

Prenatal Acute Stress Attenuated Epileptiform Activities in Neonate Mice

Behnam Heshmatian, Ph.D., Shiva Roshan-Milani, Ph.D., Ehsan Saboory, Ph.D.*

Physiology Department, School of Medicine, Urmia University of Medical Sciences, Urmia, Iran

* Corresponding Address: P.O.Box: 5756115111, Physiology Department, School of Medicine, Urmia University of Medical Sciences, Urmia, Iran
Email: saboory@umsu.ac.ir

Received: 26/Aug/2009, Accepted: 6/Apr/2010

Abstract

Objective: Development of the central nervous system (CNS) is dependent on interactions between genetic and epigenetic factors, some of which could affect the susceptibility of the developing brain to damaging insults. Gestational stress has been shown as a potential factor associated with higher risk of developing certain neurological and psychiatric disorders. This study tested the hypothesis that maternal stress influences the risk of epilepsy in offsprings.

Materials and Methods: Pregnant mice were exposed to restraint stress twice a day for three days at the start of the last week of gestation. Ten days after birth, the intact hippocampi of the newborn mice were excised and prepared for investigation. The hippocampi were bathed in low magnesium artificial cerebrospinal fluid to induce field potential, and the subsequent spontaneous seizure-like events of the CA1 neurons were recorded. Plasma corticosterone was measured using a commercial radioimmunoassay (RIA) kit and the values were expressed as $\mu\text{g}/100\text{ ml}$.

Results: Both the number of recurrent seizures and the duration of seizure activity were reduced in the stressed group compared to the controls ($p < 0.001$). Stress induced a significant rise in serum corticosterone levels in both pregnant mice and in their newborn pups ($p < 0.001$).

Conclusion: These findings suggest that acute prenatal stress, which may mimic acute stress in human pregnancy, is a likely factor affecting seizure control in childhood temporal lobe epilepsy. The underlying inhibitory mechanism may be an increase in the level of neurosteroids both in the blood and the brain.

Keywords: Epilepsy, Stress, Prenatal, Neonatal, Hippocampus, Mice

Yakhteh Medical Journal, Vol 12, No 1, Spring 2010, Pages: 81-86

Introduction

The major pathway implemented in coordinating the consequences of stress in most mammalian species is the hypothalamic-pituitary-adrenal (HPA) axis (1, 2). Neurons that express corticotrophin-releasing factor (CRF) within the paraventricular nucleus of the hypothalamus are a well known element in stress response. The typical biochemical cascade in response to stressors involves the release of CRF from the paraventricular nucleus into the hypophysial portal system, which in turn stimulates the release of adrenocorticotrophic hormone (ACTH) into the systemic circulation (2, 3). Circulating ACTH can then interact with receptors expressed in the adrenal cortex to stimulate the synthesis of steroids (steroidogenesis) and produce a marked elevation in plasma glucocorticoids (2, 4). Neural plasticity changes in any of the key nu-

clei within the HPA circuitry can occur after the primary exposure to a variety of stressors (5, 6). These neuroplastic changes can be both adaptive and beneficial in nature; however, prolonged exposure to stressful stimuli could induce pathological changes within this circuit and lead to longer lasting or elevated HPA activity. Alterations to the functions of the HPA axis occur in several clinical conditions, including epilepsy (7).

Emotions can precipitate seizures (8). It has been reported that stress-induced events such as fear, worry and frustration are commonly associated with elevated seizure occurrence (9) and several studies report that stress exacerbates epileptic seizures (10). The definition of stressful events ranges from retiring from a 45 year-long employment at an organization to police arrest for a crime, or experience of a painful procedure. Methods to at-

tenuate or cope with stress have been suggested as ways to control seizures in epilepsy patients (11, 12). Prolonged periods of stress markedly reduce the efficacy of various anticonvulsant drugs (13). Severe emotional and intellectual stresses may provoke acute seizures in people with no previous history of seizures, but it is unknown to what extent severe stress increases the risk of recurrent, unprovoked epileptic seizures (14). Although stress can influence the number of seizures reported by patients (2), the precise mechanism underlying the relationship between stress and epilepsy remains unclear.

The effect of stress on epilepsy is controversial. There are reports showing the opposite of previously described effects of stress on epilepsy. Although its underlying mechanisms are poorly understood, it is well recognized that emotional stress can be a factor affecting seizure control in temporal lobe epilepsy and other seizure syndromes. Moreover, experimental stressors, including swim stress, have anticonvulsant effects in animals (15). Both proconvulsant (16, 17) and anticonvulsant (15) effects of stress have been reported according to exacting experimental conditions.

In different experimentally-induced epilepsy models, various stressors were able to influence the responsiveness of an animal to several antiepileptic compounds (18). Consistent with these results, exposure threshold to different kinds of acute stressors such as nociceptive stimulation is markedly lower than the threshold required to evoke seizures in lithium-pilocarpine-treated rats (1). Taken together, profound neuroelectrical characteristic changes may result from exposure to various stressful conditions, which in turn can increase the susceptibility of neuronal ensembles to evoke epileptiform discharges and alter the vulnerability of hippocampal neurons to a variety of neurological insults (2). The majority of experiments on the effects of gestational stress on offspring development and behavior have been carried out in rats. There are also a few studies on rhesus and squirrel monkeys largely supporting the findings already described in rats (19). Exact comparative studies using [3H] thymidine autoradiography have shown that developing brain structures of the rat closely resemble those of the human, especially in early embryonic stages (19). However, at the end of gestation (23 days) the histological details in the rat brain are similar to those of a 16-17 week old human fetus (20). Thus, some neuronal systems present at birth in humans continue to develop in the rat for several days or weeks after parturition. Gestational stress has been associated with a higher risk of develop-

ing certain neurological and psychiatric disorders (19). Prolonged effects of stress have been linked to alterations in the activity of the HPA axis (21). Moreover, stress during pregnancy may alter brain development, and lead to altered synaptic connectivity and persistent brain function deficits (22).

Based on these studies, we propose the hypothesis that gestational maternal stress could be a risk factor for the development of perinatal brain lesions and susceptibility to epilepsy. To test this hypothesis, pregnant mice were exposed to restraint stress twice daily for the first three days of their last gestational week. We then used the intact neonatal mouse hippocampi to record the epileptiform neuronal activity. The result of this study contradicted our proposal and revealed an anticonvulsant effect of prenatal restraint stress in the neonatal mice.

Materials and Methods

Animals and the mating procedure

In this study, we used 48 virgin female C57BL/6 mice (Pasteur Institute, Tehran, Iran). At the time of arrival in the laboratory, all mice were ten weeks old and housed in groups of six per cage in our animal facility. They were kept in standard conditions as follows: with 12-hour light/dark cycles, in a $22 \pm 2^\circ\text{C}$ temperature range, and had food and water ad libitum. All experimental protocols and procedures complied with codes of the 1975 Declaration of Helsinki as reflected in the guidelines of the Medical Ethics Committee, Ministry of Health, I.R. Iran. All females were mated at 12 weeks of age with a sexually experienced male of the same genotype. Each female was paired with one male at 0900 hours, and was checked for plugging at 1500 hours. Plugged females were then immediately transferred into individual cages for their entire length of gestation. If a plug was not observed, the animal was returned to her home cage until the next morning for a new mating procedure. Pregnant mice were divided into two groups: controls and stressed ($n=24$).

Restraint stress procedure

The stressed group was subjected to a stressful procedure. Pregnant mice were exposed to restraint stress at days 15, 16 and 17 of their gestation (E15, E16 and E17 respectively). The stress exposure consisted of a 2 hour session of immobilization, twice a day at 0800 and 2000 hours. The animals were restrained in a Plexiglas tube. At the end of the stress procedure (E17), both stressed and control animals were further divided into four subgroups ($n=6$) according to the experimental days as follows: E18, P2, P6 and P10 (Table 1).

Table 1: grouping of animals in present study

groups	Sub-groups all in same sample size (n=6) *			
Control mother n=24	cmE18	cmP2	cmP6	cmP10
Stressed mother n=24	smE18	smP2	smP6	smP10
Control pups	-----	cpP2	cpP6	cpP10
Stressed pups		spP2	spP6	spP10

* *cm*: control mother, *sm*: stressed mother, *cp*: control pup and *sp*: stressed pup

At day E18 both control and stressed mothers in the E18 subgroup were decapitated at 0830 hours and their trunk blood samples were collected in 1.5 ml of EDTA-coated microcentrifuge tubes. These blood samples were kept on ice and later centrifuged for 20 minutes at 9000 rpm at 4°C. Plasma was transferred to a clean 1.5 ml microcentrifuge tube and was frozen at -20°C until its corticosterone level was determined (22). Plasma corticosterone was measured using a commercial radioimmunoassay (RIA) kit (ICN Biochemical, Costa Mesa, CA, USA) and the values were expressed as µg/100 ml. Trunk blood collection was repeated three further times in the P2, P6 and P10 in all of the respective subgroups. Pups from the P10 subgroup were decapitated and their hippocampi were extracted. The hippocampi were resected intact and bathed in low magnesium artificial cerebrospinal fluid (low Mg²⁺ ACSF) to induce the recorded spontaneous seizure-like events (SLE) from CA1 neurons. Only one male pup from each mother was subjected to the experiment in this study; the remaining were included in another behavioral experiment.

Dissection

C57/BL C57BL/6 mice from the P10 subgroup were anesthetized with halothane and decapitated. Their brains were extracted and placed in cold (2-5°C) oxygenated (95% O₂, 5% CO₂) ACSF containing (in mM) 123 NaCl, 2.5 KCl, 1.5 CaCl₂, 2 MgSO₄, 25 NaHCO₃, 1.2 NaH₂PO₄ and 15 glucose. Each hippocampus and septum was removed as a block dissection. The hippocampi were then transferred into an oxygenated ACSF at room temperature for a minimum of 1.5 hours before being placed in the recording chamber (23, 24).

Experimental environment

Epileptiform activity was achieved by perfusing the hippocampus and septal tissues with low Mg²⁺ ACSF containing (in mM): 123 NaCl, 5 KCl, 1.5 CaCl₂, 0.2 MgSO₄, 25 NaHCO₃, 1.2 NaH₂PO₄ and 15 glucose. Humidified warm oxygen flowed over the low Mg²⁺ ACSF in a 32°C chamber where this

oxygenated solution surrounded the tissues at a flow rate of 5 ml/min.

Electrophysiology

Recordings were performed from the hippocampus CA1 pyramidal neuron layer as the DG-CA3 region was positioned on a nylon mesh with the CA1 region facing upwards. A Grass S44 stimulator (Grass Technologies, USA) with a bipolar electrode to stimulate the Schaffer collaterals (1-15 V, 0.1 ms) was used along with extracellular glass electrodes (3-5 mΩ) containing 150 mM NaCl positioned at various locations in order to yield maximal evoked and spontaneous field potential amplitudes in the hippocampal CA1 stratum pyramidale neurons. Input/output relations were recorded for stimulating pulses of ten different intensities evoking subthreshold to suprathreshold population responses (23).

All single-site recordings of seizure-like events were obtained from the middle of the hippocampus, filtered with a 1 kHz low-pass filter unless otherwise stated, amplified, and recorded at a 2 kHz sampling rate using a HEKA EPC 10 amplifier (HEKA Electronics, Wiesen straze city, Germany). The signals were digitized using the ITC-1600 16-bit data acquisition system (InstruTECH, USA). Data visualization was achieved by using the Fitmaster 2.1 (HEKA Electronics, Wiesen straze city, Germany). The SLE was quantified by determining the mean numbers of recurrent seizures, seizure durations, seizure amplitudes, early seizure frequencies, late seizure frequencies, and intraburst frequencies. Number of recurrent seizures is the number of occurred SLEs during a 10-minute recording time. Seizure duration is the time elapsed between the start of the early phase and the termination of the late phase, and does not include the transition.

Statistical analysis

Data were analyzed using unpaired t tests. All results are shown as mean values ± standard error of the mean (SEM). Results were considered to be statistically significant at p < 0.05.

Results

Effects of restraint stress on corticosterone levels in C57BL/6 mice

The effects of restraint stress on corticosterone blood levels were determined in both pregnant and post-delivery mice, as well as in newborn pups. Stress significantly increased corticosterone levels

in the E18 subgroup and both in the mother and pups in the P2, P6 and P10 subgroups (Table 2).



Fig 1: Electrophysiologic recordings showing spontaneous SLE in the intact hippocampus induced by low Mg²⁺ ACSF perfusion. Top: SLE recorded from a 10 days-old mouse in the control group and (bottom) from a 10 days-old stressed mouse. Note the decrease in seizure duration, percent of seizure in total recording time as well as the SLE amplitude in the stressed animal.

Table 2: corticosterone levels in mothers and pups of control and stressed mice

groups	Corticosterone levels (µg/100 ml) In different subgroups			
	E18	P2	P6	P10
Control mother	38.93 ± 1.4	15.8 ± 1.2	7.24 ± 1.1	2.31 ± 0.6
Stressed mother	68.96 ± 2.12	32.1 ± 2.3	14.56 ± 1.85	5.16 ± 1.1
	p<0.001	p<0.001	p<0.001	p<0.001
Control pups	Not measured	2.3 ± 0.15	0.13 ± 0.013	0.087 ± 0.01
Stressed pups		7.19 ± 0.38	0.41 ± 0.05	0.27 ± 0.04
		p<0.001	p<0.001	p<0.001

Consistency and stability of SLE

Control experiments were performed on twelve hippocampi to determine the consistency and stability of the seizure activity induced by perfusing the intact hippocampus with low Mg²⁺ ACSF. In each of the 12 different hippocampi, the recurrent seizure patterns were consistent and did not change their electromorphological appearance. Recurrent seizures had a mean duration of 70.62 ± 3.4 seconds, and there were usually 3 ± 0.1 seizures every 10 minutes. Recurrent seizure events started approximately 8 ± 0.92 minutes after the administration of low Mg²⁺ ACSF and lasted for approximately 142 ± 8.55 minutes in a consistent and stable manner.

Single maximal postsynaptic population spikes were evoked by stimulating the Schaffer collaterals to monitor the viability of the hippocampi. Experiments were terminated if there was a 75% decrease in the population spike amplitude relative to its initial value. A single seizure consisted of an early and a late phase. The early phase consisted of a series of

single spikes with a mean frequency of 1.88 ± 0.06 Hz. The late phase comprised of multiple bursts with a mean burst frequency of 4.38 ± 0.25 Hz. The intraburst frequency was defined as the number of spikes per second contained in a single burst occurring during the late phase of a seizure. The mean intraburst frequency was 23.47 ± 1.21 Hz.

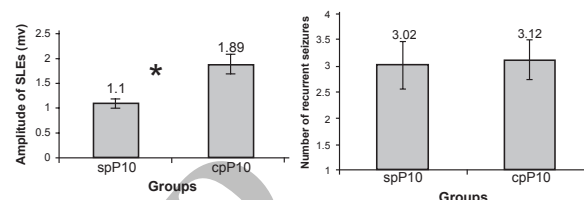


Fig 2: Effect of gestational restraint stress on Seizure-like events recorded from the *in vitro* intact mouse hippocampus. Left: amplitude of SLEs significantly decreased in spP10 (p<0.01). Right: number of recurrent seizures in 10 minutes did not change significantly (spP10: stressed pup and cpP10: control pup in the P10 subgroup).

Effects of restraint stress on seizure characteristics in C57BL/6 mice

Properties of SLEs were markedly affected by the restraint stress in this study. Seizure durations, seizure percentages and frequencies in all phases of SLEs, as well as intraburst frequencies were significantly decreased (Fig 1 and Table 3).

Table 3: Stress actions on SLEs recorded from mouse whole hippocampus in P10 subgroups of control and stressed male pups, *In Vitro*.

SLE index	Control animal	Stressed animal	P value
Seizure duration (S)	70.62 ± 3.4	43.87 ± 2.48	0.0001
Seizure percent (%)	41.94 ± 2.05	28.86 ± 0.96	0.0001
Early phase frequency (Hz)	1.88 ± 0.06	1.39 ± 0.11	0.001
Late phase frequency (Hz)	4.38 ± 0.25	2.35 ± 0.11	0.0001
Inraburst frequency (Hz)	23.47 ± 1.21	16.62 ± 0.86	0.0001

Prenatal restraint stress affected seizure-like events in the intact murine hippocampi. The amplitude of SLEs significantly decreased in the P10 subgroup (p<0.01), but the number of recurrent seizures did not change significantly (Fig 1, 2).

Discussion

In the presented study we subjected normal pregnant mice to an acute restraint stress. Then, the pups were studied for SLEs in low Mg²⁺ ACSF, which induces seizure in the intact preparations of whole murine hippocampus. The most important finding of this study is that prenatal acute stress has antiepileptic effects on hippocampal

seizure activity in newborn pups. Blood levels of corticosterone significantly increased in both the stressed mice and their pups (Table 2). Our observation that acute restraint stress increases the seizure threshold in mice is consistent with several previous studies demonstrating that acute stress is associated with anticonvulsant effects against seizures induced by pentylenetetrazol (PTZ) and other GABAA receptor antagonists (15). In this study, a 3.1-fold elevation in plasma corticosterone levels was seen at the time of seizure protection in the P10 pups which previously experienced prenatal stress. Similar increases in plasma levels of corticosterone and related steroids such as 5 α -dihydrodeoxycorticosterone (5 α -DHDOC) and allotetrahydrodeoxycorticosterone (THDOC), a GABAA receptor-modulating neurosteroid with anticonvulsant properties, have been observed previously in response to other acute stressors including foot shock (15, 25). It has been reported that both the elevation in seizure threshold and the rise in THDOC can be eliminated by pretreatment with finasteride (a 5 α -reductase inhibitor); this is consistent with the possibility that the anticonvulsant effect is mediated by 5 α -reduced neurosteroids (15, 26). In rodents, finasteride blocks both the type I 5 α -reductase isoenzyme, which is the predominant form in the brain, as well as the type II form in the prostate and gonads (15, 27). Consequently, the neurosteroids responsible for stress-induced seizure protection could be synthesized in peripheral tissues and then transported by the circulation to the brain, or they could be produced locally in the brain. In fact, because of local biosynthesis, brain THDOC levels after stressful events may be substantially higher than those of plasma (15). Because the synthesis of deoxycorticosterone (DOC), the precursor of THDOC, is increased by stress, and THDOC is well recognized for having anticonvulsant properties, THDOC is a prime candidate for being the neurosteroid responsible for the acute stress effects on seizures (15, 28).

Studies have reported that the endogenous opioid system is activated during exposure to stress. Some researchers have shown that endogenous opioids have a role in the anticonvulsant effects induced by certain kinds of stress (29). However, while endogenous opioids have inhibitory effects against seizures induced by GABA receptor antagonists and acute stresses appear to induce protective effects against these kinds of seizures, the contribution of endogenous opioids to these protective effects has not yet been elucidated (29, 30).

It is worthy to mention that several studies have reported that many stressors can potentiate animal

responsiveness to various convulsant compounds (18). In support of these findings is the observation that exposure to a variety of acute stressors (such as a 24-hour food deprivation or exposure to nociceptive stimulation) can significantly lower the threshold required to evoke limbic motor seizures in lithium- pilocarpine-treated rats (1, 2). It has also been reported that alterations of convulsive parameters and the involvement of opioid mechanisms in their mediation are critically dependent on the techniques used to induce stress (18). Our results differ from those of the aforementioned studies, possibly because of the differences between the stress models, the time of stress, as well as the seizure models. The time of stress, the stage of animal development at the time of experiencing stress, and the type of stressor and type of epilepsy model can be determinants in the presentation of stress effect on epilepsy. Unpublished data from our lab has revealed the opposite response of prenatal restraint stress on pilocarpine-induced epileptic behaviors in *in vivo* rats. Indeed, stressed rats presented with more severe tonic-clonic seizures than control rats.

Conclusion

Stressful events are associated with both excitatory and inhibitory effects on seizures depending on the exact experimental conditions used. It is well established that an imbalance between inhibitory and excitatory neural systems gives rise to epilepsy (23, 30). Some stressors can alter the excitability of neural pathways and thereby influence seizure susceptibility. Thus, the extent of seizure susceptibility during stress might therefore represent a balance between anticonvulsant (e.g. neuroactive steroids), and proconvulsant factors (e.g. glucocorticoids and CRH). Stress-induced seizures would therefore occur when the balance is shifted to favor the proconvulsant factors, surpassing the anticonvulsant action of endogenous neuroactive steroids (26). Despite the aforementioned statements, there are conflicting data on stress effect on seizures with some showing inhibitory effects (15) and others demonstrating excitatory effects (17). To address the discrepancies in findings with regard to the excitatory and inhibitory effects on seizures, we propose that additional investigations should be performed to examine the impact of different types of stressors on various animal models of epilepsy in various stages of life including prenatal, neonatal and adulthood.

Acknowledgments

This study was supported by the research council of

Urmia University of Medical Sciences, Urmia, Iran. We confirm that we have read the Journal's position on issues involving ethical publication and affirm that this report is consistent with those guidelines. The authors have no conflicts of interest to declare regarding the study presented in this paper and the preparation of this manuscript.

References

- Persinger MA, Stewart LS, Richards PM, Harrigan T, O'Connor RP, Bureau YR. Seizure onset times for rats receiving systemic lithium and pilocarpine: sources of variability. *Pharmacol Biochem Behav.* 2002; 71(1-2):7-17.
- Galic MA, Fournier NM, Martin LJ. Alpha2-adrenergic inhibition prevents the accompanied anticonvulsant effect of swim stress on behavioral convulsions induced by lithium and pilocarpine. *Pharmacol Biochem Behav.* 2004; 79(2): 309-316.
- Hermann BP, Seidenberg M, Schoenfeld J, Davies K. Neuropsychological characteristics of the syndrome of mesial temporal lobe epilepsy. *Arch Neurol.* 1997; 54(4): 369-376.
- Vazquez DM. Stress and the developing limbic-hypothalamic-pituitary-adrenal axis. *Psychoneuroendocrinology.* 1998; 23(7): 663-700.
- Mercier S, Frédéric, Canini, Buguet A, Cespuglio R, Martin S, Bourdon L. Behavioural changes after an acute stress: stressor and test types influences. *Behav Brain Res.* 2003; 139(1-2): 167-175.
- Sapolsky RM, Stein BA. Status epilepticus-induced hippocampal damage is modulated by glucose availability. *Neurosci Lett.* 1989; 97(1-2): 157-162.
- Galic MA. Elevated nociceptive thresholds in rats with multifocal brain damage induced with single subcutaneous injections of lithium and pilocarpine. *Percept Mot Skills.* 2004; 98(3 Pt 1): 825-826.
- Lathers CM, Schraeder PL. Stress and sudden death. *Epilepsy Behav.* 2006; 9(2): 236-242.
- Mattson RH. Emotional effects on seizure occurrence. *Adv Neurol.* 1991; 55: 453-460.
- Neufeld MY, Sadeh M, Cohn DF, Korczyn AD. Stress and epilepsy: the Gulf war experience. *Seizure.* 1994; 3(2): 135-139.
- Wiener P. Neuroactive steroids, relaxation, and seizure control. *Int J Neurosci.* 2003; 113(5): 631-639.
- Puskarich CA, Whitman S, Dell J, Hughes JR, Rosen AJ, Hermann BP. Controlled examination of effects of progressive relaxation training on seizure reduction. *Epilepsia.* 1992; 33(4): 675-680.
- Iancu I, Rosen Y, Moshe K. Antiepileptic drugs in posttraumatic stress disorder. *Clin Neuropharmacol.* 2002; 25(4): 225-229.
- Christensen J, Li J, Vestergaard M, Olsen J. Stress and epilepsy: a population-based cohort study of epilepsy in parents who lost a child. *Epilepsy Behav.* 2007; 11(3): 324-328.
- Reddy DS, Rogawski MA. Stress-induced deoxycorticosterone-derived neurosteroids modulate GABA(A) receptor function and seizure susceptibility. *J Neurosci.* 2002; 22(9): 3795-3805.
- Frye CA, Bayon LE. Prenatal stress reduces the effectiveness of the neurosteroid 3 alpha,5 alpha-THP to block kainic-acid-induced seizures. *Dev Psychobiol.* 1999; 34(3): 227-234.
- Edwards HE, Dortok D, Tam J, Won D, Burnham WM. Prenatal stress alters seizure thresholds and the development of kindled seizures in infant and adult rats. *Horm Behav.* 2002; 42(4): 437-447.
- Rae GA, Pereira GH, De-Lima TC. The influence of stress on convulsive parameters in the mouse. *Neurosci Biobehav Rev.* 1990; 14(4): 491-494.
- Weinstock M. Alterations induced by gestational stress in brain morphology and behaviour of the offspring. *Prog Neurobiol.* 2001; 65: 427-451.
- Bayer SA, Altman J, Russo RJ, Zhang X. Timetables of neurogenesis in the human brain based on experimentally determined patterns in the rat. *Neurotoxicology.* 1993; 14: 83-144.
- Van den Bergh BR, Mulder EJ, Mennes M, Glover V. Antenatal maternal anxiety and stress and the neurobehavioural development of the fetus and child: links and possible mechanisms. *Neurosci Biobehav Rev.* 2005; 29(2): 237-258.
- Rangon C, Fortes S, Lelièvre V, Leroux P, Plaisant F, Joubert C, et al. Chronic mild stress during gestation worsens neonatal brain lesions in mice. *J Neurosci.* 2007; 27(28): 7532-7540.
- Saboory E, Derchansky M, Ismaili M, Jahromi SS, Brull R, Carlen PL, et al. Mechanisms of morphine enhancement of spontaneous seizure activity. *Anesth Analg.* 2007; 105: 1729-1735.
- Derchansky M, Rokni D, Rick JT, Wennberg R, Bardakjian BL, Zhang L, et al. Bidirectional multisite seizure propagation in the intact isolated hippocampus: the multifocality of the seizure "focus". *Neurobiol Dis.* 2006; 23(2): 312-328.
- Barbaccia M, Roscetti G, Trabucchi M, Mostallino M, Concas A, Purdy R, et al. Time-dependent changes in rat brain neuroactive steroid concentrations and GABAA receptor function after acute stress. *Neuroendocrinology.* 1996; 63: 166-172.
- Reddy DS. Physiological role of adrenal deoxycorticosterone-derived neuroactive steroids in stress-sensitive conditions. *Neuroscience.* 2006; 138(3): 911-920.
- Rogawski MA, Reddy DS. Neurosteroids and infantile spasms: the deoxycorticosterone hypothesis. *Int Rev Neurobiol.* 2002; 49: 199-219.
- Kokate TG, Cohen AL, Karp E, Rogawski MA. Neuroactive steroids protect against pilocarpine- and kainic acid-induced limbic seizures and status epilepticus in mice. *Neuropharmacology.* 1996; 35(8): 1049-1056.
- Homayoun H, Khavandgar S, Dehpour AR. The involvement of endogenous opioids and nitric oxide pathway in the anticonvulsant effects of foot-shock stress in mice. *Epilepsy Res.* 2002; 49(2): 131-142.
- Saboory E, Ghaderi-Pakdel F, Moghanchizadeh H, Sdaghiani MM. Study of the morphine effect on frequency of hippocampal seizures in mice. *Epilepsia.* 2006; 47: 107-107.