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Peripheral nesfatin-1 reduces basal brain activity but has limited effect on epilepsy-like conditions

Ömer Faruk Kalkan[1], Osman Aktaş[1], Zafer Şahin[1], Selcen Aydin Abidin[2], Abdulhamit Yildirim[3], Ali Faruk Özyaşar[4], İbrahim Uzun[3] and İsmail Abidin[2]

[1] *Department of Physiology, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey*
[2] *Department of Biophysics, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey*
[3] *Laboratory and Veterinary Assistance Services, Maçka Vocational School, Karadeniz Technical University, Trabzon, Turkey*
[4] *Department of Anatomy, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey*

Abstract. In this study, we investigated the effects of peripheral nesfatin-1 on basal brain activity and 4-aminopyridine (4-AP)-induced epileptiform activity, and its relationship with the electrocorticogram (ECoG) power spectrum and EEG bands. Forty-nine male Wistar rats were divided into seven groups: control sham, 4-AP (2.5 mg/kg i.p.), Nesfatin-1 (1, 2, and 4 μg/kg i.p.), Nesfatin-1 (2 μg/kg) post-treatment, and Nesfatin-1 (2 μg/kg) pre-treatment. Recordings were conducted for 70 min under ketamine/xylazine (90/10 mg/kg) anesthesia. In the post-treatment group, nesfatin-1 was injected 20 min after 4-AP induction. In the pre-treatment groups, nesfatin-1 was administered following basal recordings and before 4-AP injection. 4-AP induced epileptiform activity in all animals, peaking at 30 min. Nesfatin-1 (2 μg/kg) reduced basal brain activity ($p < 0.05$) and decreased alpha, delta, and theta bands in ECoG. Post-treatment of nesfatin-1 did not affect 4-AP-induced activity ($p > 0.05$) but increased gamma band activity ($p > 0.05$). Pre-treatment of nesfatin-1 reduced epileptiform activity between 50 and 60 min ($p < 0.05$), decreased delta bands, and increased gamma bands ($p > 0.05$). We conclude that peripheral nesfatin-1 modulates normal brain activity but has limited effects on abnormal discharges.

Key words: Nesfatin-1 — Neuropeptide — Epilepsy — EEG — 4-Aminopyridine

Introduction

Neuropeptides play essential roles in neural communication and have critical functions in both physiological and pathological conditions (van den Pol 2012). In addition to their contributions to feeding, sexual behaviour, and learning, among other functions, they also have a significant impact on neurological disorders (Kovac and Walker 2013). Nesfatin-1, a neuroendocrine peptide originating from the mammalian hypothalamus, exhibits a wide range of bioactivity (Oh-I et al. 2006; Goebel et al. 2009). It is predominantly found in the arcuate and paraventricular nuclei of the hypothalamus (Goebel-Stengel and Wang 2013). Its primary role lies in the regulation of hunger and fat storage, with increased levels in the hypothalamus leading to decreased body fat (Price et al. 2008). Moreover, nesfatin-1 has further physiological functions in puberty, gastrointestinal regulation, and glucose metabolism and also it participates in pathological processes such as anxiety and panic disorder (Stengel 2015). Nesfatin-1 exerts its bioactivity in several parts of the nervous system, including the paraventricular nucleus of the hypothalamus, area postrema, dorsal motor nucleus of the vagus nerve, cerebellum, and cortex (Rupp et al. 2021). Its effects are mediated through the binding of G protein-coupled receptors, leading to Ca^{2+} influx *via* different types of Ca^{2+} channels (Brailoiu et al. 2007). This influx of calcium results in cellular activation, which varies depending on the cell type

Correspondence to: Ömer Faruk Kalkan, Karadeniz Technical University, Faculty of Medicine, Department of Physiology, 61080, Trabzon, Turkey
E-mail: ofkalkan@ktu.edu.tr

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(Brailoiu et al. 2007). For example, it may induce excitation in hypothalamic cells through glutamate drive (Gok Yurtseven et al. 2020) while inhibiting dopaminergic neurons in the limbic system *via* GABAergic transmission. The inhibition of dopaminergic neurons ultimately leads to reduced food intake (Li et al. 2014; Chen et al. 2015).

Epilepsy, a common neurological disorder, is greatly influenced by neuropeptides in its pathological processes (Kovac and Walker 2013). Clinical studies have indicated a potential link between nesfatin-1 and the pathogenesis of epilepsy. Recent research suggests that both saliva and serum levels of nesfatin-1 are elevated in patients recently diagnosed with epilepsy (Aydin et al. 2009). Furthermore, increased serum nesfatin-1 concentration has been proposed as a potential marker for identifying epileptic patients (Aydin et al. 2011). Several animal studies have also explored the relationship between nesfatin-1 and epilepsy. One study found that chronic pentylenetetrazole (PTZ) administration elevated serum nesfatin-1 levels in rats (Ergul Erkec et al. 2018) while another demonstrated that intracortical nesfatin-1 injection had a proconvulsant effect in rats (Erken et al. 2015). However, conflicting findings have emerged, with a recent study reporting that nesfatin-1 exerts an anticonvulsant effect by reducing oxidative stress in rats (Musuroglu Keloglan et al. 2023). These conflicting data have raised new research questions, prompting investigations into whether nesfatin-1 exhibits pro- or anticonvulsant effects in conditions resembling epilepsy. Furthermore, considering the clinical limitations of intracranial applications, it is imperative to explore the potential relationship between peripheral nesfatin-1 levels and conditions resembling epilepsy.

Based on nesfatin-1's ability to utilize both glutamatergic and GABAergic pathways, and its potential effect in epilepsy-like conditions, we formulated three hypotheses: i) Does peripheral nesfatin-1 injection have any effect on basal brain activity and EEG bands? ii) Does peripheral nesfatin-1 pre-treatment have any effect on 4-aminopyridine (4-AP) induced epileptiform activity and EEG bands? iii) Does peripheral nesfatin-1 post-treatment have any effect on 4-AP induced epileptiform activity and EEG bands? In line with our hypotheses, we investigated three different doses (1, 2, and 4 μg/kg), which were modified from a previous study (Arabacı Tamer et al. 2022). We proceeded with the dose that exhibited the most efficacy in basal brain activity for our epilepsy trials.

Material and Methods

Animals

In the current study, a total of 49 adult Wistar rats with an average weight of 250 g and aged 10 weeks were utilized. These rats were obtained from the Karadeniz Technical University Surgical Research Centre animal unit. They were housed in controlled conditions with a 12:12 light-dark cycle, maintained at a controlled humidity of 50 ± 10% and temperature of 22 ± 1°C. Throughout the study, the rats had *ad libitum* access to food and water. All experimental protocols adhered to the regulations outlined in the Ethical Guidelines for the Use of Animals in Research. The experimental procedures were approved by the Karadeniz Technical University Animal Studies Local Ethics Committee (Protocol No: 2022/55).

Surgical procedure for electrocorticogram (ECoG) and induction of epileptiform activity

Rats ($n = 7$ for each group) were initially anesthetized with an intraperitoneal (i.p.) injection of a ketamine-xylazine mixture at a dosage of 90 mg/kg ketamine and 10 mg/kg xylazine. Additional doses were administered as needed (Sitges et al. 2012). The animals' heads were then secured in a stereotactic frame, and a midline sagittal incision was made in the scalp. Careful craniotomy was performed to expose the right cerebral cortex, creating an oval window approximately 6–7 mm rostro-caudal to 4 mm for ECoG recordings (Harvard Instruments, South Natick, MA, USA). The dura mater was removed to ensure direct contact with the cortex and improve signal recording. A pool of warm liquid Vaseline (37°C) was applied to cover the cortical surface. Body temperature was maintained at 37°C using a homoeothermic blanket system (Harvard Homoeothermic Blanket, USA). Following the surgical procedure, the induction of epileptiform activity was initiated by administering i.p. injection of 4-AP at a dose of 2.5 mg/kg, dissolved in saline (Sitges et al. 2012).

Electrophysiological recordings

To observe ECoG recordings, we utilized two Ag-AgCl ball electrodes placed over the right neocortex. The electrode coordinates were as follows: the first electrode was positioned 2 mm lateral to the sagittal suture and 1 mm anterior to bregma, while the second electrode was placed 2 mm lateral to the sagittal suture and 5 mm posterior to bregma (Paxinos and Watson 1998). A common reference electrode was affixed to the left pinna. ECoG activities were recorded using a data acquisition system (PowerLab 16/30; AD Instruments, Castle Hill, Australia). The signals were then amplified and filtered with a bandpass of 0.1–50 Hz using BioAmp amplifiers (AD Instruments). Subsequently, the ECoG signals were digitized at a sampling rate of 1024 Hz using the Power Lab 4/SP. The digitized signals were displayed in real-time and stored for further analysis.

Chemicals and treatments

The following chemicals were utilized in our experiments: nesfatin-1, 4-AP, ketamine hydrochloride (HCl), and xylazine HCl. Nesfatin-1 was procured from Phoenix Pharmaceuticals, Inc. (Burlingame, USA). Ketamine HCl (ketalar) was obtained from Pfizer (Istanbul, Turkey), and xylazine HCl was purchased from Bayer (Istanbul, Turkey). To prepare the nesfatin-1 solution, it was initially dissolved in saline until a homogeneous mixture was obtained. Nesfatin-1 was then administered *via* i.p. injection. The sham group received injections of saline. 4-AP was dissolved in saline and administered *via* i.p. injection.

Experimental design

Electrophysiological recordings were conducted using 49 male rats, which were divided into 7 groups, each comprising seven rats, as follows: 1. Sham, injected with physiological saline; 2. Nesfatin-1 (1 μg/kg), i.p. injection; 3. Nesfatin-1 (2 μg/kg), i.p. injection; 4. Nesfatin-1 (4 μg/kg), i.p. injection; 5. Nesfatin-1 pre-treatment, injected with 2 μg/kg nesfatin-1 before 2.5 mg/kg 4-AP injection; 6. Nesfatin-1 post-treatment, injected with 2 μg/kg nesfatin-1 after 2.5 mg/kg 4-AP injection; 7. 4-AP, injected with 2.5 mg/kg 4-AP.

Prior to any injections, all animals underwent basal recordings for 10 min. ECoG recordings were obtained from the right neocortex for each animal in all groups, with a total recording duration of 70 min. For the sham group, after basal recordings, animals received i.p. saline injection, followed by an additional 60 min of ECoG recordings. In the 4-AP group, following basal recordings, animals were administered 2.5 mg/kg 4-AP, after which another 60 min of ECoG recordings were obtained. In the post-treatment group, after basal recordings, animals were administered 2.5 mg/kg 4-AP. After 20 min of recordings, a dose of 2 μg/kg nesfatin-1 was administered, followed by an additional 40 min of ECoG recordings. For the pre-treatment groups, animals received a 2 μg/kg nesfatin-1 injection. After 20 min of ECoG recordings, animals were administered 2.5 mg/kg 4-AP, followed by another 40 min of ECoG recordings. In all dose groups, after 10 min of basal recordings, nesfatin-1 was injected at different doses (1, 2, and 4 μg/kg i.p.), followed by an additional 60 min of ECoG recordings (Fig. 1).

Data analysis

The EEG power spectrum was analysed offline by using LabChart software. After notch filtering at 50 Hz, Fast Fourier Transform (FFTs) of signals of 10 min time windows of 70 min recordings were calculated as power by using Hamming windows function with a sample size of 1024 (without overlap). 0.5–50 Hz range of all FFT values transferred into a spreadsheet program (MS Excel 2010) for further analysis. Absolute powers were calculated by summation of all power values contained in a given frequency band and in total EEG (0.5–50 Hz). EEG power was computed in the selected frequency bands: delta band (0.5–4 Hz), theta band (4–7 Hz) alpha band (8–12 Hz), beta band (12–30 Hz) and gamma band (30–100 Hz; Fig. 3) (Yildirim et al. 2013).

Statistical analysis

All statistical comparisons were made using GraphPad Prism 8 software. For the analysis of epileptiform activity, absolute powers and band analysis Friedman test which

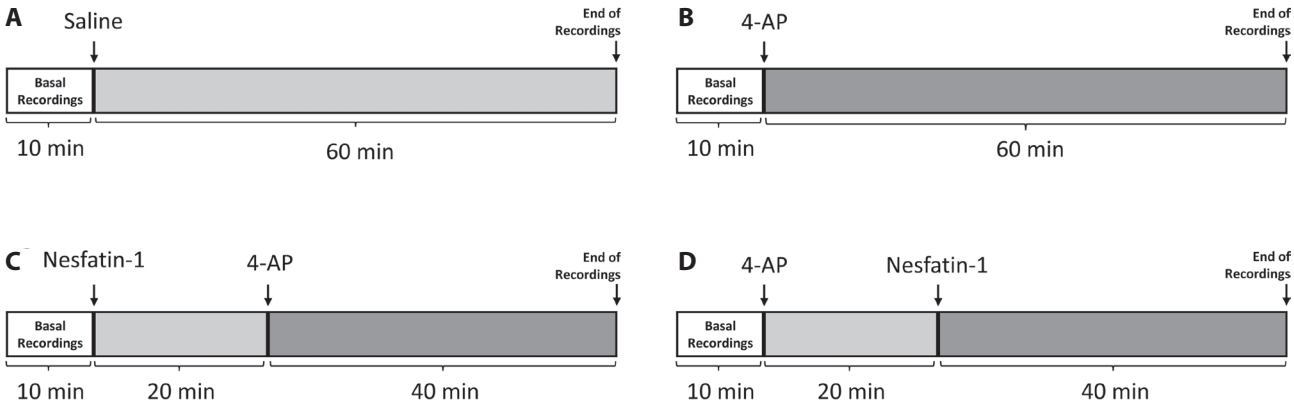


Figure 1. The time lines of *in vivo* electrophysiological recordings, obtained to investigate the effects of nesfatin-1 on excitability. **A.** The effects of i.p. injections were tested by applying saline solution alone. **B.** The induction and maturation of 4-AP-induced epileptiform activity is recorded. The effects of nesfatin-1 on epileptiform activity investigated in two different paradigms. **C.** The effects of nesfatin-1 when applied before the induction of epileptiform activity. The nesfatin-1 was applied 20 min before of 4-AP injection to evaluate potential protective role (pre-treatment). **D.** To evaluate the antiepileptic effects, nesfatin-1 was administered 20 min following 4-AP (post treatment).

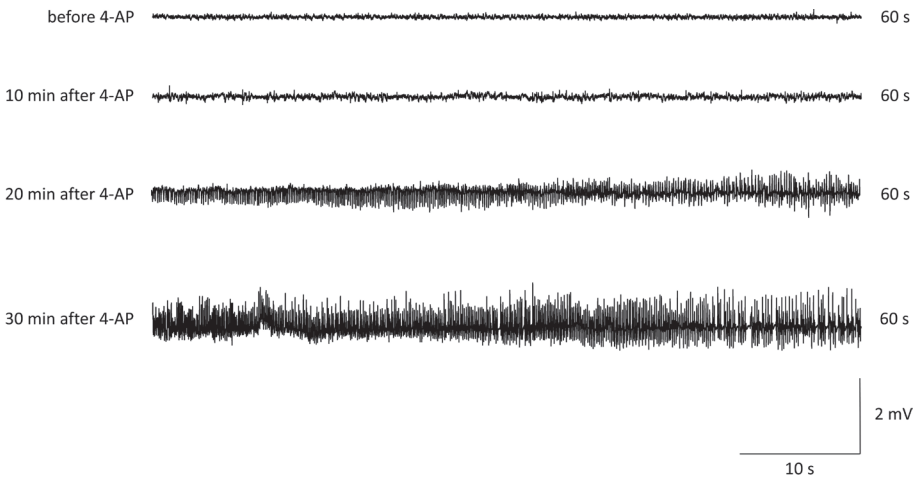


Figure 2. Raw ECoG traces of basal activity and following 4-AP injection presented at different time points for 60 s. 4-AP injection (2.5 mg/kg, i.p.) reliably produced a hyper excitable state.

is nonparametric equivalent of repeated measures of ANOVA and for the pairwise comparison Dunn's multiple comparison tests were used. All time points were compared to basal activity (0–10 min). To identify the difference between three different doses of the nesfatin-1, Kruskal-Wallis test with Dunn's multiple comparison test were assessed. To investigate the effects of pre- and post-treatment of nesfatin-1 on epileptiform activity, nonparametric Mann-Whitney U tests were made. Data were presented as means ± SEM and accepted as significantly different when $p < 0.05$.

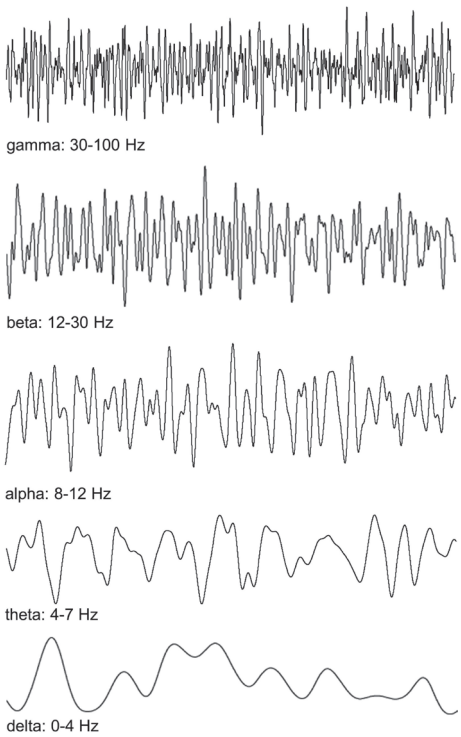


Figure 3. Comparison of EEG bands (gamma, beta, alpha, theta, delta) and their corresponding frequency intervals.

Results

4-AP-induced epileptiform activity

We did not observe any epileptiform activity from the animals during the 10 min basal recordings obtained before induction of epileptiform activity. We induced epileptiform activities by injecting 2,5 mg/kg 4-AP (i.p). Epileptiform discharges began around 10th min after 4-AP injection and had a fully developed pattern after ~30 min (Fig. 2 and Fig. 4A). Non-parametric Friedman test revealed that there was a significant increase in powers after 4-AP injection ($p < 0.0001$) and Dunn's test showed comparison between basal activity (0–10 min) and the other time windows. 4-AP-induced epileptiform activity bands were shown in the graph (Fig. 2 and Fig. 4A). Furthermore, 4-AP did not affect the delta band during recordings (Fig. 4B). However, it resulted in an increase in the power of the theta and alpha bands between the 50th and 60th min of recordings (Fig. 4C,D). Additionally, it led to an increase in the beta band from the 40th minute onwards (Fig. 4B) and an increase in the gamma band from the 20th minute of recordings (Fig. 4E).

Effects of nesfatin-1 on basal brain activity and EEG bands

To evaluate the effects of different doses of nesfatin-1 on ECoG activity, we recorded baseline activity for 10 min. Subsequently, we injected all animals with three different i.p. doses (1, 2, and 4 μg/kg) and continued recording for another 60 min. One-way ANOVA revealed significant differences between three doses at 20–30th min ($F_{2,18} = 7.396$; $p = 0.0045$), 30–40th min ($F_{2,18} = 8.002$; $p = 0.0036$), at 40–50th min ($F_{2,18} = 9.192$; $p = 0.0020$) and at 50–60th

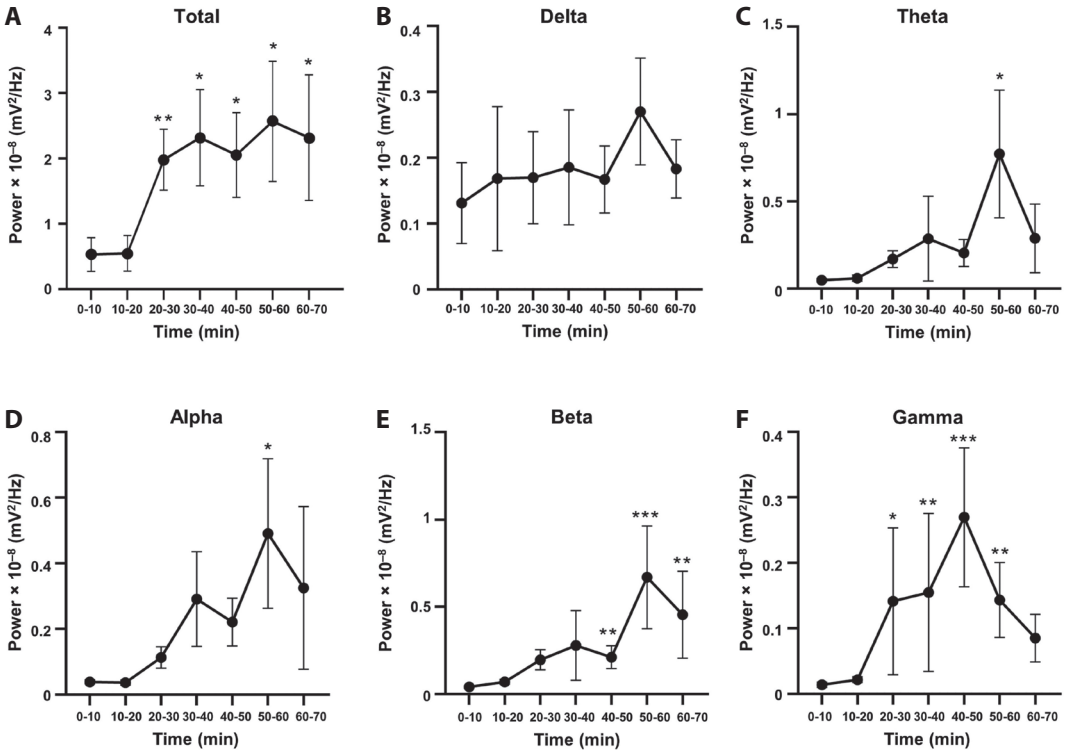


Figure 4. A. Effects of 4-AP on EEG bands compared with baseline values by using Friedman test with Dunn's multiple comparison test ($n = 7$). The absolute powers of all time windows following 4-AP injection were significantly higher than that of the baseline activity, before 4-AP. * $p < 0.05$, ** $p < 0.01$. **B–F.** Delta, theta, alpha, beta and gamma wave powers.

min ($F_{2,18} = 11.42$; $p = 0.0008$). The administration of doses at 1 and 4 μg/kg did not elicit any significant effect on basal brain activity during the 60-min recordings in animals (Fig. 5). However, we observed a reduction in basal brain activity following the injection of 2 μg/kg nesfatin-1 ($p < 0.05$) from the 30th min until the end of the ECoG recordings, in comparison to the same time intervals in the Nesfatin-1(1 μg/kg) and Nesfatin-1 (4 μg/kg) groups (Fig. 5). Furthermore 1 and 4 μg/kg nesfatin-1 injection did not cause any change on EEG bands (Fig. 6A,C) nevertheless repeated measures of ANOVA revealed 2 μg/kg nesfatin-1 injection induced reduction of alpha, delta and theta bands ($p < 0.05$) during recordings (Fig. 6B).

Effects of nesfatin-1 pre-treatment on epileptiform activity

No epileptiform activities were observed in any of the animals during the 10-min basal recordings obtained before the induction of epileptiform activity. We administered 2 μg/kg nesfatin-1 (i.p.) prior to 4-AP injection. Epileptiform activities were induced by injecting 2.5 mg/kg 4-AP (i.p.) after 20 min of drug delivery. Epileptiform discharges began at the 10th min following 4-AP injection and peaked after 30 min. The nonparametric Mann-Whitney U test showed that Nesfatin-1 pre-treatment group only resulted in a reduction in epileptiform activity between the 50th and 60th min ($p < 0.05$) compared to the 4-AP group (Fig. 7A).

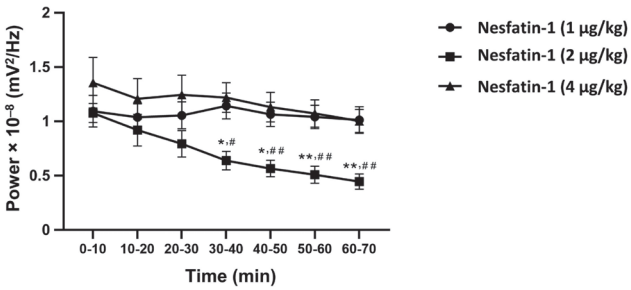
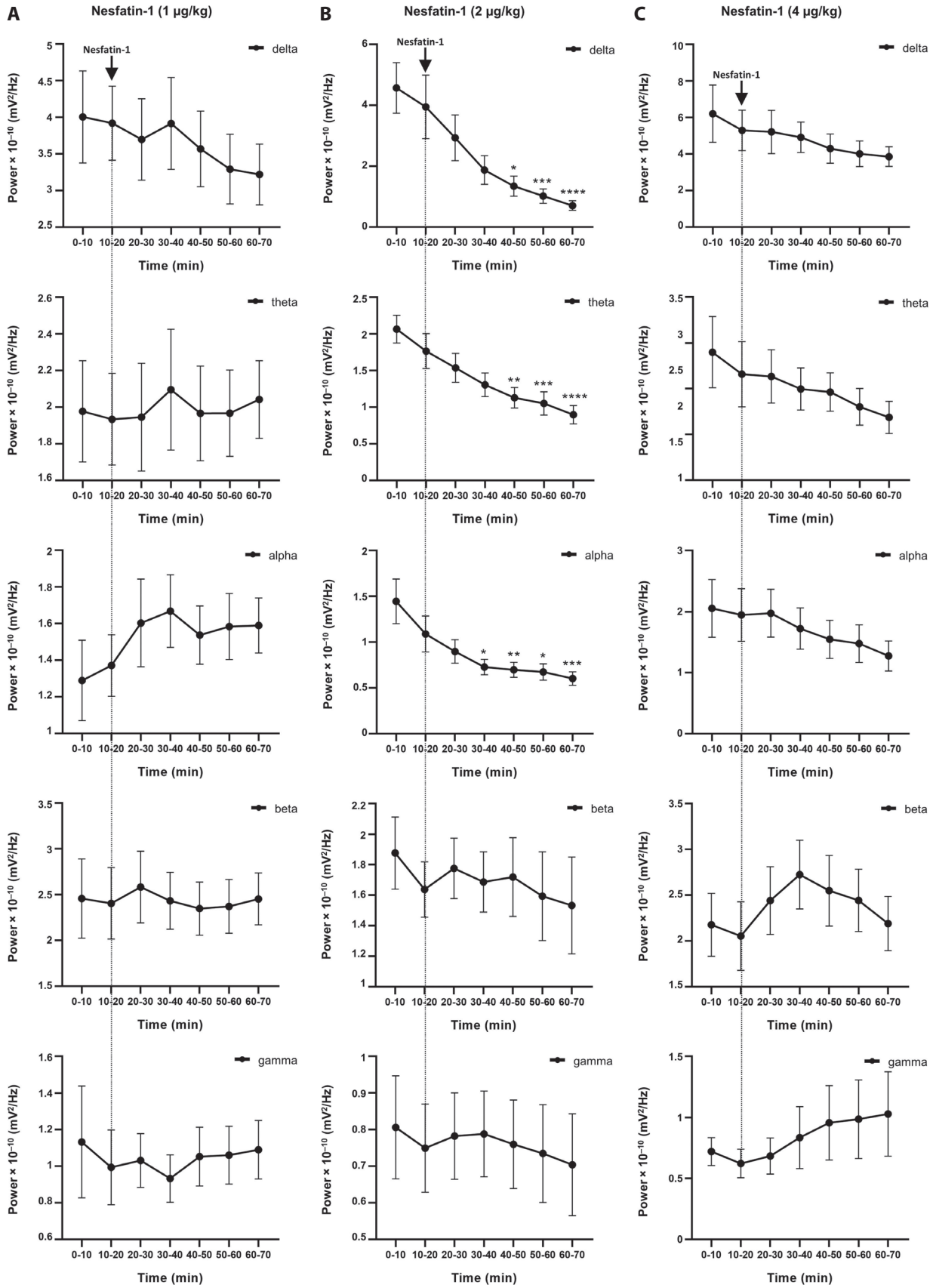


Figure 5. Evaluation of effects of nesfatin-1 on basal ECoG activity. Each dose was compared with its own 10-min baseline activity. Following 10 min of baseline recording different doses of nesfatin-1 (1, 2 and 4 μg/kg) were administered to different groups. Same data points of different doses were compared with Kruskal-Wallis test with Dunn's multiple comparison test ($n = 7$). 1 and 4 μg/kg nesfatin-1 did not change the total power ECoG. 2 μg/kg of nesfatin-1, on the other hand decreased the total power of ECoG. * $p < 0.05$, ** $p < 0.01$ *vs.* Nesfatin-1 (1 μg/kg) group; # $p < 0.05$, ## $p < 0.01$ *vs.* Nesfatin-1 (4 μg/kg) group.


A
Nesfatin-1 (1 µg/kg)
B
Nesfatin-1 (2 µg/kg)
C
Nesfatin-1 (4 µg/kg)
Nesfatin-1
delta
theta
alpha
beta
gamma
Power × 10⁻¹⁰ (mV²/Hz)
Time (min)
0-10 10-20 20-30 30-40 40-50 50-60 60-70
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◄ **Figure 6.** All nesfatin-1 doses were administered after a 10-min basal recording period. Each dose was compared with its own 10-min baseline activity. **A.** 1 μg/kg nesfatin-1 did not have significant effect on band powers. **B.** 2 μg/kg nesfatin-1 reduced the power of alpha, delta and theta band powers. **C.** 4 μg/kg nesfatin-1 did not have significant effect on band powers. Friedman test with Dunn's multiple comparison ($n = 7$); * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Effects of post-treatment of nesfatin-1 on epileptiform activity

We administered 2 μg/kg nesfatin-1 (i.p.) at the 20th min following 4-AP injection. Nesfatin-1 did not induce any change in EEG power compared to the 4-AP group (Fig. 7B).

Effects of pre- and post-treatment of nesfatin-1 on EEG bands

To assess the impact of nesfatin-1 on EEG bands during epileptiform activity, we administered 2 μg/kg nesfatin-1 (i.p.) under two distinct conditions. Firstly, we investigated the post-treatment effects of nesfatin-1. Epileptiform activity was induced by injecting 4-AP, and at the 20th min of ECoG recordings, nesfatin-1 was administered to the animals. We observed no significant changes in the alpha, beta, delta, and theta bands following nesfatin-1 post-treatment (Fig. 8A). However, there was a notable increase in gamma band activity between the 40th and 50th min of ECoG recordings (Fig. 8A) (repeated measures ANOVA, $p < 0.05$). Secondly, we examined the effects of pre-treatment with nesfatin-1 on EEG bands. Nesfatin-1 was injected at the 10th min of ECoG recordings, followed by administration of 4-AP after 20 min. Our findings revealed no significant alterations in the alpha, beta, and theta bands following nesfatin-1 pre-treatment (Fig. 8B). However, there was a reduction in the mean power of delta bands between the 60th and 70th min of recordings (Fig. 8B) ($p < 0.05$). Additionally, pre-treatment with nesfatin-1 induced an increase in gamma band activity between the 40th and 50th min of recordings (Fig. 8B) ($p < 0.05$).

Discussion

In this study, we investigated the effects of peripheral nesfatin-1 injection on basal brain activity, as well as the effects of both pre- and post-nesfatin-1 treatment on 4-AP-induced epileptiform activity. Furthermore, we explored the effects of nesfatin-1 on EEG bands under both basal and abnormal discharge conditions.

We observed that administration of 2 μg/kg nesfatin-1 reduced basal brain activity and the mean power of alpha, delta, and theta bands in ECoG recordings. Post-treatment with nesfatin-1 showed no significant effect on 4-AP-induced epileptiform activity, but it did lead to a transient increase in the absolute power of gamma bands. Pre-treatment with nesfatin-1 had a limited impact on 4-AP-induced epileptiform activity, resulting in a reduction of abnormal discharges for a 10-min period in ECoG recordings. Additionally, nesfatin-1 pre-treatment reduced delta band activity and increased gamma band power for a brief period during recordings.

Neuropeptides are chemical messengers that are synthesized and released by neurons (van den Pol 2012). They have both excitatory and inhibitory effect on synaptic transmission. Nesfatin-1 is a neuropeptide synthesized in the hypothalamus of mammals and can cross the blood–brain barrier without saturation (Pan et al. 2007). In our study, we examined the effects of three different doses (1, 2, and 4 μg/kg) of nesfatin-1 i.p. administered. Surprisingly, only the dose of 2 μg/kg showed a significant effect, resulting in a reduction of basal brain activity. Previous studies that also administered nesfatin-1 peripherally have reported a dose-

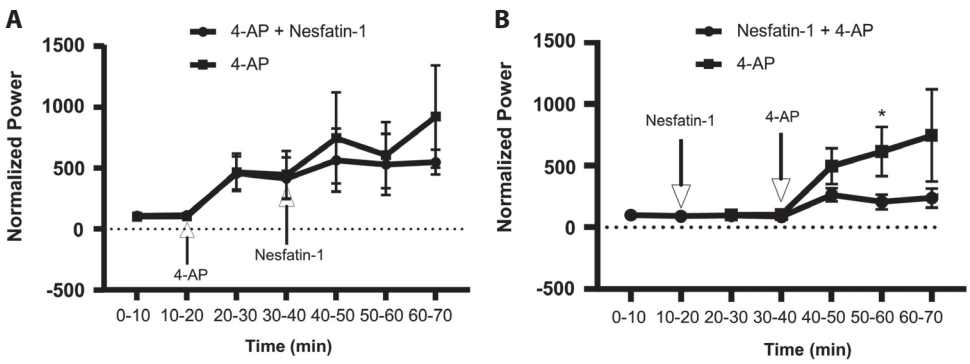

Figure 7. A. Evaluation of effects of pre-treatment with nesfatin-1 on epileptiform activity. Nesfatin-1 (2 μg/kg) administered before 4-AP injection to test whether nesfatin-1 pathway could disrupt the epileptic synchronization. Compared to the 4-AP group (2,5 mg/kg 4-AP i.p.), nesfatin-1 reduced the normalized power of epileptiform activity only between 50th and 60th min of recordings. **B.** Nesfatin-1 did not have significant effect on induced epileptiform activity. Compared to the 4-AP group, application of 2 μg/kg nesfatin-1 following the induction of epileptiform activity (20 min after 4-AP) did not significantly change the normalized ECoG power. Mann-Whitney U test ($n = 7$); * $p < 0.05$.

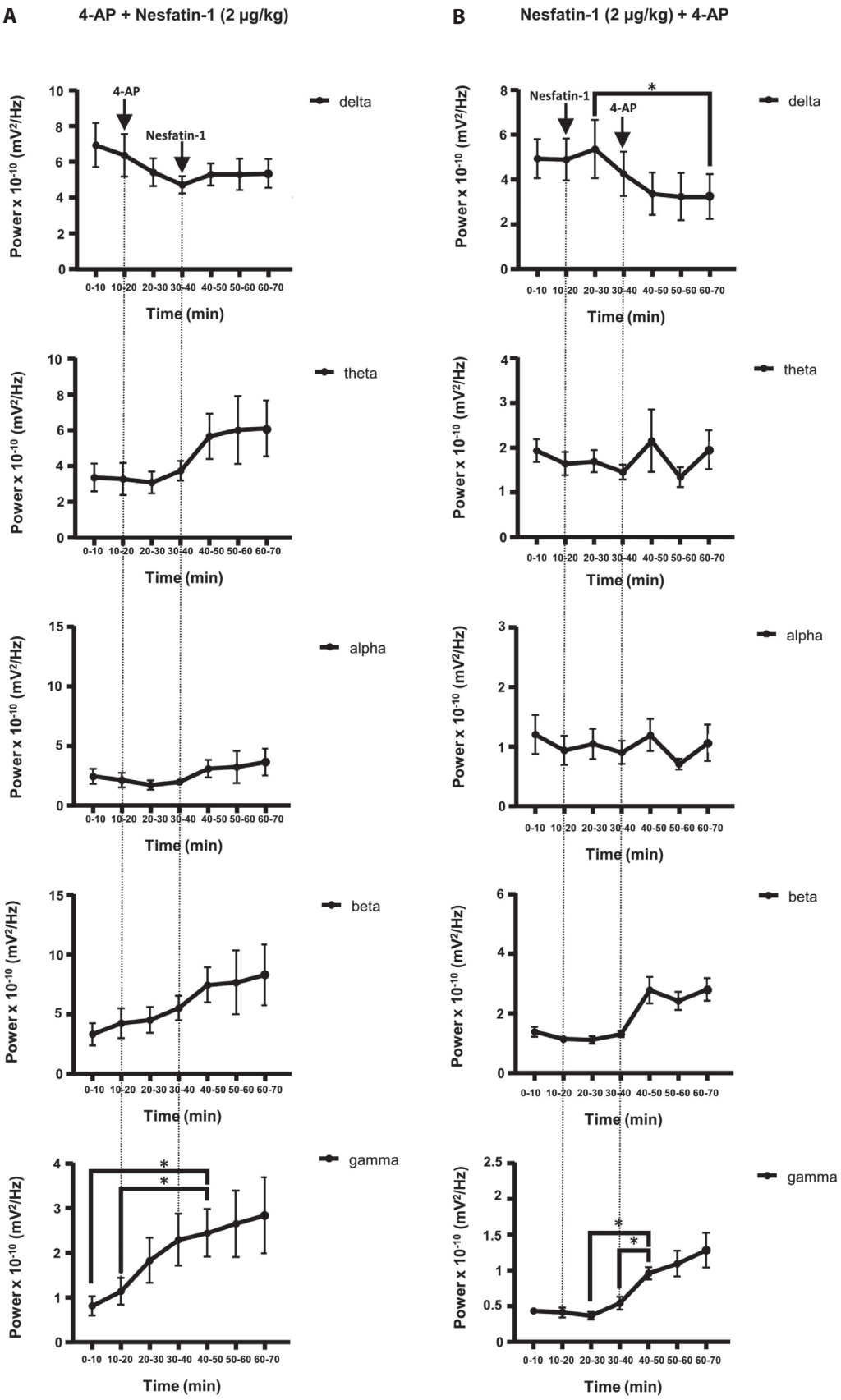

Figure 8. A. Compared to the 4-AP group (2,5 mg/kg 4-AP i.p.), 2 µg/kg nesfatin-1 had no effect on the power of alpha, beta, delta and theta band powers. However, it increased the gamma band powers between 50th and 60th min of recordings. **B.** Pretreatment with nesfatin-1 (2 µg/kg) reduced delta band power at 60th min and increased gamma band power at the 40th min of recordings. Friedman test with Dunn's multiple comparison ($n = 7$); * $p < 0.05$.

dependent effect of nesfatin-1 (Su et al. 2010; Tanida and Mori 2011). However, in our experiments, we did not observe a dose-dependent reduction. We concluded that while nesfatin-1 can permeate the blood-brain barrier without saturation, the dose of 2 μg/kg may represent the physiological concentration necessary for modulating basal brain activity, with higher concentrations potentially being hindered by various synaptic mechanisms. Nesfatin-1 exerts its effects by binding to G-protein-coupled receptors, triggering calcium influx and cellular excitation (Brailoiu et al. 2007). However, contrary to our expectations, we observed a reduction in basal brain activity instead of excitation. Given the known influence of the cannabinoid system on epilepsy and brain activity, it's possible that nesfatin-1's mechanism of action intersects with cannabinoid signalling pathways, resulting in the observed suppression of brain activity (Senn et al. 2020). It is also known that there is a relationship between nesfatin-1 and cannabinoid receptors and nesfatin-1 induces inhibition of feeding (Kaya et al. 2019). The interaction between cannabinoid receptors and nesfatin-1 may offer an explanation for why peripheral administration of 2 μg/kg nesfatin-1 resulted in a reduction of basal brain activity. This suggests a complex interplay between nesfatin-1 signalling and cannabinoid pathways, underscoring the need for further investigation into their combined effects on brain function. The brain electrical signals detected by EEG have been shown to represent the postsynaptic potentials of pyramidal neurons in the neocortex (Pillai and Sperling 2006). EEG waves can be subdivided to alpha, beta, delta, theta and gamma (Pillai and Sperling 2006). Theta and delta waves are not seen in wakefulness and more related to sleeping brain (Pillai and Sperling 2006). Our experiments revealed a decrease in delta, theta, and alpha waves, which likely underlie the observed reduction in total basal brain activity. Previous research has demonstrated the bioactivity of nesfatin-1 in different brain areas, including the neocortex (Stengel 2015) suggesting that i.p. administration of nesfatin-1 may modulate cortical pyramidal neuron activity. However, further studies are warranted to elucidate the precise mechanisms underlying this modulation.

4-AP is an effective convulsant that causes abnormal discharges in the rat brain *in vivo* conditions (Fueta and Avoli 1992). We observed epileptiform-like activities in the ECoG recordings induced by 4-AP, with some animals exhibiting long-lasting ictal discharges. These findings are consistent with those reported in previous studies, further supporting the validity of our experimental model (Kalkan et al. 2024). 4-AP application induces epileptiform activity by blocking different types of K^+ channels (Gonzalez-Sulser et al. 2012). As a result of this blockage, cytosolic calcium $[Ca2^+]_i$ increases (Qian and Saggau 1999). Increased $[Ca2^+]_i$ causes both excitatory and inhibitory transmission (Qian and Saggau 1999). We found that 2 μg/kg nesfatin-1 pre-treatment had a limited effect on 4-AP-induced epileptiform activity. Although there appears to be a reduction from the 40th min of recordings, we found the only significant difference between the 50th and 60th min of recordings. Furthermore, 2 μg/kg nesfatin-1 post-treatment had no effect on 4-AP-induced epileptiform activity. We used 2.5 mg/kg i.p 4-AP to induce epileptiform activity (Sitges et al. 2012). Indeed, abnormal discharges that initiated by a potent K^+ channel blocker may not be limited by an endogenous peptide like nesfatin-1. This may also explain the limited bioactivity of nesfatin-1 in neocortex. In a recent study, researchers found anticonvulsant effect of nesfatin-1 in the penicillin-induced epileptic activity (Musuroglu Keloglan et al. 2023). However, they injected nesfatin-1 intracranial and did not find a direct inhibitor effect of nesfatin-1, instead they found an oxidative stress-mediated anticonvulsant effect (Musuroglu Keloglan et al. 2023). Central administration of nesfatin-1 and usage of penicillin as a convulsant agent may explain how they found a limited anticonvulsant effect. Gamma rhythm is a pattern of neural oscillation in humans and correlated with large-scale brain network activity (Guan et al. 2022). Altered gamma activity has been observed in many cognitive disorders such as epilepsy and schizophrenia (Hughes 2008). We found that, nesfatin-1 altered gamma activity. Altered gamma activity in epilepsy conditions is coherent with the literature. However, we should clarify that how nesfatin-1 enhanced gamma activity by performing further experiments. A recent study showed that nesfatin-1 hyperpolarizes dopamine neurons of the ventral tegmental area (VTA) by inducing an outward potassium current (Dore et al. 2020). It is also known that dopamine signalling plays a major role in the control of epilepsy-like conditions (Bozzi and Borrelli 2013). In the current study we tried to investigate if pre-treatment of nesfatin-1 activates these dopaminergic signalling and this activation has a contribution to epilepsy-like conditions. We found that nesfatin-1 pre-treatment reduced 4-AP-induced epileptiform activity between 50th and 60th min of ECoG recordings. This data indicates a limited effect of nesfatin-1. Limited extra hypothalamic bioactivity of nesfatin-1 may explain the limited contribution. Furthermore, nesfatin-1 pre-treatment caused an increased gamma activity and reduced delta activity. Decreased delta activity may explain the reduction of epileptiform activity between 50th and 60th min of recordings (De Stefano et al. 2022).

In conclusion our results suggest that peripheral nesfatin-1 injection reduces basal brain activity and has limited effect on epilepsy-like conditions.

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