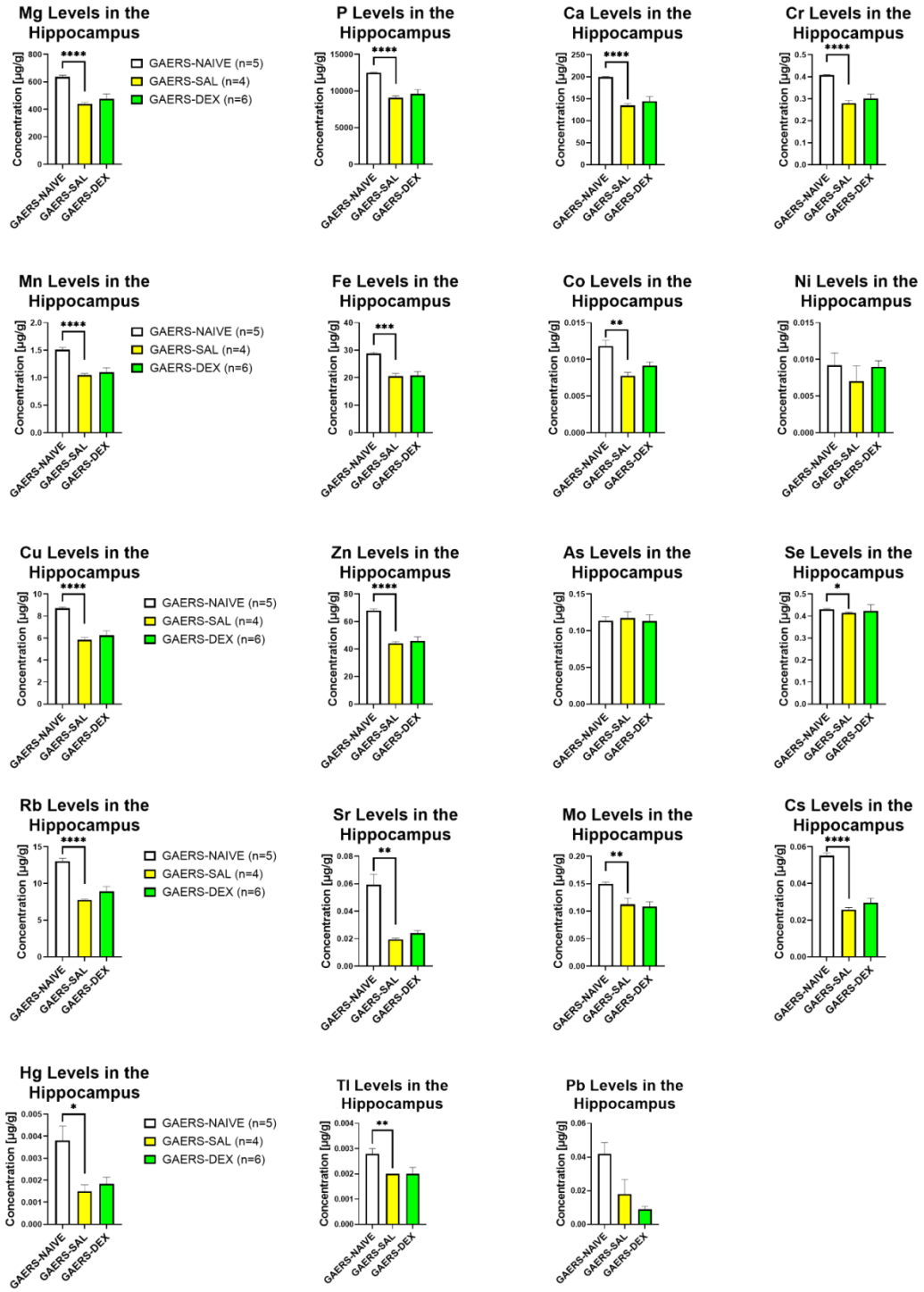

Figure 2. Macro and trace elements in the hippocampi [µg/g]: All data are expressed as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001 denote significant differences. Significant higher levels of Mg, Mn, Fe, Sr, Cu, Mo, P, Hg, Cr, Co, Zn, Se, Rb, Cs, and Tl were observed in the GAERS-NAÏVE group compared to the GAERS-SAL group (p<0.05). Data are expressed as mean ± SEM.

3. DISCUSSION

The induction of absence status epilepticus with the α2AAR agonist dexmedetomidine did not alter the macro and trace element levels in the cortex and hippocampus of rats compared to the control/operated group (administered with saline solution). Interestingly, rats implanted with cannulas for intracerebroventricular injection showed decreased levels of almost all macro and trace element levels (As, and Ni were the exceptions; Ca levels were not altered in the cortex, but in the hippocampus they were also reduced with cannula implantation).

The pathophysiology of epilepsy involves a certain dysfunction of receptors or ion channels. Absence epilepsy is known to be specifically associated with certain ion channels, such as T-type calcium channels and hyperpolarization-activated cyclic nucleotide-gated (HCN) channels [21-26]. Other ion channels such as N-methyl-D-aspartate (NMDA) receptors [27] and GABAA [28] are also reported to be involved. Changes in the function of ion channels can lead to changes in neuronal excitability, making neurons more prone to firing action potentials, which are the electrical signals that neurons use to communicate, and in epilepsy there is increased neuronal excitability.

Macro elements such as Zn, Cu and Mg play essential roles in modulating the activity of ion channels. Receptor channels are recognized to be allosterically modulated by macro and trace metals such as Zn, Cu, and other transition metals [29]. Zn is associated with both proconvulsant and anticonvulsant (anti-seizure) properties due to its neuromodulatory actions on NMDA, GABAA and AMPA receptors [30, 31]. Specifically following simultaneous release of glutamate and Zn from pre-synaptic neurons, immediate inhibition of NMDA glutamate receptors due to Zn binding to allosteric sites on these ion channels and downstream pathways is associated with NMDA-induced excitotoxicity [32]. For its part, Cu is involved in the function of certain enzymes that regulate ion channels activity and neurotransmitter release. Together with ion channels, Cu is also linked to glutamatergic and adrenergic neurons, especially in regions such as the hippocampus, olfactory bulb, and locus coeruleus [33, 34]. Similarly, Mg is an antagonist on NMDA channels as well as modulates GABA receptors, and lower Mg levels are associated with seizures [20, 35].

There are few reports on Fe accumulation in models of convulsive status epilepticus. In rats that underwent kindling, increased Fe levels were found in the hippocampus during both the acute and chronic phases of kindling, when spontaneous recurrent seizures occurred [36]. Other experiments in the same study, in vitro experiments on brain slices of neurons exposed to Fe, demonstrated increased Fe uptake, particularly when epileptiform activity was induced [36]. Fe accumulation has also been confirmed in pentylenetetrazol-induced epileptogenesis model and in the kainic acid-induced or pilocarpine-induced convulsive status epilepticus model [37, 38], suggesting direct association between Fe deposits and convulsive seizures.

So far, Zn, Mg and Fe levels appear to be strongly associated with convulsive status epilepticus, but interestingly no reports on the absence status exist. Our experiments did not reveal any noticeable changes associated with the induction of absence status. Likewise, existing literature offers limited information on macro and trace element levels in DEX-induced absence status. However, studies indicate that DEX may confer protection against Fe-associated oxidative injuries [39-41].

Marked changes occurred simply with the intracerebroventricular implantation of a guide cannula in the lateral ventricle. Cortical and hippocampal levels of macro and trace elements decreased significantly for virtually all of them. Intracerebroventricular delivery as known, facilitates the circumvention of the blood-brain barrier, allowing for direct delivery of high drug concentrations to the central compartment of the brain. A plastic drug reservoir can be implanted subcutaneously in the scalp for ICV drug infusion [42]. These findings advocate for continuous rather than pulsatile delivery of small-molecule therapies via ICV. Thus, incorporating responsive drug release through a seizure detection or prediction unit may yield diminished effectiveness [43].

The initial thought that comes to mind is that there appears to be an influence of material used to manufacture the cannulas on the levels of macro and trace elements in the surrounding tissues. Contamination caused by simple contact of samples with various materials, including stainless steel needles, is well recognized, leading to inaccurate macro and trace element results [44]. However, this typically results in increased levels and in our study we found lower levels in NAÏVE (non-injected) rats than in injected ones. On the other hand results in our study with the decreased levels of trace elements, let alone not only excludes trace metal contamination but suggests a possible leaching of the tissues by the administration of the solution (DEX or SAL). In the literature not many reports have been found published so far. It is also important to consider since we have 4-5 animals in each groups this might be our limitation with the results of the levels of trace elements.

This finding that cannula implantation causes drastic changes in macro and trace element levels in the cortex and hippocampus raises the question of whether the method used is suitable for epilepsy research, as guide cannulas are used for chronic injection of drugs to investigate therapeutic targets or injection of drugs or other chemicals to induce epilepsy models. Possibly, the leaching of tissues due to the bolus injections through guide cannulas should be considered, and lower rates of infusion can recover these substantial leachings. This aspect needs to be further investigated and should be considered in future research, since many studies in the field of epilepsy involve the implantation of brain cannulas.

4. CONCLUSION

In conclusion, our study shows that no distinct impact of DEX-induced absence status on trace element levels were found in the cortex and hippocampus, showing deviation from convulsive forms of epilepsies. The unaltered trace element levels in these brain regions prompt further exploration into the mechanisms underlying absence statuses and their potential differences from convulsive statuses. Additionally, the unexpected influence of cannula placement on trace element levels raises important questions about the reliability of current intracerebroventricular cannula implementation methodologies. In addition to the complexities of epilepsy research, these findings highlight the need for continued investigation and refinement of experimental techniques to enhance the accuracy and reliability of results in epilepsy research.

5. MATERIALS AND METHODS

5.1. Animals

In the experiments, male GAERS rats (age, 3–4 months; weight, 250–350 g), known to have spontaneous SWD activities, were used. They were obtained from the breeding colony of the Experimental Animals Unit of Acibadem Mehmet Ali Aydinlar University. The rats were housed in a temperature-controlled room (21 ± 3 °C) with a 12-hour light/dark cycle (lights on at 8 a.m.). Rats were placed individually in a cage and received food and water ad libitum. All procedures performed on rats were approved by the Ethics Committee for Experimental Animals of Acibadem Mehmet Ali Aydinlar University (Protocol no: 2023/48) and were in compliance with EU Directive 2010/63/EU on the protection of animals used for scientific purposes and the ARRIVE guidelines.

5.2. Stereotaxic surgery

GAERS-DEX and GAERS-SAL rats were anesthetized with ketamine hydrochloride (100 mg/kg, IP, Alfamine 10%; Alfasan International B.V.) and xylazine (10 mg/kg, IP, Alfazyne %2; Alfasan Int., Netherlands). Each animal was placed in a stereotaxic cage (Stoelting Model 51600, Stoelting Co., IL, USA). A longitudinal incision was made over the skull and four stainless steel screws with specifically made insulated wires were implanted bilaterally over the frontoparietal cortex for cortical EEG recordings. The electrodes were connected by insulated wires to a micro-connector for EEG recording. A guide cannula was implanted in the lateral ventricle at the coordinates (AP, −1.0 mm; ML, −1.4 mm; and V, −4.1 mm) according to Paxinos and Watson [45]. The electrodes and wires were covered with dental acrylic and fixed to the skull.

5.3. EEG recordings and analysis

After implantation of electrodes and cannulas by stereotaxic surgery, the rats were left to rest for one week. After a one-week recovery, a 3-hour record of the rat's baseline activity was made (CONT-Basal). The following day, the 40-min baseline EEG activity was recorded in the EEG groups. Then, while the GAERS control group (CONT-SAL) were injected with 5 µl of saline solution (SAL), the drug groups received dexmedetomidine in saline (DEX-2.5 µg). After intracerebroventricular injections of dexmedetomidine, 3-hour EEG recordings were obtained. SWD complexes, which are usually identified by their duration greater than 1 sec, with a sharp wave train followed by a slow wave (7-11 Hz) with an amplitude of at least twice the EEG background amplitude, were analyzed. The EEG was amplified using a BioAmp ML 136 amplifier, PowerLab 8S system (PowerLab 8S, ADI Instruments, Oxfordshire, UK) with band-pass filters settings at 1–40 Hz, and recorded and analyzed using the LabChart 7 software.

5.4. Tissue isolation

The rats were sacrificed at the 15th minute of the induction of second period of DEX-induced prolonged seizure events [7] and placed into isoflurane. GAERS-SAL rats were sacrificed at approximately the same time. The cortices and hippocampi were carefully isolated for further analysis. The same procedures were

performed for animals that did not undergo stereotaxic surgery (GAERS-NAIVE). After EEG experiments, rats were decapitated under isoflurane anesthesia. The isolated tissue samples were used for macro and trace element analysis by ICP-MS.

5.5. ICP-MS analysis of macro and trace elements in rat cortices

5.5.1. Sample pretreatment

Samples of the isolated cortices were weighed in acid-washed Eppendorf tubes and dried at 80 °C (drying oven) until constant weight. Then, the samples (approximately 1 mg) were transferred to 15 mL polypropylene tubes and 250 µL of high-purity HNO3 (≥ 69%, TraceSELECT Fluka, Honeywell, Muskegon, MI, USA) and 50 µL of high-purity H2O2 (30%, Suprapur, Supelco, Merck Millipore, Darmstadt, Germany) were added to digest the sample for 72 hours at room temperature and 1 hour at 60 °C. After sample digestion, the volume was adjusted with ultrapure water and internal standards (IS) solution to a final volume of 10 mL. Sample blanks were obtained using the same procedure. For analytical quality control, two certified reference materials (CRM) were used: Mussel tissue (ERM-CE278K) and Skimmed Milk Powder (ERM-BD151), both from the European Commission's Joint Research Centre (JRC). The CRM were analyzed under the same sample pretreatment and analytical procedure as the samples. The obtained solutions were stored at 4 °C until further analysis.

5.5.2. Macro and trace element analysis

Analysis of sample solutions was performed by inductively coupled plasma mass spectrometry (ICP-MS). The instrument was an iCAP Q (Thermo Fisher Scientific, Waltham, MA, USA), equipped with a Meinhard TQ+ concentric quartz nebulizer, a Peltier-cooled high-purity quartz baffled cyclonic spray chamber, and a demountable quartz torch with a 2.5 mm i.d. quartz injector. The interface consisted of two (sampler and skimmer) Ni cones. High-purity argon (99.9997%) was used as nebulizer and plasma gas. Sample solutions and calibration standards were presented to the ICP-MS instrument using a CETAC ASX-520 autosampler (Teledyne CETAC Technologies, Omaha, NE, USA). Before each analytical run, the instrument was tuned for maximum sensitivity and signal stability and for minimal formation of oxides and double-charge ions. The main operational parameters of the instrument were: nebulizer gas flow, 1.14 L/min; auxiliary gas flow, 0.79 L/min; plasma gas flow, 13.9 L/min; radiofrequency generator power, 1550 W; and dwell time, 1-10 ms. The elemental isotopes 7Li, 25Mg, 27Al, 31P, 43Ca, 52Cr, 55Mn, 57Fe, 59Co, 60Ni, 65Cu, 66Zn, 75As, 82Se, 85Rb, 88Sr, 111Cd, 121Sb, 133Cs, 137Ba, 205Tl and 208Pb were measured for analytical determination and the elemental isotopes 6Li, 45Sc, 71Ga, 89Y, 103Rh, 193Ir and 209Bi were monitored as IS [46].

5.6. Statistical analysis

Statistical analysis was performed using GraphPad Prism software. To evaluate differences between groups, the unpaired t-test was used. Significance levels were set at $p < 0.05$.

Acknowledgements: None.

Author contributions: Concept – M.Y..; Design – M.Y.; Supervision – M.Y., A.A., F.O.; Resources – M.Y., A.A., F.O.; Materials – M.Y., A.A., F.O.; Data Collection and/or Processing – M.Y., G.D., R.A.; Analysis and/or Interpretation – M.Y., G.D., R.A.; Literature Search – M.Y., G.D., R.A.; Writing – M.Y., G.D., R.A., A.A.; Critical Reviews – M.Y., A.A., F.O.

Conflict of interest statement: The authors declared no conflict of interest.

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