

Letter to the Editor

The Effects of the Enriched Environment on Sympathetic Skin Response in Pentylene-tetrazol-Kindled Rats^{*}

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Epilepsy is a neurodegenerative disease that interrupts the normal electrical activity of the brain and promotes abnormal wiring in this organ. Epileptic seizures are often associated with significant changes in the functioning of the autonomic nervous system (ANS). Autonomic signs such as flushing, sweating and piloerection often accompany partial seizures and auras. Patients with epilepsy often display impairments in attention, memory, mental processing speed, executive functions, mood, personality and interictal depression^[1].

The damaging effects of seizures on cognition have been extensively studied in various kindling models. Pentylene-tetrazol (PTZ) induced kindling is an accepted animal model for the study of epilepsy and its consequences on memory^[2].

Cognitive, emotional and physical behaviors involve changes in peripheral autonomic activity. Electrodermal activity (EDA), the so-called galvanic skin response or sympathetic skin response, reflects sympathetic tone, and is therefore frequently used as an indirect measure of attention, cognitive effort, or emotional arousal^[3]. EDA is a multisynaptic sympathetic reflex that may be evoked by a variety of internally generated or externally applied arousal stimuli. It is recorded as an index of psychological processing properties of stimuli, such as significance, novelty or emotional relevance, and effortful processing^[4].

Skin conductance level (SCL) is a parameter of EDA. A high SCL may result from increased eccrine sweat gland activity and sympathetic activity^[5]. There are merely a few studies related to EDA and epilepsy during the ictal^[1,6] and interictal period^[7] in epileptic patients.

Environmental enrichment in laboratory animals means exposure to housing conditions that offer enhanced stimulation of the sensory, cognitive and motor systems of the brain, in comparison to the

standard housing conditions that are considered as impoverished. Environmental enrichment has been shown to induce neural plasticity at several levels in the brain, which include modifications in structure and circuitry, improvements in cognitive function, and mostly favorable alterations in brain chemistry^[8].

There are a lot of studies investigating psycho-physiological system with EDA both in humans^[5] and on rats^[9]. The studies on attention and arousal system were also conducted in humans, but were very limited on rat^[9]. The aim of this study was to investigate sympathetic activity and attention level with EDA in pentylene-tetrazol-kindled rats during the interictal period and to determine if environmental enrichment can alter pituitary-adrenocortical responsiveness and sympathetic response in epileptic rats. And this is an only study in literature regarding EDA and epileptic rats.

We used twenty-eight male adult Wistar rats (240-280 g) in this study. The rats were obtained from the Hakan Çetinsaya Experimental and Clinical Research Center of the Erciyes University and experimental protocols were approved by the Animal Care and Use Committee of the Erciyes University. The rats were randomly divided into four groups: the control group in standard conditions (CSC) ($n=7$), the control group in enriched conditions (CEC) ($n=7$), the epileptic group in standard conditions (ESC) ($n=7$), and the epileptic group in enriched conditions (EEC) ($n=7$). Serum physiologic and PTZ (35 mg/kg, ip) were injected into the control or epileptic groups, respectively, once every alternate day for a 5-week period. One week after the last injection, EDA was recorded from plantar surface of the posterior extremities of each rat using Ag/AgCl electrodes during the interictal period. Tonic and phasic skin conductance levels (SCL) were also recorded.

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PTZ (Sigma, St. Louis, MO, USA, 35 mg/kg in 0.9% saline, i.p.) was applied for chemical kindling. Injections were administered at 9.00 am every alternate day for a period of 38 days (i.e., a total of 19 injections). Immediately after each injection, seizure activity was observed for 45 min in a clear Plexiglas box with a matted floor and scored according to Racine, modified by Becker et al., as follows: Stage 0, no response; Stage 1, ear and facial twitching; Stage 2, myoclonic jerks without rearing; Stage 3, myoclonic jerks, rearing; Stage 4, turn over onto side position, clonic-tonic seizures; Stage 5, turn over onto back position, generalized clonic-tonic seizures. In the present study, animals were considered to be fully kindled if they had at least three consecutive tonic-clonic seizures, reaching the stage 4 or 5, during the 5-week testing period.

Rats were reared for 1 month in two housing conditions: standard cage (SC) or enriched cage (EC). The EC group was housed separately in 7 groups in a large cage (100 cm × 50 cm × 70 cm). The cage contained wood shavings, a shelter (a house-shaped toy with a concave opening), plastic colored toys and small constructions. The shelter was kept in the cage throughout the enrichment period while the toys and constructions were changed once a week. Likewise, the feeding boxes and water bottles were moved to different points in the cage once a week in order to encourage foraging and explorative behaviors.

The SC group were housed in standard cages (two or three rats per cage) (40 cm × 26 cm × 18 cm) containing wood shavings without objects. Feeding boxes and water bottles were kept in the same position throughout the test period. The animals received usual care by raising staff with no particular and prolonged manipulation.

EDA was measured using the MP30 system (MP30; Biopac Systems Inc., Santa Barbara, CA) and the physiological recordings took place in a dimly lit, electrically and acoustically shielded experimental room. Before the recordings, rats were subjected to a habituation period of 5 min. EDA was recorded, employing a constant voltage technique and sampling the absolute, direct current skin conductance at the rate of 20 samples per second, from the plantar surface of the posterior extremities of each rat using Ag/AgCl electrodes during the interictal period. Animals were conscious during recording and multipurpose gel ("Sigma Gel") was used between the skin and the electrodes.

Electrodes were connected to the MP30 system. The incoming signals of skin response were converted to digital signals via an MP30 data acquisition unit and processed with off-line analysis.

Tonic EDA: A period of 2 min was allowed at the start of recording in order to register non-specific SCL ($\mu\text{mho}/\text{cm}^2$) during a no-stimuli period.

Phasic EDA: The 15 auditory stimuli were presented at the end of the tonic EDA period (no-stimuli period, Figure 1). All were 1-s, 90 dB, 1000-Hz tones with 50-ms rise and fall times. They occurred at pseudo-random intervals ranging from 30 s to 65 s, averaging 45 s^[5]. The mean SCL values were calculated also off-line for phasic EDA. All the statistical analyses were performed using SPSS 16.0 for Windows software. Differences were considered to be significant with a probability of less than 0.05.

Figure 1 shows the numbers of epileptic seizure at Stage 5 seizures in standard and enriched cages. There was a significant decrement in the number of Stage 5 seizures in the enriched cage (8.28 ± 1.79) compared to that in the standard cages (4.71 ± 1.38) in epileptic rats ($z = -3.16, P < 0.002$).

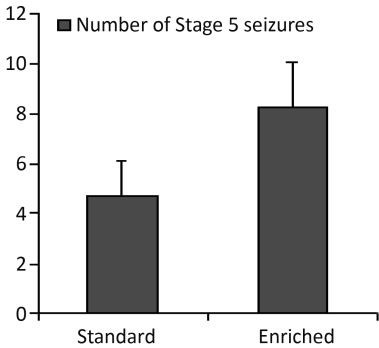


Figure 1. Comparison of Stage 5 seizures in epileptic group under standard and enriched conditions.

Table 1 indicates the mean SCL values in all groups. The effects of group (control and epileptic group) and housing conditions (standard and enriched cages) on tonic and phasic SCL values were compared using two-way ANOVA. There were main effects for group [$F(1,24) = 21.76; P = 0.000$] and cage [$F(1,24) = 5.08; P = 0.034$]. An interaction effect (group × cage) was also observed [$F(1,24) = 13.02; P = 0.001$]. An independent *t*-test was applied in order to analyze group effect and the result showed that there were significant differences between the control and the epileptic groups for tonic ($t = 3.66, P = 0.001$) and phasic SCL ($t = 5.43, P = 0.000$). According

Table 1. The Mean SCL Values in Control and Epileptic Groups (mean±SE)

Cage	Control Group (n=14)		Epileptic Group (n=14)	
	Standard (n=7)	Enriched (n=7)	Standard (n=7)	Enriched (n=7)
SCL (μmho)				
Tonic	27.53±3.72	15.93±0.89	11.17±0.45	13.85±0.87
Phasic	25.32±2.57	16.62±0.97	9.69±0.47	11.60±1.11

to these findings, PTZ-induced epileptic rats had diminished electrodermal (SCL) and sympathetic activity compared with the control group.

Low SCL in epileptic rats may result from decreased eccrine sweat gland activity and sympathetic activity. According to the study by Lazarova and his colleagues, selective depletion of forebrain noradrenaline has been shown to potentiate various types of experimentally induced seizures and they suggested that alpha-2 type adrenoceptors were involved in the control of PTZ-induced seizures in rats^[10]. Sirrou et al. evaluated the conduction properties of somatic and sympathetic sudomotor fibers in patients with epilepsy and found that mean sympathetic skin response (SSR) latencies were significantly prolonged in epileptic patients. At least one SSR abnormality was shown in 20 (33.3%) patients; six of them had absent SSR from the hand or/and the foot. These results demonstrated some subclinical abnormalities of the sympathetic sudomotor function in the considerable number of epileptic patients^[6]. Berilgen et al. investigated the function of autonomic nervous system during the interictal period in epileptic patients. They measured SSR at the beginning and the third month of antiepileptic treatment. In patients with partial epilepsy, SSR latency in the upper extremity (1.3+/-0.2 s) was longer than that in controls (1.2+/-0.3 s) at baseline (P=0.05), and was significantly reduced (1.1+/-0.3 s) after treatment (P<0.05). The sympathetic dysfunction was observed in the patients with partial epilepsy and parasympathetic dysfunction in the patients with generalized epilepsy^[7]. As we noted, our results are in consistent with the results from these studies. Although Poh et al. found that there was an increased sympathetic activity in epileptic patients, their study was performed during ictal epileptic seizure^[11].

SCL is mainly used as an indicator of psycho-physiological arousal and sustained attention^[9]. SCL was lower in the epileptic animals than in the control group. There are many studies on epilepsy and cognitive function. However, most of these studies

were conducted in humans and behavior profiles revealed greater attention problems in children. We were unable to find any study on direct attention differences in epileptic rats. And findings from our present study revealed that the epileptic rats had decreased attention which was in consistent with findings from studies in humans.

The rats in an enriched environment are exposed to more stimuli in quantity and diversity than the rats in the standard cages. In the enriched environment, rats can climb and manipulate a number of tools available that are changed frequently and the video recordings showed that they were very active, particularly during dark periods, climbing, swinging and handling the objects available in rather skilled ways. Therefore, we believe that an enriched environment may cause intermittent hormonal activation in a physiological way.

When we put control group’s animals from standard cage to enriched environment, their stress level and sympathetic activities decreased. The rats housed in enriched cages had lower tonic and phasic SCL values than those in standard cages (tonic SCL; *t*=3.0, *P*=0.01; phasic SCL; *t*=3.15, *P*=0.008). This also might have been related to adaptation to stimuli. The epileptic animals in the enriched cage had higher tonic SCL and stress levels compared with the epileptic animals in the standard cage (*t*=-2.7, *P*=0.019). This improvement may result from induced neural plasticity with a novel enriched environment and decreased habituation in the epileptic group to novel objects. We suggest that an enriched environment may have improved the sympathetic activity in the epileptic group.

When the groups were compared according to their housing condition, tonic and phasic SCL values were significantly different in animals between the CSC and ESC groups. Epileptic rats housed in standard cages had lower tonic and the phasic SCL than the control group rats housed in standard cages (tonic, *t*=4.36, *P*=0.001; phasic *t*=5.96, *P*=0.000). The phasic SCL values were significantly different in

animals between the CEC and EEC groups. Epileptic rats housed in an enriched cage had lower phasic SCL than control group rats housed in an enriched cage (phasic $t=3.39$, $P=0.005$). No significant differences for the tonic SCL values (Figure 2) was observed.

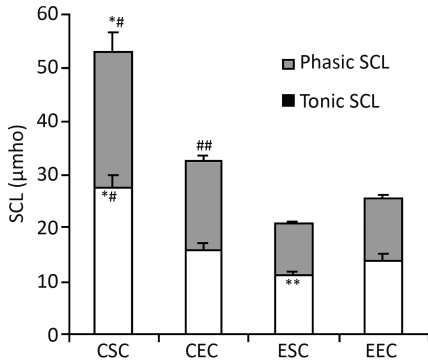


Figure 2. Comparison of SCL values of groups according to housing conditions. CSC: control group in standard conditions, CEC: control group in enriched conditions, ESC: epileptic group in standard conditions, EEC: epileptic group in enriched conditions. *:CSC-CEC, **:ESC-EEC, #:CSC-ESC, ##:CEC-EEC.

This is the only EDA study in rats with epilepsy housed in enriched conditions. Diminished EDA during interictal period is a possible sign of autonomic instability that could play a role in the pathophysiology of epilepsy. Diminished EDA may also be a sign of the decrease of the attention level of the epileptic rats during the interictal period. The enriched environment may improve the attention level of the epileptic rats and we believe that this improvement may result from induced neural plasticity with a novel enriched environment.

Conflict of interest statement

None of the authors has any conflict of interest to disclose.

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