

Anxiolytic and antidepressive effects of electric stimulation of the paleocerebellar cortex in pentylenetetrazol kindled rats

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Anxiety and depression are component of interictal behavioral deteriorations that occur as a consequence of kindling, a procedure to induce chronic epilepsy. The aim of this study was to evaluate the possible effects of electrical stimulation (ES) of paleocerebellar cortex on anxiety and depressive-like behavior in a PTZ kindled epilepsy model. Kindling was induced *via* pentylenetetrazol (PTZ) (25.0 mg/kg IP daily) during three weeks. Locomotion in open field, elevated plus-maze (EPM) and Porsolt forced swimming test have been used for the assessment of anxiety and depression-like behavior. ES (100 Hz) has been delivered to V–VII lobules of vermal cortex of kindled rats. ES of paleocerebellum reversed kindling-induced reduction of crossings of central squares, increased rearings, and decreased the number of defecations in open field. The duration that kindled animals spent in the open arms of the EPM increased in post- ES period, and the number of enterings into the closed arms of the EPM decreased. The duration of the immobility response in the swimming test in kindled rats was reduced after ESs of paleocerebellum. In all: ES of paleocerebellar structures suppressed anxious and depressive-like behavior in PTZ-kindled rats.

Key words: anxiety, cerebellum, depression, kindling, epilepsy

INTRODUCTION

The involvement of cerebellar structures in the development of anxiety was recently shown and an inverse relationship between anxiety and volume of the cerebellum was established (Chung et al. 2010). Analysis of wide spectrum of behavioral and psychiatric consequences of the cerebellar degeneration revealed the unusual prevalence of depression (68%) with 35% patients with major depression (Schmahmann et al. 2007). Those patients have had an overall rate of psychiatric disorder of 78%, including personality change, anxiety and psychotic disorders (Schmahmann et al. 2007). Abnormalities of cortical-limbic-cerebellar white matter networks may contribute to the treatment-resistant depression (Peng et al. 2013). An increased volume of anterior cerebellar lobe was

observed in men suffering from major depression opposite to the data on degenerative cerebellar deteriorations as causative for emotional behavior disturbances (Yucel et al. 2013). It should be added that a role of the cerebellum in the modulation of aggression and mood appears in children with the posterior fossa syndrome following surgery involving the vermis, and during clinical and experimental neurosurgical manipulation (Strata et al. 2011).

Following this large amount of observations supporting a role of the cerebellum in emotional processes, the hypothesis of the “limbic cerebellum” (vermis and fastigial nucleus) as part of Papez’ circuit has been formulated (Bernston and Torello 1982). Hence, a role of cerebellum in emotional behavior is strongly supposed (Koziol et al. 2014), and recent findings demonstrate that long-term potentiation (LTP) of synapses in the cerebellar cortex occurs in relation with associative fear learning, similar to data reported from hippocampus and amygdala (Strata et al. 2011).

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Searching for the substrate at cellular level which is in charge for the cerebellar role in emotional behavior, it has been discovered that mouse mutant with an inactivated Purkinje cell-specific gene, *Pcp2(L7)*, that encodes a GoLoco domain-containing modulator of Gi/o protein-coupled receptors revealed an increased duration of immobility in the forced swimming test (Walton et al. 2012). Besides, cerebellar histopathological abnormalities have been well documented in autism although findings of structural differences, as determined by magnetic resonance imaging, have been less consistent. In most cases the involvement of VI-VII lobules have been revealed in children with autism suffered during 3–4 years (Webb et al. 2009, Tsai et al. 2012). The contribution of V–VII lobules has been demonstrated for the precipitation of modulative effects of cerebellar vermal cortex electrical stimulation (ES) upon penicillin-induced epileptic activity in rat's cortex (Godlevsky et al. 2012).

The data on the role of cerebellum in emotional behavior, especially anxiety posed forward the question on possible role of benzodiazepine receptors located in cerebellar structures as targets. Hence, it has been shown in PTZ kindled rats that the benzodiazepine receptor density in cerebellar cortex was reduced almost by half and that this effect persisted for 6 months after the termination of kindling (Bazyán et al. 2001). Strengthening of the anticonvulsive effect of diazepam in conditions of ES of cerebellar structures was established earlier (Godlevskii et al. 1999). In particular, strengthening of the diazepam's anticonvulsive effect was observed following prior serial stimulation of the paleocerebellar cortex, which suggests an activation of the endogenous system of ligands of benzodiazepine receptors in conditions of such stimulation (Godlevskii et al. 1999). Hence, it is reasonable to investigate whether animals kindled with PTZ have increased anxiety and depressive-like behavior, signs of severely impaired emotional behavior (Szyndler et al. 2002).

It should be stressed that in our investigations both anxiety and depression represented components of kindling-induced chronic deteriorations of behavior as a consequence of sustainable development of seizures and was investigated in 24 h and more longer (months) from the last kindled administrations of PTZ (Kalynchuk 2000, Szyndler et al. 2002). Hence, sustainable interictal maintenance of behavioral deteriorations is based on neuroplastic kindling-specific mechanisms, which are quite distinct from the classi-

cal anxiety precipitated by PTZ 10 min after IP administration in a dosage of 20 mg/kg, when the effects on behaviour are directly induced by the PTZ molecule (Chimakurthy and Talasila 2010).

The main goal of this research was to investigate the effects of ES of paleocerebellar cortex on anxious and depressive-like behavior, which were induced in rats by means of PTZ-induced kindling.

METHODS

Animals

Fifty-five 6–8 months old Wistar male rats were used. Animals were kept in standard conditions (constant temperature 23°C, and relative humidity 60%, 12 h dark/light cycles, standard diet and tap water were given *ad libitum*) in accordance to international laws and policies [European Community Council Directive 86/609, OJ L 358, 18/12/1986 P. 0001-0028; National Institute of Health Guide for Care and Use of Laboratory Animals, US National Research Council, 1996 P.21-55].

Surgery and stimulation (ES)

A bipolar stimulating electrode was placed in the V–VII lobules (culmen, declive, pyramis) of the vermal cerebellar cortex (it was insulated except the tip of the nichrome wire, diameter of 0.15 mm and an inter-electrode distance of 0.25–0.3 mm) under ketamine anesthesia (100.0 mg/kg, IP). The electrodes were fixed on the cranial surface with a fast-hardening dental cement.

Electric stimulations (ESs) were conducted using a electric stimulator ESU-2 (universal electrical stimulator, FSU) with impulse frequency 100 Hz, monophasic pulse duration 0.25 ms, intensity 50 μ A, and ES duration 2.5 s. The stimulations were repeated every 2.5–3.5 minutes. A total of 10 and 20 stimulations were conducted in the two stimulation groups. Sham stimulations were made in form of connection the animal electrodes to the stimulator without delivering electrical current.

Kindling modeling and experimental groups

Kindling was induced in rats using a subthreshold dosage of PTZ (25.0 mg/kg, IP) starting on the 10–14th

day following surgery. A total of 21 injections with the epileptogen was carried out. Testing of behavioral reactions was conducted at 09:00 AM–12:00 PM, 24 hours after the last kindling administration of PTZ. Those animals, which demonstrated generalized clonic-tonic seizures as a response to each of the last three times of PTZ administration, were used for further observation.

Hence, the following groups of rats were formed: (i) kindled rats with 20 ESs of paleocerebellum (13 animals); (ii) kindled rats with 10 ESs of paleocerebellum (10 animals); (iii) kindled rats with implanted electrodes, sham stimulated (16 animals); (iv) intact rats with implanted electrodes – control group (16 animals).

All groups were tested in the following order: first, the open field test; second, the EPM test; and third, the Porsolt test. The first test started 15 min after the moment of the last ES exposure. The duration of observations for each rat in the three tests did not exceed 30 min.

The Open-Field Test

The apparatus, which was used for performing this test, was made of wood covered with impermeable formica, had a white floor with the area of 100×100 cm (divided with black lines into 25 equal squares, 20×20 cm each) and white walls, 40 cm high. The illumination in the test room provided 7 lux inside the apparatus (Ramos et al. 2003). Each rat was placed in the center of the open field, which was novel to the animal, and the following variables were scored for 5 min (Markel 1981, Lapin 2000): the total number of crossed squares; the number of crossed central squares (away from the walls); rearings and the total number of fecal boli (defecation) (Markel 1981, Lapin 2000, Ramos et al. 2003). The floor of the apparatus was cleaned with a wet sponge and dry paper before usage and between animals.

The Elevated Plus Maze (EPM)

The apparatus made of wood covered with a layer of black formica had four elevated arms (100 cm from the floor), each 50 cm long and 10 cm wide. The arms were arranged in the form of a plus sign, with two opposite arms being enclosed by 40 cm high walls and the other two being open, all four being connected by a central

Table I

Histological verification of electrodes location in vermal lobules				
Experimental groups	Lobules			
	V	VIa	VIb	VII
Rats with 10 ES (<i>n</i> =10)	2	4	3	1
Rats with 20 ES (<i>n</i> =13)	1	5	5	2
Sham stimulated kindled (<i>n</i> =16)	3	4	5	4
Intact rats with implanted electrodes (<i>n</i> =16)	3	6	4	3

Note: in case of lobule V locations were at the border between V and VI a, while in case of VII – at the border between VII and VI b

open platform (10.5×14.0 cm). The open arms were surrounded by a raised ledge (1 mm thick and 5 mm high) to prevent rats from falling off the arms. The central platform was under 7 lux of illumination (Ramos et al. 2003). Each rat was placed on the central platform facing an open arm, and the behaviors that followed have been observed for 5 min. Both the number of entries and the time spent (with all four paws) inside each type of arm were recorded (Lapin 2000). The amount of time spent in the open arms and number of open arm entries was interpreted as anti-anxiety behavior.

Porsolt forced swimming test

Depression-like behavior was studied in applying the Porsolt test. One hour before the last (21st) PTZ administration the animal has been placed in a glass cylinder (23/50 cm) that was 2/3 filled with water at 25°C temperature for 15 minutes; then it was removed out of the cylinder and left to dry during one hour. 24 hours later the animal was placed in the water for the second time. The depression-like state was evaluated during the next 5 minutes by duration of immobility. The immobility response was defined as total absence of swimming movements, while the animal was lying passively and motionless on the water surface (Markel 1981, Lapin 2000).

Both direct visual observation and video-recordings were used for quantifying the behavior of the rats.

Histological control of electrode location

Upon completion of the experiment a histological control of the electrodes placement was carried out; for that purpose an electrocoagulation was performed in the area of their fixation, applying direct current with amplitude of 5 μ A during 30 s, and using electrodes as anodes. Hystological control of location of electrodes revealed their presence in medial part of vermal cortex with the most of them in being located in VI lobule: 7 out of 10 in the group with 10 ES and 10 out of 16 in the group with 20 ES (Table I).

Statistical analysis

Values were compared using one-way analysis of variance followed by *post-hoc* *t*-tests. Values are presented as mean \pm standard error of the mean, with findings of $P < 0.05$ considered significant.

RESULTS

First 1–3 injections of PTZ did not result in seizures but were followed by subtle manifestations, which compose a typical number of “minor signs” of absence epilepsy: freezing of animals along with tremor of vibrissae, staring, tilting of head and slight breathing acceleration were noted (Coenen et al. 1992). Those minor behavioral deteriorations were getting more intense, and occurred more often and lasted longer during the next 1–3 PTZ administrations and seizure twitches appeared along with the precipitation of clonus of body muscles. Typical rearings with clonus of forelimbs and falling of animals accompanied the next 2–4 administrations with the epileptogen. Precipitation of generalized tonic-clonic fits was observed in most rats after 7–15 injections. During seizures animals were falling on their side and displayed the post-seizure depression. The following 2–4 injections PTZ injections were effective in the induction of generalized seizures in all experimental animals.

Open Field Test

The number of crossed squares of the kindled animals in the open field was 31.3% less than in sham-operated animals ($F_{3,54} = 3.37$, $P = 0.025$), while the number of crossed central squares was 4.1 times less compared to rats of the control group ($F_{3,54} = 6.49$, $P = 0.000$)

(Fig. 1; I and II). Measurement of those indices in kindled rats after 20 ESs of paleocerebellar cortex revealed the absence of significant difference when compared with the control rats, while they exceeded the data in kindled sham-stimulated rats by 33.0% ($t = 1.979$, $P > 0.05$) and 3.4 times respectively ($t = 3.0003$, $P < 0.05$). Besides, after 10 ESs a reduction 52.1% of crossed central squares was found when compared with sham-stimulated kindled group ($t = 1.112$, $P > 0.05$).

The number of rearings was smaller in the kindled rats compared to the intact animals by 3.0 times ($F_{3,54} = 5.82$, $P = 0.002$) (Fig. 1; III). The 20 ESs of paleocerebellar cortex group showed a 2.43 times increase of rearings in comparison with the kindled sham group ($t = 4.030$, $P < 0.05$). Also the 10 ESs group showed a higher number of rearings compared to the kindled sham group by 1.81 times ($t < 2.121$, $P > 0.05$), while it was lower by 33.7% than in intact rats ($t = 2.121$, $P > 0.05$) (Fig. 1; III).

The kindled animals showed an increased number of defecations that exceeded the respective values in the control group 2.1 times ($F_{3,54} = 3.31$, $P = 0.027$), ($t = 2.599$, $P > 0.05$) (Fig. 1, IV). In addition, the number of defecations was decreased (by 47.1%) in the 20 ESs group as compared to kindled sham group ($t = 2.599$, $P < 0.05$) (Fig. 1, IV).

Elevated Plus Maze

The kindled animals spent less time (2.7 times) in the open area of the EPM in comparison to the intact control

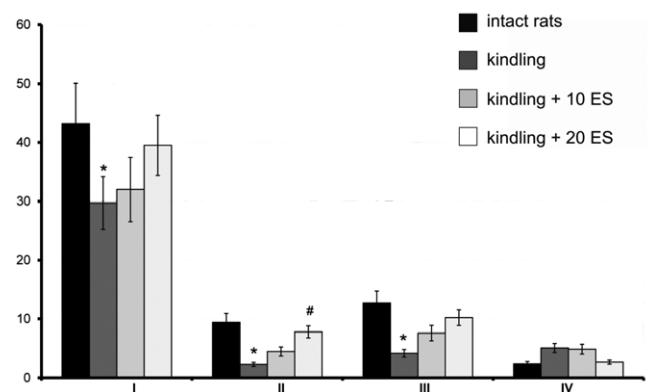


Fig. 1. Open field test intact and kindled rats behavior and effects of paleocerebellar ES in kindled rats. Abscissa: (I) number of crossed squares; (II) number of crossed central squares; (III) rearings; (IV) number of boluses. Ordinate: absolute values of the studied indices. * $P < 0.05$ – in comparison to the control (intact sham-operated rats) group; # $P < 0.05$ – in comparison to the value in kindled animals.

animals ($F_{3,54}=13.42$, $P=0.000$) (Fig. 2A; I). The time the rats spent in the dark arm of the EPM was 24% higher as compared to the control group ($F_{3,74}=3.74$, $P=0.017$) (Fig. 2A, II). 10 ESs of the cerebellar cortex in the kindled rats were accompanied by 1.77 times increase of the time spent in open arms when compared with the corresponding index in the sham-stimulated kindled animals ($t=2.433$, $P>0.05$), and stayed 28.6% lower than the time registered in the control rats ($t=2.970$, $P<0.05$) (Fig. 2A; III). Following 20 ESs of the paleocerebellar cortex the time period that kindled animals spent in the open arms of the EPM increased by 2.2 times compared to the sham-stimulated kindled group ($t=4.116$, $P<0.05$), and it did not differ from that in the intact shame group ($t=1.717$, $P>0.05$) (Fig. 2A; V).

The number of entries in the closed arms of the

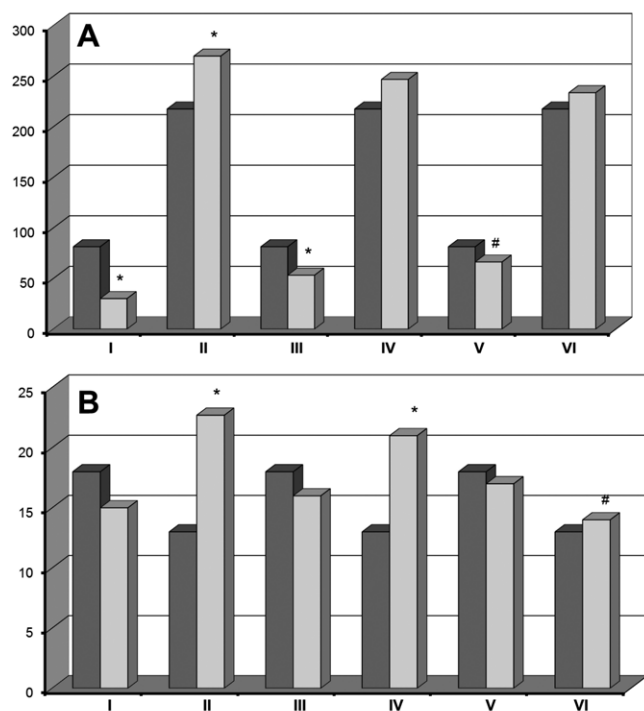


Fig. 2. Impact of ES of the paleocerebellar cortex on the intact and kindled rats' behavior in the elevated plus maze test. (A) abscissa: time of stay in open (I, III, V) and closed (II, IV, VI) arms of the EPM. Ordinate – seconds. (B) abscissa: number of enterings into open (I, III, V) and closed (II, IV, VI) arms of the EPM. Ordinate – absolute values of investigated indices. In both (A and B) fragments: first column – intact (control) rats, and second column – for sham stimulated kindled rats (I), for kindled rats with 10 ESs (III and IV) and for kindled rats with 20 ESs (V and VI). * $P<0.05$ – in comparison to the control group; # $P<0.05$ – in comparison to the kindled sham-stimulated rats.

EPM of the kindled rats exceeded 1.75 times that one in the shame intact animal group ($F_{3,54}=5.60$, $P=0.002$) (Fig. 2B; II). This index remained high after 10 ESs of the cerebellum cortex and exceeded 1.45 times respective index in the shame intact rats ($t=4.699$, $P<0.05$) (Fig. 2B; IV). Following 20 ESs of the paleocerebellar cortex the number of enterings into the closed arms of the EPM by the kindled animals was 37.9% lower than in shame-stimulated kindled rats ($t=5.454$, $P<0.05$), and it did not differ from the respective index in the control group ($P>0.05$) (Fig. 2B; VI). There were no significant differences between the groups in the values of number of enterings into opened arms of EPM ($P>0.05$) (Fig. 2B).

Forced Swimming Test

The duration of the immobility response in kindled rats was 38.1% higher ($F_{3,54}=9.98$, $P=0.000$) than in rats of the control group (Fig. 3; II). After 10 ESs and 20 ESs of the paleocerebellar cortex the immobility duration remained 41.0% ($t=4.647$, $P<0.05$) and 12.8% higher ($t=0.902$, $P>0.05$) than that in the control group. It is noteworthy that after 20 ESs the immobility duration in kindled animals was significantly 30.2% shorter in comparison with the kindled sham group ($t=3.498$, $P<0.05$) (Fig. 3; IV).

DISCUSSION

The obtained data showed that by repeated administration of a subthreshold PTZ dosage chemical kindled rats showed behavioral disorders during the interictal period. Namely, 24 h after the completion of kindling anxiety and depressive-like behavior were observed. A reduction in the number of central squares crossings in the open field pertained to the number of crossing by the intact rats. That result along with the shortening of the time that the kindled animals spent in the open arms of the EPM (as well as increase in the number of enterings into the closed arms of EPM might be in favor of increased anxiety in the kindled rats.

This result might be opposite to earlier (Runke and McIntyre 2008) data, which support the role of the initial anxiety level for proneness to kindling development. These authors showed that the animals with an initially high anxiety level manifested a faster kindling development. Those “fast” rats spent a longer time

period in the open arms of the EPM, and had a higher emotional tension according to the number of defecations in the open field (Runke and McIntyre 2008). And vice versa, rats with the opposite behavioral indices demonstrated slow velocity of kindled seizures precipitation (“slow” kindled rats). It might be supposed that in both cases, when “fast” and “slow” types of rats are used in the course of PTZ-induced kindling formation an increase in anxiety and emotional tension is observed during interictal period. It should be noted that an aggravation of anxiety in animals with PTZ kindling that was revealed in the current study corresponds to the (Zarraga-Galindo et al. 2011) data, which showed that PTZ-induced kindling in Wistar rats was characterized by reduction of the number of crossed squares in the open field test, decrease of the vertical rearings, and increase in the defecations number.

Electrical kindling of the amygdala was followed by both anxiolytic and anxiogenic effects depending on the initial anxiety level in rats (Adamec et al. 2005). Prolonged (150 ESs) kindling of the septum was accompanied with an anxiolytic effect (Thomas and Gunton 2011). Such differences between PTZ and ES kindling can be explained by the interaction of PTZ with benzodiazepine receptors that facilitates activity reduction of the later in the process of kindling (Bazyan et al. 2001).

Paleocerebellar cortex, as a target for decrease of the level of anxiety in kindling rats is of special interest as far as cerebellum is characterized by an almost twofold reduction of the density of benzodiazepine receptors in the course of PTZ kindling (Bazyan et al. 2001). Exposure to the high frequency (100 Hz) 20 ESs of the V-VII vermal lobules (culmen, declive, pyramis) of the kindled rats was followed by the net increase in the number of central squares crossings, and rearings. Besides, emotionality was reduced – the number of defecations decreased. Also in the EPM test 20 paleocerebellar ESs caused an increase in the time spent in the open arms, as well as a reduction of the number of enterings into the closed arms. Hence, the paleocerebellar ES possesses antianxiety activity as far as the increase in the central squares crossings and an increase in the open arm activity (duration of stay and/or number of entries) reflects an anti-anxiety behavior (Markel 1981, Lapin 2000, Ramos et al. 2003).

PTZ-kindled rats showed increased immobility duration, suggesting an increase in the severity of depression-like behavior. Opposite to the “slow” kin-

dled rats, the animals with the initial high depression-like behavior score showed accelerated kindling (Barbakadze et al. 2010, Kanner et al. 2012). It is worth noting that the above mentioned role of benzodiazepines involvement in the cerebellar ES-induced anxiolytic effects contradicts the role that the benzodiazepines play in the forced swimming depression: diazepam and other agonists prolong the duration of the immobility response in the forced swimming test (Nagatani et al. 1987). Nevertheless, this contradiction should be reconsidered from the point of view of the PTZ-kindling induced “reconstruction” of the benzodiazepine receptors density in the brain structures (Bazyan et al. 2001). It is also important to note that some antidepressant compounds, such as magnesium, which reduces the duration of the immobility response, are able to involve both mechanisms of NMDA receptors blocking and activating of benzodiazepine receptors (Poleszak 2008). PWZ-029, a compound with moderate inverse agonist functional selectivity at GABA A receptors containing $\alpha 5$ subunits eliminates depression in the Porsolt test (Savic et al. 2008).

Another prominent role in the control of anxiety and depressive disorders might be played by the adenosine system. Adenosine caused inhibition upon parallel fibers-induced excitation of Purkinje cells (Wall et al. 2007, Kovács and Dobolyi 2013, Kamikubo et al. 2013). The role of adenosine in cerebellar-dependent anxious behavior is supported by data on a very certain functional links between adenosine – and benzo-

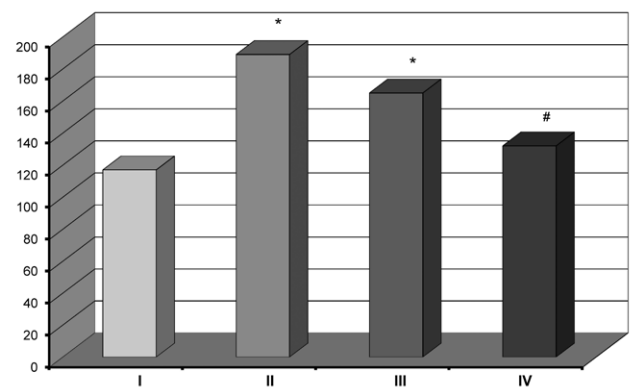


Fig. 3. Duration of depression in Porsolt forced swimming test in intact and kindled rats and effects of paleocerebellar cortex ES. Abscissa: I – intact (control) group of rats, II – kindled animals; III – 10 ESs, and IV – 20 ESs of the paleocerebellum in the kindled animals. Ordinate – seconds. * $P < 0.05$ – in comparison to the control group; # $P < 0.05$ – in comparison to the kindled animals.

diazepine effects in cerebellar cortex: agonists of adenosine receptors are able to alleviate signs of benzodiazepine-induced withdrawal symptoms (Kaplan et al. 1992, Listos et al. 2008).

It should be stressed that regimen of ES, which have been explored in our investigation, might be regarded as such one which is able to induce synaptic fatigue. Meanwhile, the time – course of activation of adenosine elaboration *via* electrical stimulation of cerebellar cortex is different from well known regimen of synaptic activation (Wall and Dale 2007). These authors have shown that bigger numbers of electrical stimuli along with longer trials (10 s trains at 20 Hz) are of need to cause the release of adenosine in the cerebellar cortex. Other data which support a most possible adenosine role in observed effects of cerebellum came from data on the enhancement of antiepileptic effects of cerebellar ES in rats maintained on ketogenic diet (Godlevskii et al. 2012). It was shown that ketogenic diet engendered antiepileptic action via activation of purinergic brain system (Masino et al. 2009, 2011).

Finally it should be noted that pharmacological blockage of the CB1 cannabinoid receptor increased anxiety-like behavior in rats including reduced open arm exploration in the elevated plus maze and increased withdrawal-related manifestations (Navarro et al. 1997, Haller et al. 2002). The density of cannabinoid receptors in cerebellar cortex is comparatively higher than in other brain structures (Navarro et al. 1997). That is why an involvement of cannabinoid receptors in the regulation of anxious behavior should not be excluded.

Hence, multifunctional influences from paleocerebellum along with reorganized sensitivity and distribution of receptors in the brain structures contribute to both antianxiety and antidepressive effects of cerebellar ES.

CONCLUSION

Electrical stimulations of (100 Hz) of V–VII lobules of vermal cortex of pentylene-tetrazol-kindled rats abolished kindling induced anxiety and depression-like behavior during the interictal period.

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