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

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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

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: $50 \pm 10\%$, 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 intraperito-neally (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 groom-ing, head washing and body grooming.

2.9. Resident-intruder test

Evaluation of aggressive behavior was carried out according to a

previously described method (Ibi et al., 2008; Ruzza et al., 2015). The mice called "residents" were first housed individually in transparent plastic cages (24 cm×12 cm ×8 cm) for 5 days. To cause the aggressive encounter, the "intruder" mouse was placed inside the home cage of the resident mouse and behaviors of the resident mouse against the intruder were videotaped over 5 min with a camera positioned above the testing cage. The duration of aggressive behavior including attacking/biting, tail rattling, aggressive grooming, sideways posturing and pushing under were observed and analyzed by an experimenter blinded to the treatment and housing conditions.

2.10. Determination of clonic seizure threshold

In order to measure the clonic seizure threshold in mice, we used a method that was previously described in our studies (Amini-Khoei et al., 2015a, 2015b). Briefly, a winged infusion set (30 gauge) was used to infuse the PTZ (0.5%) at a constant rate of 1 ml/min into the tail vein of the freely moving subject. Infusion was halted when forelimb clonus followed by full clonus of the body (began with running and then loss of righting ability) was observed. The minimal dose of PTZ (mg/kg mouse weight) needed to induce a clonic seizure was considered as the index of seizure threshold. As such, seizure threshold is dependent on the dose and time of PTZ administration.

2.11. Statistics

SPSS (version 21) and GraphPad prism (version 6.1) statistical softwares were used for data analysis and figure creation. Comparisons between the groups were assessed using t-test and one-way ANOVA followed by Tukey's post hoc test. A value of $P < 0.05$ was considered for significance.

3. Results

3.1. Effects of different housing conditions and GBP treatments on the seizure threshold dose of PTZ

As shown in Fig. 1A, t-test revealed that SIS significantly decreased the seizure threshold in comparison with SC mice ($t=10.65$ $df=10$, $P < 0.001$). Acute treatment with different doses of GBP 60 min before the test significantly increased the seizure threshold in the PTZ-induced seizure in SC and IC animals (F (7, 52) =14.831, P < 0.001, Fig. 1B). The Tukey's post hoc test showed that acute injection of GBP significantly increased the seizure threshold in SC mice at doses 3 and 5 mg/kg (P < 0.05 and P < 0.001, respectively). Also, acute administration of GBP was effective in the IC group only at a dose of 5 mg/kg (P < 0.001) when compared to IC control counterpart. Fig. 1C shows that chronic treatment with different doses of GBP significantly increased the seizure threshold in SC and IC animals (F (7, 48) =13.87, P < 0.01, Fig. 1C). Post test analysis showed that chronic treatments with GBP had no significant effect on the seizure threshold in SC mice, while a chronic dose of 5 mg/kg produced anticonvulsant effects in comparison with the IC control counterpart (P < 0.001).

3.2. Effects of different housing conditions and GBP treatments on the number of head-dips in the hole-board test

One-way ANOVA analysis showed that there are significant differences between the groups in the number of head-dips in both the acute and chronic scheduled experiments (F (7, 43) =25.7, Fig. 2A, P < 0.001, F(7, 54) =12.3, P < 0.001, Fig. 2B). As shown, SIS significantly decreased the number of head dips in comparison to SC animals (P < 0.001). Tukey's test showed that acute or chronic administration of GBP 5 mg/kg significantly increased the number of head dips of IC mice when compared to IC control group (P < 0.001 for both). However, administration of doses 1 and 3 mg/kg had no significant

effect in IC mice ($P > 0.05$). Furthermore, our results showed that there are no significant differences between acute and/or chronic GBP treatment in SC mice when compared to control SC group (P > 0.05).

3.3. Effects of different housing conditions and GBP treatments on the EPM

In the EPM, the percentage of time spent in the open arms and percentage of open arms entries were evaluated as variables relevant to anxiety-like behaviors. ANOVA analysis revealed that there are significant differences between the groups in the open arm time (F (7, 42) =23.7, P < 0.001, Fig. 3A) for acute and (F (7, 47) =19.2, P < 0.001, Fig. 3B) for chronic GBP treatment. One-way ANOVA revealed that acute and/or chronic GBP treatment led to significant differences in open arm entries of SC and IC mice (F (7, 42) =25.7, P < 0.001, Fig. 3C) for acute and (F (7, 47) =16.6, P < 0.001, Fig. 3D) for chronic administration. In comparison with SC mice, SIS remarkably decreased the percentage of time spent in the open arms ($P < 0.01$) as well as the percentage of open arms entries ($P < 0.001$) in IC mice. Furthermore, acute or chronic administration of GBP 5 mg/kg significantly increased the percentage of time spent in the open arms (P < 0.01) as well as the percentage of open arms entries (P < 0.01) of IC mice when compared to the IC control counterpart. The administration of doses 1 and 3 mg/kg of GBP had no significant effect in the EPM of IC mice ($P > 0.05$). Additionally, no significant difference was observed in the EPM between acute and/or chronic GBP treatment in SC mice when compared to the control SC group (P > 0.05).

3.4. Effects of the different housing conditions and GBP treatments on locomotion and anxiety-like behaviors in open field test

Results obtained from one way ANOVA (Fig. 4) showed that there are significant differences in the time spent in the central zone (F (7, 46) =35.66, P < 0.001, Fig. 4A, acute treatment and F (7, 52) =43.17, P < 0.001, Fig. 4B, chronic treatment), distance moved (horizontal activity) (F (7, 46) =29.3, P < 0.001, Fig. 4C, acute treatment and F (7, 52) =27.21, P < 0.001, Fig. 4D, chronic treatment) as well as the number of rearings (vertical activity) (F (7, 46) =23.3, P < 0.001, Fig. 4E, acute administration and F (7, 52) =17.06, P < 0.001, Fig. 4F, chronic administration) in both acute and chronic treatment with GBP. As shown in Fig. 4, isolation housing leads to a significant decrease in the time spent in central zone of OFT when compared to the SC group ($P < 0.001$). In addition, GBP 1 and 3 mg/kg failed to increase the central zone time spent of IC mice ($P > 0.05$). However, administration of GBP 5 mg/kg increased the time spent in the central zone for both acute and chronic treated groups ($P < 0.001$ for both). Also, social isolation significantly increased the distance moved (horizontal activity) in IC mice in comparison with SC mice ($P < 0.001$). Furthermore, treatment with GBP 5 mg/kg significantly decreased the horizontal activity in the both acute ($P < 0.001$) and chronic ($P < 0.01$) treated IC groups when compared to the corresponding IC group. However, GBP 1 and 3 mg/kg administrations had no effect on this behavior in IC animals ($P > 0.05$).

The isolation housing significantly increased the number of rearing in OFT when compared to the SC group ($P < 0.001$). As shown, elevation in the number of rearings in the IC mice was significantly decreased after acute and chronic administration of GBP 5 mg/kg ($P < 0.001$ for acute and $P < 0.01$ chronic treatment). Additionally, treatments with GBP 1 and 3 mg/kg had no effect on the number of rearings in either IC or SC group ($P > 0.05$). In addition, there are no significant differences among SC groups in the OFT ($P > 0.05$).

3.5. Effects of the different housing conditions and GBP treatments on grooming activity time in the splash test

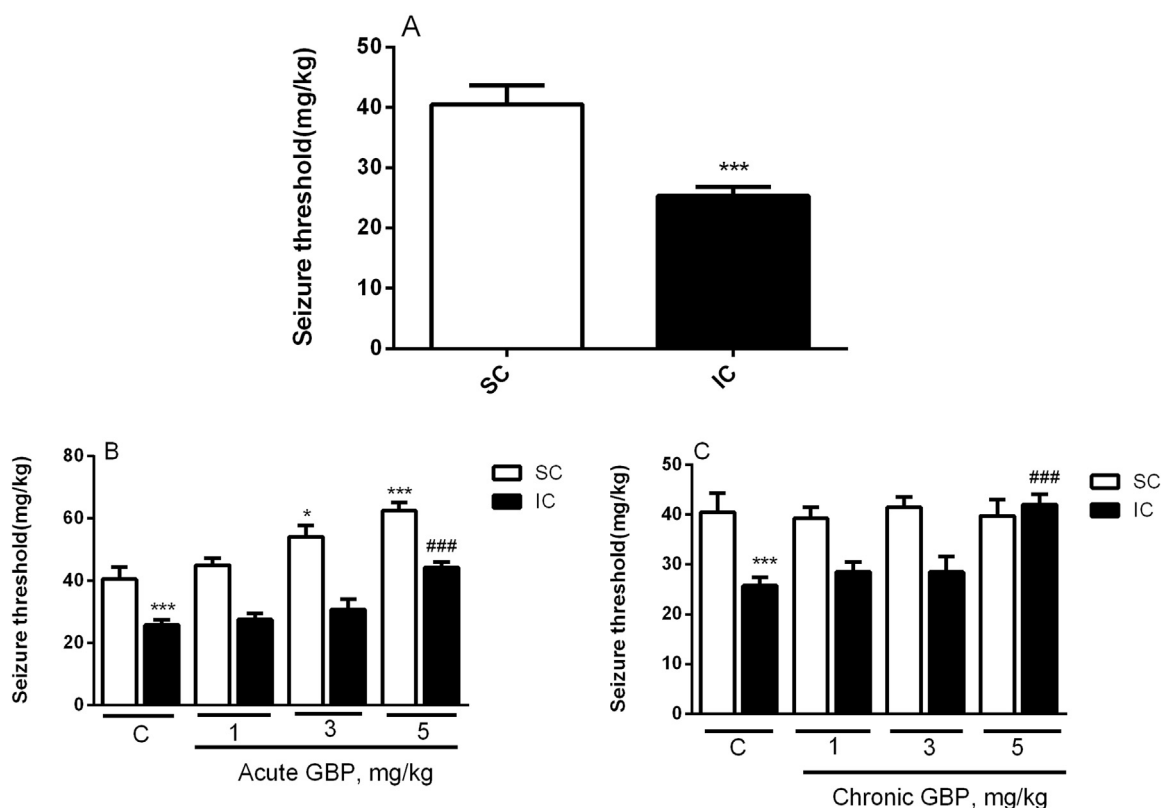There are significant differences in the grooming activity time in the

Fig. 1. Effects of different housing conditions (A), acute (B) and chronic (C) administration of GBP (1, 3 and 5 mg/kg, i.p.) on the seizure threshold in PTZ-induced convulsion). Values are presented as mean ± S.E.M, n =7. *$P < 0.05$ and ***$P < 0.001$ compared to control SC mice, ###$P < 0.001$ compared to control IC mice.

splash test in both the acute and chronic scheduled tests between IC and SC groups (F (7, 50) =18, $P < 0.001$, Fig. 5A, acute treatment and F (7, 48) =21.42, $P < 0.001$, Fig. 5B, chronic treatment). As shown in Fig. 5, IC significantly decreased the grooming activity time in comparison to SC mice ($P < 0.001$). Tukey's test showed that grooming activity time was significantly increased by treatment with acute and chronic GBP 5 mg/kg in IC mice compared to the IC control counterpart ($P < 0.001$ and $P < 0.01$, respectively). Doses 1 and 3 mg/kg GBP failed to create significant grooming activity differences in IC mice ($P > 0.05$). In addition, there are no significant differences among SC groups ($P > 0.05$).

3.6. Effects of the different housing conditions and GBP treatments on the aggressive behavior

Results of one-way ANOVA for resident-intruder test showed that there are significant differences in aggressive behavior in both acute and chronic scheduled tests between IC and SC groups (F (7, 50) =27.2, $P < 0.001$, Fig. 6A, acute treatment and F (7, 50) = 40.80, $P < 0.001$, Fig. 6B, chronic treatment). As shown, resident IC mice displayed a higher duration of attack time against intruder mice than SC mice ($P < 0.001$). Moreover, administration of acute and chronic GBP 5 mg/kg significantly attenuated the duration of the attacks by IC mice in comparison with IC control counterpart ($P < 0.01$ and $P < 0.05$, respectively), whereas GBP 1 and 3 mg/kg either acute or chronic failed to reduce this behavior ($P > 0.05$). Statistical analysis revealed that there
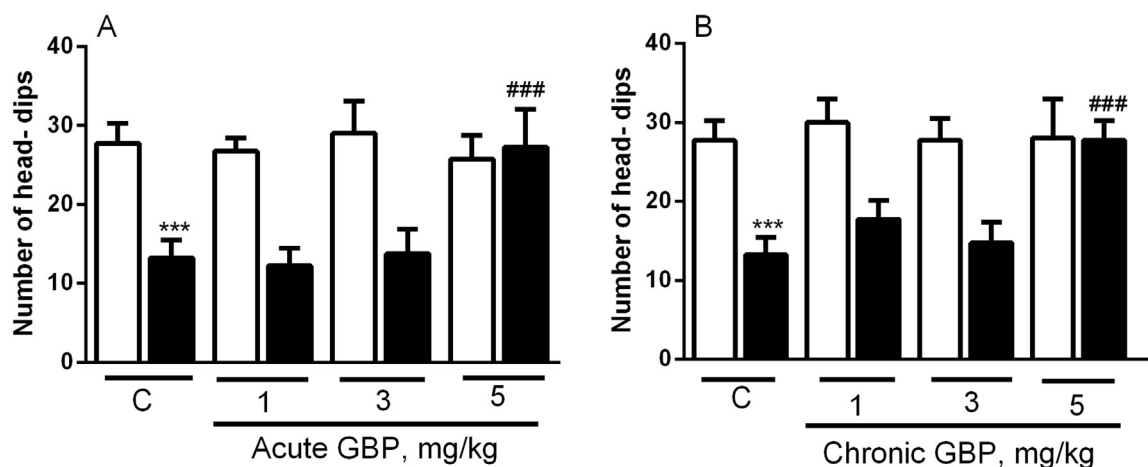

Fig. 2. Effects of different housing conditions and also acute and chronic administration of GBP (1, 3 and 5 mg/kg) on the number of head-dips in the hole board test. Values are expressed as the mean ± S.E.M, n=7. ***$P < 0.001$ compared to control SC group, ###$P < 0.001$ compared with the control IC group.
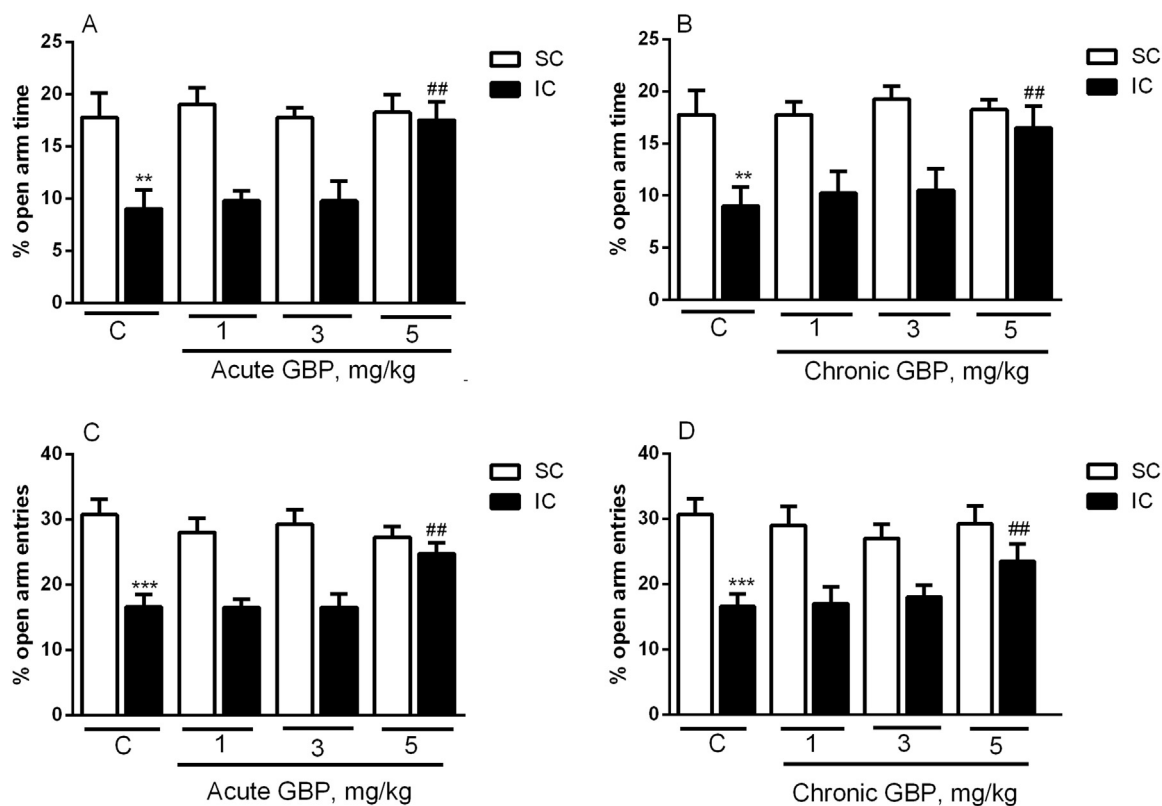
Fig. 3. Effects of different housing conditions and GBP treatments on the percent of open arm time (A) and percent of open arm entries (B) in the EPM. Values are presented as mean ± S.E.M, n=7. **$P < 0.01$ and ***$P < 0.001$ compared to control SC mice, ##$P < 0.01$ compared to control IC mice.

are no significant differences amongst control, acute and chronic treatments in SC mice ($P > 0.05$).

3.7. Effects of different housing conditions and GBP treatments on the immobility time in the FST and TST

ANOVA investigation demonstrated that there are significant differences between the groups in the immobility time of FST in GBP treatments (acute and chronic) in two groups of animals (SC and IC) (F (7, 48) =15.45, $P < 0.001$, Fig. 7A, acute treatment and F (7, 48) =10.20, $P < 0.001$, Fig. 7B, chronic treatment). Findings showed that SIS significantly increased the immobility time in IC mice in comparison with SC mice in the FST ($P < 0.01$). Tukey's test showed that administration of acute (but not chronic treatment) GBP 5 mg/kg significantly decreased the immobility time in IC mice when compared to IC control group ($P < 0.05$). However, administration of doses 1 and 3 mg/kg of GBP in acute as well as chronic regimen had no significant effect on immobility time of FST in IC mice ($P > 0.05$). Furthermore, no statistical significant differences were seen in SC mice in the FST between GBP groups with control counterpart ($P > 0.05$).

On the other hand, in TST there are significant differences between the groups in the immobility time in acute and chronic GBP treatments SC and IC mice (F (7, 50) =121.1, $P < 0.001$, Fig. 8A, acute treatment and F (7, 47) =173.3, $P < 0.001$, Fig. 8B, chronic treatment). Results demonstrate that SIS significantly increased immobility time in comparison with social condition housing ($P < 0.001$). Tukey's test showed that administration of acute (but not chronic) GBP 5 mg/kg significantly reduced the immobility time of IC animals when compared to control IC counterpart ($P < 0.001$). However, administration of lower doses in acute treatments and all applied doses of GBP in chronic treatments had no significant effect in immobility time of IC mice in the TST ($P > 0.05$). In addition, no significances were observed in SC mice between GBP and control mice ($P > 0.05$).

4. Discussion

In this study, we showed that early SIS provoked a variety of behavioral abnormalities relevant to depression, anxiety and aggressiveness. In addition, behavioral alterations in adult IC mice correlated with increased seizure susceptibility to PTZ. Results of this study also demonstrated that administration of GBP (acute and chronic) reversed the proconvulsant effects of early SIS as well as psychiatric comorbidities. However, the same treatment with GBP produced no behavioral alteration in the social condition counterparts.

Adolescence is considered as a sensitive period of life of which exposure to chronic stress in this period potently increases the risk of psychiatric difficulties as well as seizure disorders in later life (Amiri et al., 2014; Andersen and Teicher, 2008; Huang, 2014). Consistent with previous studies, our results revealed that 28 days of SIS (PND: 21–49) exerted proconvulsant effects along with psychiatric comorbidities including depressive, aggressive and anxiety-like behaviors in IC mice (Fone and Porkess, 2008; Matsumoto et al., 2003). It is well-accepted that psychiatric comorbidities in people with seizure disorders not only affect the quality of life but also, complicate the drug therapy (Maguire and Salpekar, 2013). Social environments such as social isolation, play a key role in triggering the incidence of psychiatric comorbidities in patients with epilepsy (McCagh et al., 2009). Our results revealed that early SIS is able to induce co-occurrence of psychiatric difficulties with seizure disorders suggesting that early SIS is a useful animal model to investigate the underlying mechanisms involved in psychiatric complications in seizure disorders.

Considering that depression is the most frequently occurring comorbid psychiatric disorder in patients with epilepsy, we showed that IC mice exhibited behaviors related to core symptoms of human depression including self-care difficulties and despair behavior. An increase in the immobility time in the FST and TST has been considered as an index reflecting the despair behavior in mice (Castagné et al., 2011). Also, a decrease in the grooming activity time
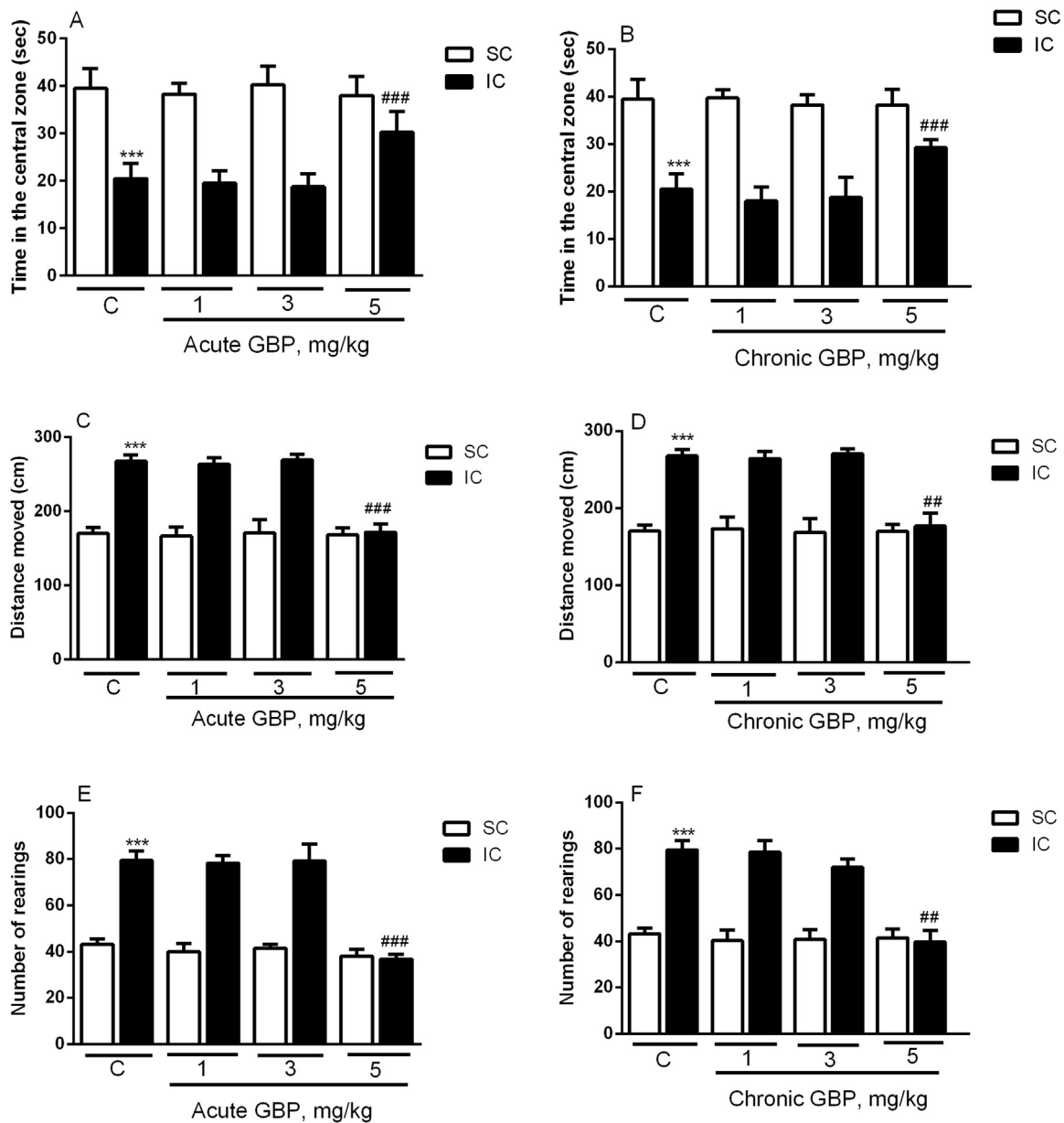
Fig. 4. Effects of different housing conditions as well as acute and chronic administration of GBP (1, 3 and 5 mg/kg) on the recorded parameters in the OFT: The time spent in the central zone (A), the distance moved (horizontal activity) (B) and number of rearings (vertical activity) (C). Values are expressed as the mean ± S.E.M, n=7. ***P < 0.001 compared with SC control group, ##P < 0.01 and ###P < 0.001 compared with the IC control group.
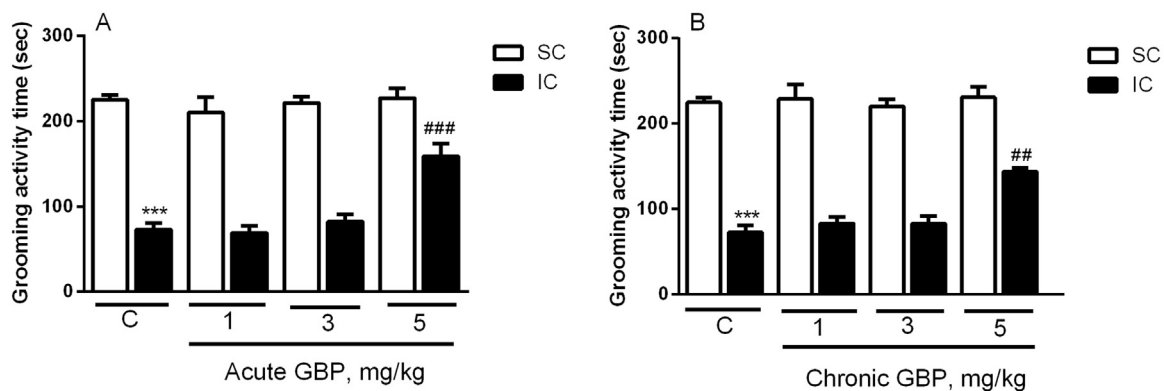

Fig. 5. Effects of different housing conditions and also acute and chronic administration of GBP (1, 3 and 5 mg/kg) on grooming activity time in the splash test. Values are expressed as the mean ± S.E.M, n=7. ***P < 0.001 compared with SC control group and ##P < 0.01 compared with the IC control mice.
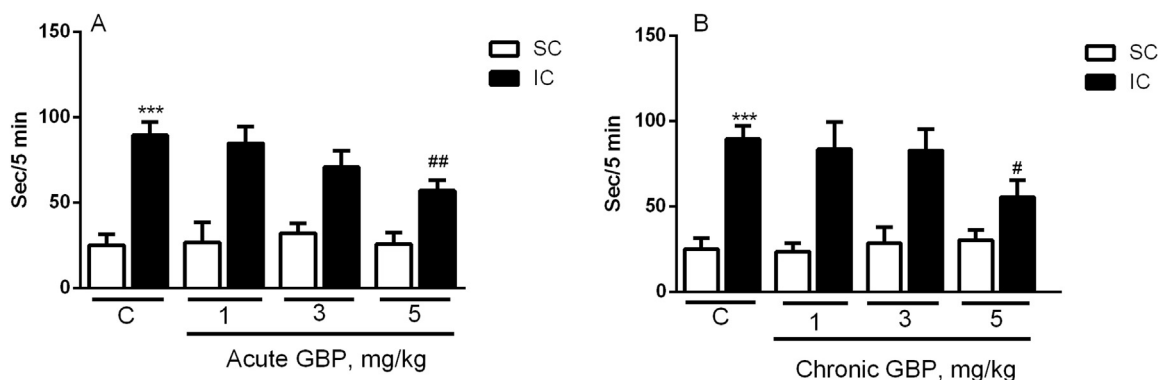
Fig. 6. Effects of different housing conditions (SC and IC) with acute and chronic administration of GBP (1, 3 and 5 mg/kg) on the duration of the attacks in resident-intruder test: Values are expressed as the mean ± S.E.M, n=7. ***P < 0.001 compared to SC control group, ###P < 0.001 compared to IC control counterpart.

in the splash test has recently been reported as an appropriate index to evaluate the motivation state in both mice and rats (Surget et al., 2008; Willner, 2005). There are pieces of evidence suggesting that GBP possesses therapeutic properties in patients with unipolar and bipolar disorders and improved mood status in epileptic patients (Dimond et al., 1996; Piedad et al., 2012). In this study, our results demonstrated that administration of both acute and chronic doses of GBP reversed the SIS-induced depressive-like behaviors in IC mice while the same treatments did not alter the behavior of SC animals. Acute (and not chronic) treatment with GBP reduced the immobility time in the FST and TST indicating that GBP is able to decrease despair behavior in IC mice. Also, both acute and chronic GBP increased the grooming activity time in the splash test suggesting that GBP promotes self-care behavior in IC animals. Using FST, the antidepressant-like effect of GBP on non-stressed rats was reported in a recent study and similar to our results the authors observed that the effect of GBP appeared after 21 days of chronic treatment (Czubak et al., 2008). Anxiety is also a prevalent comorbid disorder in people suffering from seizure disorders and there are numerous ways anxiety may be associated with epilepsy (for review see Goldstein and Harden (2000)).

Evidence from numerous clinical and preclinical studies indicate that GBP has anxiolytic effects (Perucca and Mula, 2013). In this study, we showed that both acute and chronic GBP is able to modulate the SIS-induced anxiety-like behaviors in the HBT and OFT as well as EPM while such effects were not observed in SC animals. In the OFT, IC mice exhibited hyper-locomotion (increase in both vertical and horizontal activity) along with a decrease in the time spent in the central zone of apparatus indicating increased anxiety-like behaviors in response to a novel environment (Kulesskaya and Voikar, 2014). Administration of GBP reversed the anxiety -like behaviors and normalized the hyper locomotion in IC mice as indicated by an increase in the percentage of entries and spent time in the open arms in the EPM. Gabapentin also

reversed the anxiety-like behavior of IC mice in the HBT by increasing the number of head-dips as an index of emotional response relevant to anxiety (Takeda et al., 1998).

Although the exact mechanism(s) through which GBP exerts its therapeutic effects is not clear, it has been reported that this drug modulates the high voltage-gated calcium channels through binding to the α2-δ subunit. Accumulating evidence suggests that therapeutic effects of GBP are associated with modulation of excitatory neurotransmission (Sills, 2006). In this regard, recent studies showed that GBP effectively prevents hyper-excitability following cortical damage in neonatal mice and protects against epileptiform activity in adulthood (Andersen and Teicher, 2008; Noebels et al., 2012). Similar results were reported by Rossi and colleagues indicating that GBP exerts protective effects against neuronal damages induced by lithium-pilocarpine seizures (Rossi et al., 2013). In this respect, previous research has shown that while early SIS enhances excitatory neurotransmission (Whitaker et al., 2013), it also diminishes the activity of inhibitory neural systems (Matsumoto et al., 2007). Thus, the modulatory effects of GBP may be associated with the attenuation of excitatory conditions in the brain. Additionally, the results of our study revealed that GBP exerts its antidepressant-like effect mainly by an increase in the climbing activity and not swimming time in the FST. It is well documented that climbing activity in the FST is mainly associated with an increase in the adrenergic neurotransmission indicating that the antidepressant-like effect of GBP is partly mediated by the adrenergic system (Detke et al., 1995; Schramm et al., 2001). In this case, GBP failed to produce an antidepressant-like effect when animals were pretreated by propranol (a beta adrenergic receptor antagonist) (data not shown). Surprisingly, recent research has shown that GBP effectively alters the adrenergic and glutamatergic neurotransmission in the locus coeleurus (LC), a brain structure disrupted in the depressed patients, and plays a key role in the regulation of emotions and mood (Bernard
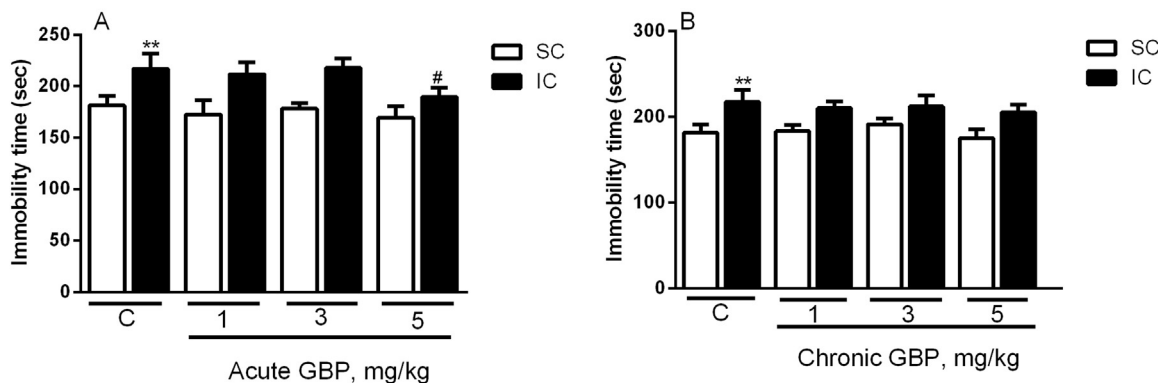

Fig. 7. Effects of SC and IC and also acute or chronic administration of GBP (1, 3 and 5 mg/kg) on the immobility time is FST. Values are expressed as the mean ± S.E.M, n=7. **P < 0.01 compared with SC control group and ##P < 0.01 compared with the IC control counterpart.
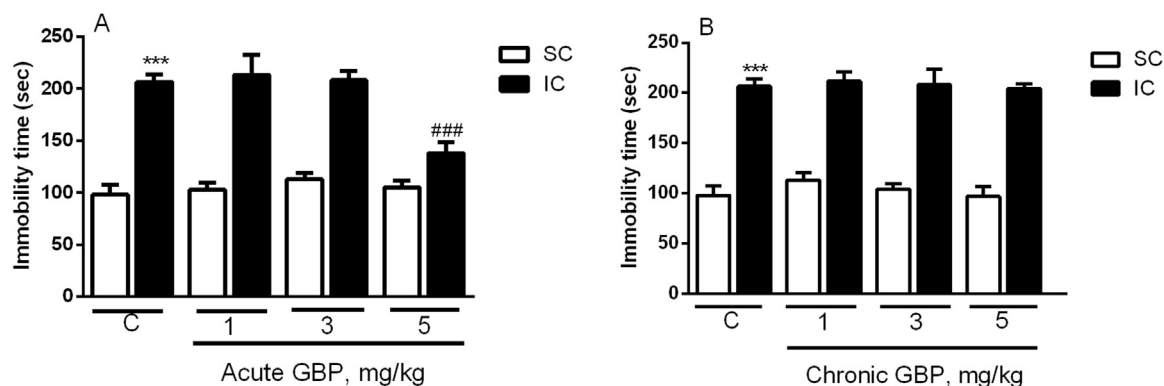
Fig. 8. Effects of different housing conditions and also acute or chronic administration of GBP (1, 3 and 5 mg/kg) on the immobility time is TST. Values are expressed as the mean ± S.E.M, n=7. ***P < 0.001 compared with SC control group and ###P < 0.001 compared with the IC control counterpart.

et al., 2011; Suto et al., 2014).

In regards to the anti-epileptic properties of GBP, higher doses of acute GBP produced anticonvulsant effects in SC mice while lower doses exerted the same effects in the IC mice. Although early SIS increased the susceptibility of adult IC mice to the PTZ, both acute and chronic administration of GBP reversed this effect to a greater extent. Similar treatment had no effect on the seizure activity in the SC mice indicating that sub-effective doses of GBP in the SC mice (physiological conditions) exerts potent anticonvulsant effects in the IC mice (chronic stress conditions). Our results were in line with recent findings reporting the protective effects of GBP against detrimental effects of seizure activity mostly during brain development (Cilio et al., 2001; Goldstein and Harden, 2000). In addition, we found that the administration of GBP from adolescence (PND: 28) to adulthood (PND: 50) produced no adverse effects in SC mice. Also, we did not observe behaviors such as aggression, anxiety, motor function and seizure activity in the SC mice indicating that GBP is a safe drug when administered chronically during developmental stages of the brain. Aggression and violent behavior were also reported in people with seizure disorders (Kanner, 2004; Mori et al., 2014). Our results demonstrated that GBP effectively succeeded to mitigate the offensive and aggressive behaviors of IC mice in the resident-intruder test. It has been well accepted that SIS induces aggressiveness in rodents (Toth et al., 2012). As it is believed that GBP modulates the neurotransmission mostly under excitatory conditions, it is possible that the modulatory effects of GBP on the behavioral profile of IC mice are related to its effect against hyper-excitation following early SIS. In consistent with our results, Cilio et al. found that acute or daily administration of GBP had no effect on the cognitive abilities of animals in all ages and also GBP showed protective effects against kainite-induced seizures (an hyper-excitation condition) and aggressiveness in animals (Cilio et al., 2001).

It is important to note that early SIS has a great negative effect on different neurotransmission systems, and is able to induce a variety of behavioral abnormalities in rodents. In this regard, our recent studies showed that SIS is able to enhance excitatory neurotransmission through N-Methyl-D-aspartate (NMDA) receptors and nitrergic system. These effects of SIS on excitatory neurotransmission are associated with behavioral abnormalities and increased seizure susceptibility in rodents (Amiri et al., 2016; Haj-Mirzaian et al., 2016). Furthermore, recent studies have reported that early SIS is able to suppress the inhibitory neurotransmission through the downregulation of neurosteroid biosynthesis, which results in GABAergic neurotransmission deficits (Agís-Balboa et al., 2007; Pibiri et al., 2008). In this regard, Matsumoto and colleagues have reported that dysfunction in neurosteroid biosynthesis is associated with affective behavioral deficits and susceptibility to seizure in socially isolated mice (Matsumoto et al., 2003, 2007). Thus, both excitatory and inhibitory neurotransmission are affected by SIS and play a role in the pathophysiology of behavioral

deficits and increased seizure susceptibility in SIS rodents.

5. Conclusion

Taken together, in this study we found that chronic administration of GBP during adolescence not only had no adverse effect on the behavioral profile of SC mice but also reversed the seizure susceptibility and psychiatric comorbidities in IC mice. Results of this study provided preliminary evidence that GBP (at lower doses than therapeutic dose) despite having anti-epileptic properties also possesses protective effects against long-term consequences of psychological stress. In future studies, we are interested in investigating through which underlying mechanisms GBP exerts the protective effects against negative consequences of early SIS.

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