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# Protective effects of gabapentin against the seizure susceptibility and comorbid behavioral abnormalities in the early socially isolated mice

Shayan Amiri[a,b,c,1], Arya Haj-Mirzaian[a,b,1], Hossein Amini-Khoei[d,e], Ali Razmi[f], Armin Shirzadian[a,b], Maryam Rahimi-Balaei[g], Carl O. Olson[c], Arman Mohsenzadeh[a], Mojgan Rastegar[c], Mohammad-Reza Zarrindast[a], Mahmoud Ghazi-Khansari[a,b,*]

[a] Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, P.O. Box, 13145-784, Tehran, Iran
[b] Experimental Medicine Research Center, Tehran University of Medical Sciences, Tehran, Iran
[c] Department of Biochemistry & Medical Genetics, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, MB, Canada
[d] Medical Plants Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran
[e] Department of Physiology and Pharmacology, School of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran
[f] Department of Pharmacology and Applied Medicine, Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, Karaj, Iran
[g] Department of Human Anatomy and Cell Science, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, MB, Canada

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ABSTRACT

Adolescence is a pivotal period of brain development during lifespan, which is sensitive to stress exposure. Early social isolation stress (SIS) is known to provoke a variety of psychiatric comorbidities as well as seizure risk. Psychiatric comorbidities present challenging dilemmas for treatment and management in people with seizure disorders. In this study, we aimed to investigate whether gabapentin (GBP) as an anti-epileptic drug is able to alleviate the seizure activity as well as comorbid behavioral abnormalities in socially isolated mice. Results showed that early SIS induced proconvulsant effects along with depressive, aggressive and anxiety-like behaviors. Whereas the administration of both acute and chronic GBP at sub-effective doses produced no alterations in the behavioral profile of socially conditioned counterparts the same treatments effectively reversed the seizure susceptibility to pentylenetetrazole and behavioral deficits in isolated mice. Results of the study indicate that 1) Early SIS could be considered as an animal model of psychosocial stress to investigate the psychiatric comorbidities in seizure disorders, 2) Chronic administration of low dose GBP prevented the shaping of behavioral abnormalities in adulthood, 3) Chronic administration of low dose GBP produced no negative behavioral effects in socially conditioned mice suggesting the safety of the drug, 4) Gabapentin at low doses may be considered as an agent for management of epilepsy in individuals with psychiatric comorbidities.

## 1. Introduction

The developing brain is susceptible to environmental influences and exposure to stressful conditions profoundly affects brain development (Lupien et al., 2009). Adolescence is a pivotal state in cortico-limbic development and is associated with maturation of behavioral and cognitive abilities (Andersen and Teicher, 2008; Paus, 2005). Ample evidence indicates that experiencing psychological stress during adolescence potently predisposes the development of psychiatric difficulties in adulthood (Fone and Porkess, 2008). Evidence is accumulating that epilepsy is associated with incidence of psychiatric disorders (Kanner et al., 2012; Thapar et al., 2009). Psychiatric comorbidities in seizure disorders are accompanied by notable burden in morbidity, management of patients, treatment and quality of life (Fazel et al., 2013; McCagh et al., 2009). Anxiety and depression are of most prevalent psychiatric comorbidities which occur which occur 4–5 more often in subjects with seizure disorders than in normal populations (Maguire and Salpekar, 2013). Surprisingly, although there is a large body of evidence indicating the importance of psychiatric comorbidity in seizure disorders, fewer studies have focused on the treatment of such comorbidities (Kanner, 2003; Swinkels et al., 2005).

Recently, we showed that early social isolation stress (SIS) provoked seizure risk along with affective behavioral dysfunctions in adult mice (Amiri et al., 2014). Early life stress is known to increase the risk of epileptogenesis and occurrence of psychiatric comorbidities (Huang, 2014; Jones et al., 2014). It has been suggested that applying animal

* Corresponding author at: Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, P.O. Box, 13145-784, Tehran, Iran.
*E-mail address:* ghazikha@sina.tums.ac.ir (M. Ghazi-Khansari).
[1] Please note that the first two authors are considered as the first author

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models such as early SIS provides conditions to investigate the psychopathological similarities which are observed in humans (Nestler and Hyman, 2010).

There is a large body of evidence, which indicates that anti-epileptic drugs have positive effects on mood and behavior (Perucca and Mula, 2013; Russo et al., 2013). Gabapentin (GBP), as an anti-epileptic drug with normothymic properties, is structurally similar to γ-aminobutyric acid (GABA). Increasing lines of research suggest that GBP has therapeutic effects in mental disorders including mood and anxiety disorders (Perucca and Mula, 2013). Gabapentin is known to reduce the neural excitability in the central nervous system via binding to α2-δ subunit of voltage-gated calcium channels leading to a decrease in excitatory neurotransmission. Also, GBP has an ability to increase ambient GABA levels in the brain indicating its effects on alteration of GABA synthesis or release (Brickley and Mody, 2012; Honmou et al., 1995). However, the mechanism of action of GBP is not clear well (Sills, 2006).

In this study, as early SIS induces proconvulsant effects along with affective behaviors in mice, we aimed to investigate whether GBP is able to attenuate the proconvulsant effect of SIS along with psychiatric comorbidities in male mice. GBP was used in this study because it is a safe drug with minor side effects and has a high therapeutic index (Arif et al., 2009; Perucca and Mula, 2013).

## 2. Material and methods

### 2.1. Animals and housing conditions

Male NMRI mice weighing 10–12 g on postnatal day 21 (PND: 21) (Pasteur Institute, Tehran, Iran) were used. Animals were housed under standard conditions (temperature: 22 ± 2 °C, humidity: 50 ± 10%, 12-h light–dark cycle, and free access to food and water) for 4 weeks in two conditions: social condition (SC) and isolated condition (IC). Socially conditioned mice were housed (6 per cage) in Plexiglas cages (25 cm×25 cm ×15 cm) while IC mice were housed individually in Plexiglas cages (24 cm×17 cm ×12 cm) in a separate room. Cages of IC mice were cleaned weekly. All experiments were conducted during the period between 09:00 a.m. and 02:00 p.m. Each experimental group consisted of 6–8 mice. All procedures in this study were carried out in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (NIH publication # 80-23) and institutional guidelines for animal care and use (Department of Pharmacology, School of Medicine, TUMS).

### 2.2. Drug and treatment procedure

GBP was a gift from the Alborz Bulk pharmaceutical company, Iran. Firstly, we determined the effective and sub-effective doses of GBP (in both SC and IC mice) using pentylentetrazole (PTZ)-induced clonic seizures paradigm. In order to investigate the acute effects of GBP on behavioral profile of the mice, doses 1, 3, 5 mg/kg were injected 60 min prior to the behavioral tests at PND: 50–54. GBP was freshly dissolved in saline, being prepared immediately and administered intraperitoneally (i.p.) with a volume of 5 ml/kg body weight.

In order to examine the chronic effects of GBP on the behavioral profile of the mice various doses of GBP (1, 3, 5 mg/kg) were used after 1 week of housing (PND: 28). For this purpose, GBP dissolved in drinking water and administered for 21 days (PND: 28–50). After the treatment, experimental animals were subjected to behavioral tests at PND: 50–54.

### 2.3. Open-field test (OFT)

The open-field test was used to evaluate the locomotion and anxiety behavior (Kulesskaya and Voikar, 2014). The open-field apparatus was made of white opaque Plexiglas (50 cm×50 cm ×30 cm), which was dimly illuminated. Each mouse was placed gently on the center square (30 cm×30 cm), and behaviors were recorded by a camera for 5 min and were analyzed by an experimenter blind to the treatments and conditions. The surface of the apparatus was cleaned with 70% ethanol after testing each mouse. The distance moved (horizontal activity), the number of rearings (vertical activity) and also time spent in the central zone were evaluated.

### 2.4. Hole-board test (HBT)

The hole-board test was used to evaluate the anxiety of subjects and carried out based on our recent study (Amiri et al., 2015). The apparatus consisted of a white Plexiglas square (50 cm×50 cm) with 16 equidistant holes (3 cm in diameter) and was positioned 50 cm above the floor. Mice were placed in the center of the board and the number of head-dips was counted in a 5-min period. The apparatus was cleaned with 70% ethanol after testing each subject.

### 2.5. Elevated plus maze (EPM)

The EPM is an appropriate test to assess the effects of both anxiogenic and anxiolytic agents in rodents (Ducottet and Belzung, 2005). The apparatus was made of black opaque Plexiglas and consisted of two open (30×5 cm) and closed (30×5×15 cm) arms, which were connected by a platform area (5×5 cm). The testing room was dimly illuminated and animals were individually placed in the center of the EPM facing to closed arm and each behavioral session was videotaped for a 5-min period. The total time spent in the open arms, and number of entries into the open arms reported as percentages.

### 2.6. Forced swimming test (FST)

The test was directed using a method which was previously described (Porsolt et al., 1977). In brief, mice were separately placed in an open cylinder-shaped flask (diameter: 10 cm, height: 25 cm), containing 19 cm water at 23 ± 1 °C. Mice were permitted to swim for 6 min and the immobility time was recorded throughout the last 4 min of the test. Each mouse was judged to be immobile when it ceased struggling and stayed floating motionless in the water, making only those movements necessary to keep its head above water.

### 2.7. Tail suspension test (TST)

The total duration of immobility induced by tail suspension was measured based on the method described by Steru et al. (1985). Briefly, a mouse was suspended 50 cm above the bench by adhesive tape placed 1 cm from the tip of the tail. The mouse was considered immobile only when it hung passively and completely motionless. Immobility time was recorded during a 6-min period by an experimenter blind to the animal condition.

### 2.8. Splash test

Splash test was carried out to evaluate the motivational and self-care behaviors according a previously described method (Ducottet and Belzung, 2004). In this test, grooming behavior of mice, which can be considered as an indirect measure of palatable solution intake, was measured. A 10% sucrose solution was squirted on the dorsal coat of animals in their home cage and mice were videotaped for 5 min. The total grooming activity time was recorded during 5 min after the sucrose vaporization. Grooming activity consists of nose/face grooming, head washing and body grooming.

### 2.9. Resident-intruder test

Evaluation of aggressive behavior was carried out according to a