# Limited Exposure to Social Isolation does not Affect the Spike Frequency and Amplitude of Penicillin-induced Epileptiform Activity in Adolescent Rats

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## Abstract

**Objective:** It is known that the stress experienced during this period increases the risk of seizures. This study, it was investigated the effect of limited social isolation (SI) stress experienced in early life on penicillin-induced epileptiform activity.

**Methods:** Wistar Albino male rats (n=21) 28 days postpartum (PND21) were randomly divided into 3 groups (n=7); control group (C), SI group for 28 day (SI28), SI group for 14 days (SI14). SI stress was established by keeping rats in cages alone for 6 hours per day. Following the experimental protocol, rats were anesthetized with urethane (1.25 g/kg). The epileptiform activity was induced with Penicillin-G (500 IU, i.c.) and ECoG was recorded for 3 hours.

**Results:** SI stress was no effect on the spike frequency and amplitude of penicillin-induced epileptiform activity. There was no difference in penicillin-induced epileptiform activity whether the SI was 14 or 28 days.

**Conclusion:** SI stress per day for 6 hours was no effect on penicillin-induced epileptiform activity early in life. After the rats were exposed to SI stress for 6 hours a day, housing in a standard cage may have reduced or eliminated the effects of isolation providing social interaction. In order to better interpret this finding, we suggest that possible changes with different durations of SI should be investigated with further studies.

**Keywords:** Social isolation, stress, epilepsy, penicillin

## INTRODUCTION

Stress experienced early in life affects neuronal function and brain networks.[1] There are experimental animal models to investigate the effects of stress on humans.[2,3] Social isolation (SI) is one of the methods of creating social stress in animals.[4] SI stress after weaning has been reported to cause changes in different brain regions.[5]

The Coronavirus disease-2019 (COVID-19) pandemic has caused an unprecedented number of people to be affected by quarantine or isolation.[6] A strong association has been shown between SI and anxiety, depression in children and adolescents during the COVID-19 pandemic.[7] It is stated that children and adolescents are more likely to experience high rates of depression and anxiety after isolation.[8]

Epilepsy is a neurological disease closely related to stress and anxiety.[9,10] In studies in humans and animals, it has been shown that the risk of increased seizure susceptibility increases due to SI stress.[11,12] The current COVID-19 pandemic can influence the seizure frequency in adult people with epilepsy.[12] The impact of COVID-19 and quarantine isolation on the course of epilepsy and the incidence of new-onset seizures remains unclear.[13] For epilepsy, which most commonly begins in the first two decades of life, adolescence is a period of great change that takes place both in epilepsy itself and in many other areas.[14] Increasing knowledge about the interaction between early life stress, seizures, and epileptogenesis is thought to provide a basis for new treatment strategies for epilepsy.[15,16] However, although SI affects seizure development, its effect on the brain has not yet been clarified.[8,9]

It is important to investigate the effect of this isolation, which is mandatory exposure with the COVID-19 pandemic, especially childhood-onset epilepsy. Therefore, in this study, the effect of 6 hours of daily SI stress exposure on epileptiform activity was investigated in adolescent rats.

## METHODS

### Animals and Experimental Groups

This study was done at postnatal 21 days (PND21) old Wistar Albino male rat (n=21). The ethical approval of the study was taken from the Local Ethical Committee of Ondokuz Mayıs University (ethical no: 2018/25, date: 20.04.2018). All animals are purchased from, Ondokuz Mayıs University Laboratory Animal Center which has a 12-hours light/dark cycle at the ambient temperature of 21±1 ºC. The rats were acclimated one week before starting the experimental procedure. The rats were given free access to food and water. The experimental procedure started at PND28 and lasted 4 weeks (Figure 1). The rats were randomly divided into 3 groups (n=7); control group (C), SI group for 14 days, and SI group for 28 days.

### Social Isolation Procedure

SI involved removing the experimental animal from the home cage and placing it into an isolated cage with dimensions 25×42×15 cm. Every day rats were exposed to stress between 08:00-14:00 for 6 h in a separate cage. Rats were exposed to SI for 14 days or 28 days.[11,17,18] After completing their SI, rats spent the rest of the day in standard rat cages. During this period, the C group (per cage of 3-4 rats) spent their time in the standard cage.[19] These animals were exposed to the same environmental stimuli as group housed rats but were deprived of social contact.

### Electrocorticography Recordings

After the 28-day experimental procedure, on the PND57, the rats were anesthetized with urethane (1.25 g/kg, i.p.) and placed in the stereotaxic apparatus. Skin and subcutaneous tissue were removed from the skull. Two screw electrodes were placed on the left somatomotor cortex and a ground electrode was placed on the nasal sinus. Two bipolar Ag-AgCl ball electrodes were placed in the somatomotor cortex of the left hemisphere.[20]

ECoG activity was then monitored with an eight-channel recorder (PowerLab, 8/SP, AD Instruments, Castle Hill, NSW, Australia). Penicillin G (I.E. Ulagay, Turkey) 500 IU potassium was dissolved in normal saline, administered as a single dose and 2 µL was injected using a Hamilton microsyringe (type 701N; Aldrich, Milwaukee, WI, USA) into the 1 mm beneath the brain surface taking the bregma reference point. After 2 minutes of basal activity, penicillin was injected and epileptiform activity was recorded for 180 min. The frequencies and amplitudes of ECoG activity were analyzed offline.[20]

### Statistical Analysis

Statistical comparisons were made using Statistical Package for Social Sciences. In multiple comparisons, one-way analysis of variance was used after it was determined that the obtained data fit the normal distribution (Shapiro-Wilk test). Tukey Kramer post-hoc tests for multiple comparisons were performed. For all statistical tests, $p<0.05$ was considered statistically significant. The results are presented as the means±standard error of the mean.

## RESULTS

### Effect of Social Isolation on Penicillin-induced Epileptiform Activity

Approximately 30 min after the penicillin injection, epileptiform activity reached a constant level and this activity lasted for about 3h. The means of spike frequency and amplitude of penicillin-induced epileptiform activity in the control group were 78.13±8.13 spike/min and 1189.13±26.75 µV, respectively.

There was no found significant difference that the spike frequency and amplitude of penicillin-induced epileptiform activity in the SI28 and SI14 groups were compared to the C group ($p>0.05$) (Figure 2a and 2b). The means of the spike frequency and amplitude of epileptiform activity were 82.75±7.75 spike/min and 1011.31±81.50 µV in the 100 min after the injection of penicillin respectively in SI14. In SI28, the spike frequency and amplitude of epileptiform activity were 78.38±3.81 spike/min and 1095.62±83.37 µV, respectively, within 100 minutes after penicillin injection. In addition, there was seen a decrease in amplitude of epileptiform activity in the SI groups compared to the control group although it was not statistically significant (Figure 2b).

## DISCUSSION

In this study, we investigated the effect of SI on penicillin-induced epileptiform activity. According to our results, 6 hours a day of SI in early daily life did not change the frequency or amplitude of epileptiform activity. In addition, we did not detect any difference on epileptiform activity between 14 days of SI or 28 days of SI.

Studies in the literature show that SI induces stress and reduces seizure threshold.[21,22] In experimental animals, the daily SI period exposed during the experimental procedure can be as short as 1 hour, or it can be for the whole day, ie 24 hours.[23,24] Amiri et al.,[24]

**MAIN POINTS**

- It is known that the stress experienced during this period increases the risk of seizures.
- We are investigated the effect of limited social isolation stress experienced in early life on penicillin-induced epileptiform activity.
- Social isolation stress was no effect on the spike frequency and amplitude of epileptiform activity.

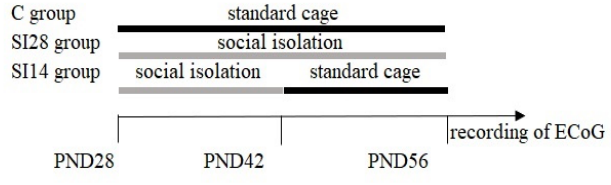


**Figure 1.** The schematic timeline of the experimental procedure
PND: Postnatal day, C: Control group, SI28: SI group for 28 days, SI14: SI group for 14 days

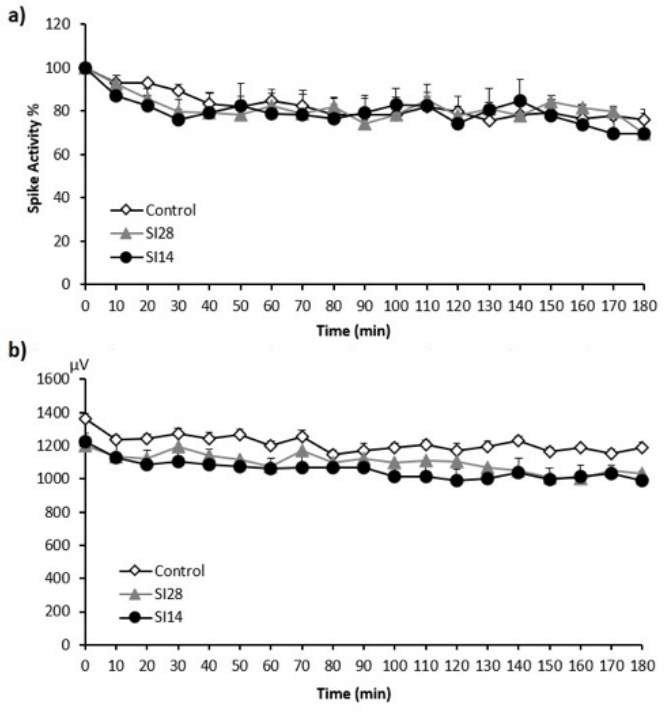


**Figure 2.** a) The spike frequency % of penicillin-induced epileptiform activity did not statistical significance change in between the groups. b) The mean of amplitude of penicillin-induced epileptiform activity did not statistical significance change in between groups ($p>0.05$). The results are presented as the means±standard error of the mean (SEM)
SI28: SI group for 28 days, SI14: SI group for 14 days

found that SI stress significantly decreased the seizure threshold in comparison with the social condition for PN21-PND50 in mice (all day SI). In our study, SI was applied for 6 hours a day, not all day. Furthermore, a previous study showed that applied stress during limited hours 180 or 15 minutes found that stress increased vulnerability to kindling.[25] However, in the study of Ali et al.,[25] stress was applied between PND 2nd and 14th days. In addition, Lai et al.[26] showed that the neonatal isolation (from PND day 2 (P2) to P9) plus status epilepticus induced rats had greater cognitive deficits and decreased seizure threshold. Lai et al.,[26] showed that applied stress to a much earlier period of life compared to our study. Therefore, we recommend that stress before PND 21 days has a more negative effect on epileptogenesis. This may be due to the partial or complete deprivation of maternal care of the offspring in the stress models applied before PND21.[1] Rau et al.,[27] showed that early life stress increases excitability but in their studies, the isolation was housed after PND21 until P101-P115. The SI applied in their study is longer than our study. Here, we think that affects our results especially the daily exposure time to SI and total duration of SI in the early stages of life.

In the present study, we showed that SI of rats for a limited time of 6 hours daily did not affect penicillin-induced epileptiform activity. Here, we suggest that the short duration of SI may have eliminated the possible negative effects of SI. In our study, rats spending the rest of the day in the normal cage after isolation, thus re-socializing, may have a curative effect on isolation. The previous study found that re-socialization can revert both long-term chronic SI stress induced anxiety and social memory impairment.[28] On the other hand, studies are showing the negative effect of SI on epilepsy. Another reason for the different results in our study may be that the epileptiform activity was directly evaluated, not the seizure susceptibility of the rats in our study.

Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter found in the cerebral cortex.[29] Enhancement of GABAergic inhibition is useful for the treatment of pathological conditions including epilepsy.[30] GABA has two major receptors; $GABA_A$ acts as a chlorine channel and a baclofen-sensitive metabotropic receptor $GABA_B$.[31] The convulsant properties of penicillin are associated with antagonism of inhibitions mediated by $GABA_A$ receptor within the mammalian central nervous system.[32] Studies are showing that SI is associated with epilepsy and that the GABAergic system plays a role in this relationship.[33,34] Early life stress causes prolonged neuronal hyperexcitability in some limbic regions with less effective inhibition by GABA.[35] Neurochemical, molecular, and electrophysiological evidences demonstrate that SI is associated with alteration in the structure and function of $GABA_A$ receptors.[36] Another study demonstrated that SI induced a decreased behavioral response to systemically administered GABA-mimetic drugs in mice.[37] In previous showed that long-term SI beginning PND28 and lasting 7 weeks increased seizure susceptibility to a $GABA_A$ antagonist picrotoxin in mice.[33] In our study, we did not see the effect of SI stress on penicillin-induced epileptiform activity, although penicillin is an agent that acts on GABA antagonism. This may be because penicillin is an acute seizure model and thus only 3 hours of epileptiform activity was recorded.

### Study Limitations

In addition, animal studies have limitations arising both from the problems associated with modeling neuropsychiatric disorders in the laboratory and from the disadvantages of animal models of epilepsy themselves.[38] For example, many epilepsy patients have a latency period of months or even years of no seizure activity after transient brain injury reported early in life.[39]

## CONCLUSION

According to our findings, SI applied for a limited time does not affect the frequency and amplitude of penicillin-induced epileptiform activity. The fact that the rats were in a normal cage after isolation may have prevented the effects of SI stress from appearing. In future studies, we suggest investigating the effect of exposure to SI stress at different durations on epileptiform activity.

### Ethics

**Informed Consent:** This study was done at postnatal 21 days (PND21) old Wistar Albino male rat (n=21).

**Ethics Committee Approval:** The ethical approval of the study was taken from the Local Ethical Committee of Ondokuz Mayıs University (ethical no: 2018/25, date: 20.04.2018)

### Authorship Contributions

Surgical and Medical Practices: F.B.A., F.A., Concept: S.M.K., Design: S.M.K., L.Ş. Data Collection or Processing: S.M.K., F.B.A., Analysis or Interpretation: S.M.K., F.B.A., L.Ş., M.A., E.A., Literature Search: S.M.K., L.Ş., M.A., E.A., Writing: S.M.K., F.B.A., L.Ş., F.A., M.A., E.A.

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