Effects of AgRP Neurons Activation in the Hypothalamic Arcuate Nucleus on Experimental Epilepsy

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ABSTRACT

Anticonvulsant effects of Neuropeptide Y and GABA, which are closely related to AgRP neurons, are known. However, a possible role of AgRP circuits in the mechanism of epilepsy is not reported. We hypothesized that acute and chronic stimulation of AgRP neurons may affect in a kainic acid-induced experimental epilepsy model. For this purpose, electrophysiological and behavioral parameters in epilepsy were investigated in AgRP-ires-cre transgenic animals with acutely and chronically stimulated AgRP neurons.

INTRODUCTION

According to the report published by the International League Against Epilepsy in 2017; A seizure is the appearance of temporary signs and/or symptoms due to abnormal, excessive, or synchronized neuronal activity in the brain. Epilepsy is a neurological disease characterized by recurrent and unexpected seizures caused by excessive neuronal excitability. The central melanocortin system is one of the best characterized neuronal pathways involved in the regulation of energy homeostasis. The modulatory effect of Agouti-related peptide (AgRP) neurons in the arcuate nucleus of the hypothalamus with gamma aminobutyric acid (GABA) is important for energy metabolism. It is known that energy metabolism is impaired in epilepsy.

Due to the anticonvulsant effects of GABA, Neuropeptide Y and Ghrelin associated with AgRP neurons. It was hypothesized that these neurons may play a role in epilepsy. We presumed that acute and chronic stimulation of AgRP neurons may affect kainic acid (KA)-induced experimental epilepsy model.

METHODS

Behavioural Tests

- Feeding Behaviour
- Activity Test-24 hours
- Open Field Test
- Object Location Test
- Novel Object Recognition Test

KA: 15mM-100 nl

Racine scaling > 3

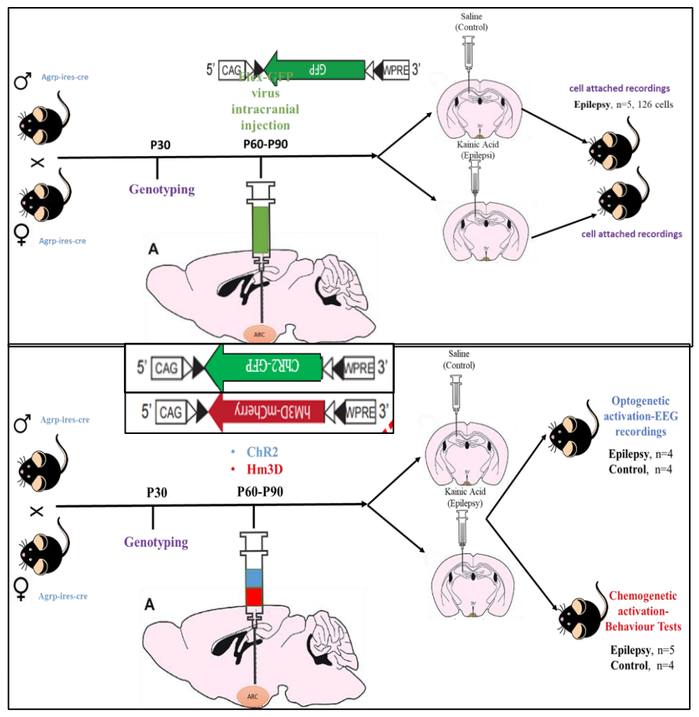


Figure 1: Experimental design.

RESULTS

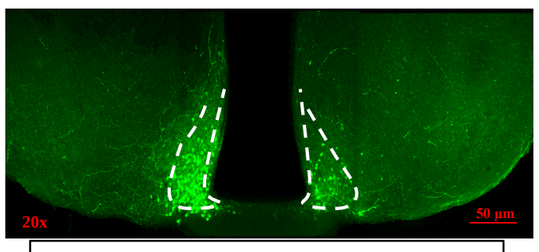


Figure 2: GFP injection into the ARC region in AgRP-cre mice.

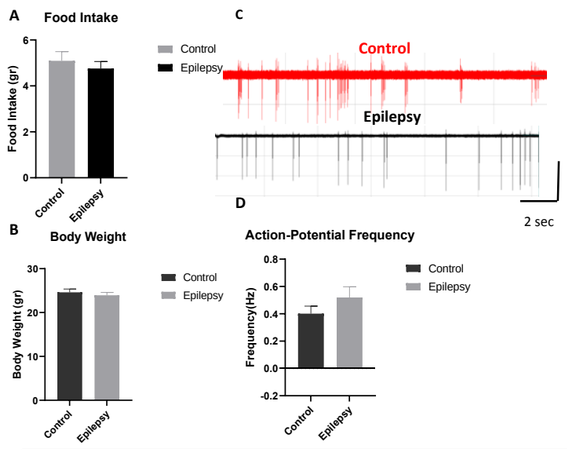
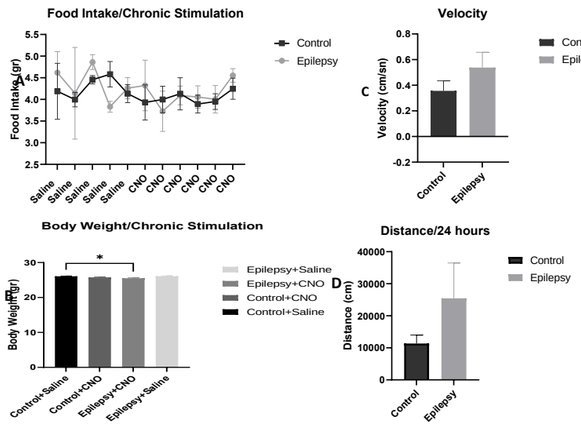
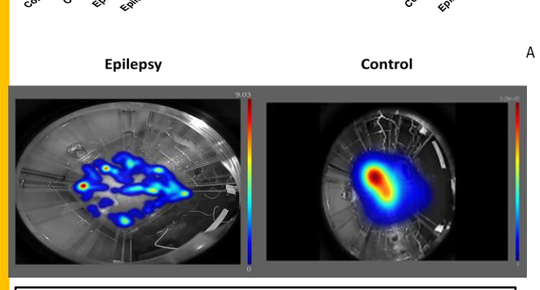


Figure 3: Graphs of epilepsy and control groups for food intake (A) and body weight (B) in AgRP-GFP animals. There was no significant difference between the groups. Traces (C) and graphs (D) of action potential frequency of epilepsy and control group in AgRP-GFP experiment. No significant difference was found between the groups (Epilepsy n=5,126 cells; Control n=4,106 cells).





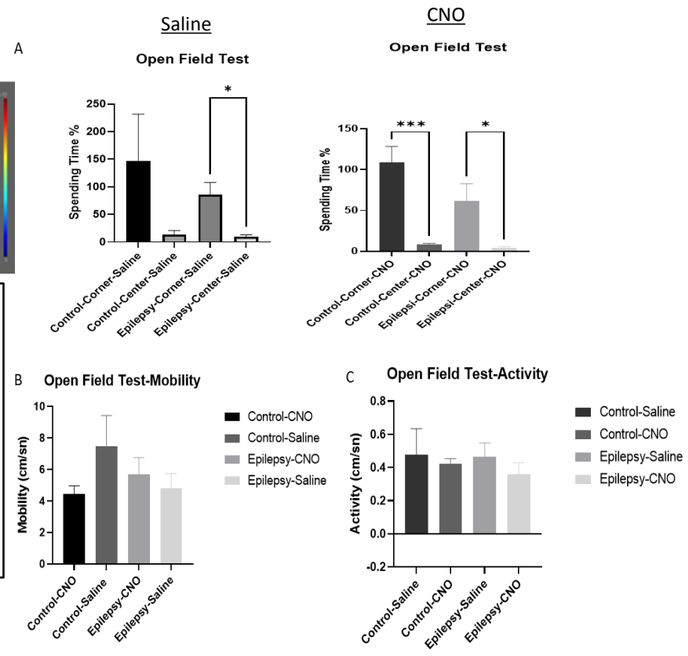


Figure 4: Chronic activation of AgRP neurons. Food intake (A) and body weight (B) graphs of AgRP-chemogenetic animals. A statistical difference was seen between the days given clozapine- N-okside (CNO) to epileptic animals and the days given Saline to control animals in body weight (B; *,p<0.05). Although no significant difference could be found between the velocity (C) and distance (D) of the animals after 24 hours of activity recording, it can be seen that the epilepsy animals have higher velocity and make more distance numerically.

Figure 5: The effect of chronically activated AgRP neurons on anxiety-like behaviors. In the Open Field test, it has been observed that epilepsy animals exhibited anxious behavior when given saline (A; *, p<0.05). On the other hand, it was observed that control and epilepsy animals showed more anxious behaviors in the presence of AgRP neurons that were continuously stimulated chemogenetically (A; *, p<0.05; ***, p<0,001). On the other hand, no statistical difference was found between the experimental groups in terms of mobility (B) and activity (C).

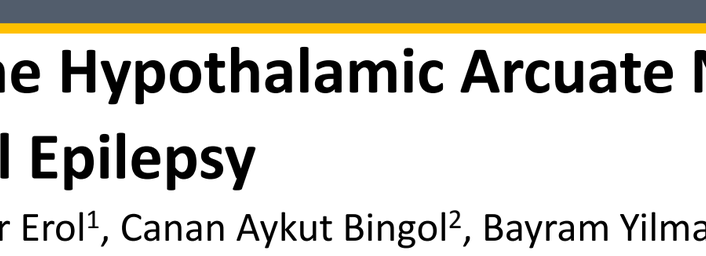


Figure 6: In the object location test (A) and novel object recognition tests (B) based on hippocampus function, it was shown that chemogenetically stimulated AgRP neurons did not make a difference between the epilepsy and control groups.

Figure 7: Acute excitation of AgRP neurons. EEG recordings were taken from experimental animals with ferrules and tripolar electrodes attached to their skulls. With this recording, 30 minutes were baseline, then 10 hz blue light (480 nm) was stimulated for 10 minutes. After the stimulation, the recording was taken for 30 minutes. Body weight (A) and food intake (B) were followed up for the groups. Although no statistically significant difference could be found between the epilepsy and control groups in frequency data (C). A significant difference was found per minute and epileptic animals had numerically larger amplitudes (D) before stimulation. In addition, EEG traces are as in the figure (E). Finally, we showed that we put the optogenetic ferrules in the right places with their confocal images (F).

Conclusions

The present study has shown that the activation of AgRP neurons produced results similar to the control group in epileptic animals. GABA release of these neurons can be an important starting point.

It is also thought that hunger signals affect energy metabolism so that the brain works to maintain its own homeostasis instead of causing seizures.

Finally, this AgRP activation may have acted via MCRs rather than GABA in the melanocortin pathway. Considering previous studies, investigating whether this neuron group affects epilepsy via energy metabolism or GABA neurons will take this study to the next step.

References

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Poster # PC09