PHYSIOLOGY

Biorhythmologic Aspects of Seizure Activity

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Seasonal and circadian rhythms of neuronal and organism resistance to convulsive effect of strychnine and penicillin were studied *in vivo* on mice and rats and *in vitro* on cultured mouse cerebellar sections. Resistance was assessed by the latency of seizures in mice and neuronal response to convulsants in sections. In the night and morning time (0:00-9:00) seizure resistance in mice increased: it manifested in longer latency and lower mortality compared to those in the day and evening time (12:00-21:00). Seizure resistance was minimum in autumn and maximum in winter. Neurons in cerebellar section were most resistant to the convulsive effect of penicillin in autumn and winter and least resistant in spring and summer. Circadian rhythms of cerebellar neuron resistance to convulsants were opposite, which attests to reciprocal relations between epileptogenic and antiepileptic (cerebellar) cerebral structures.

Key Words: *circadian and seasonal rhythms; epileptic seizure; cerebellar section; convulsion resistance of neurons and animals*

The accumulated experimental data and clinical observations show that not only physiological, but also pathological processes change regularly during the day and depend on the season. Such environment factors as day-night changes, climatic and geomagnetic conditions affect the incidence of epileptic seizures (ES). The cases are described when ES appeared in patients only during a certain period of year (seasonal epilepsy). Some authors describe the peak of epileptic activity in summer or early spring [7]. In children, fever-related convulsions appear most frequently during November-December and June-August [15].

Generalizations made on the basis of clinical observations were experimentally corroborated on the models of generalized seizures in mice and rats. Seasonal variations of the effects of some anticonvulsants were established: some agents were ineffective in March and April [9].

Seizure activity is characterized by not only seasonal, but also circadian periodicity. Experiments on rats and mice, where ES were induced by electrical stimulation of epileptogenic cerebral structures (hippocampus, amygdaloid complex, and motor cortex) showed that temporal seizures are most pronounced in the daytime, while parietal seizures occur at nights, and they do not coincide in phase with limbic seizures [12,13]. Limbic seizures in humans and rats occurred predominantly in the daytime, which attests to involvement of the circadian control system in their genesis [14].

The circadian and seasonal rhythms of functional activity of various systems manifested not only at the organism level, but can be also seen in neurons *in vitro*. Previous experiments on neuronal model (cultured mouse cerebellar sections) revealed circadian and seasonal oscillations of impulse activity (IA) of neurons *in vitro* correlating with rhythms of motor activity *in vivo* [1]. ES are usually modeled *in vitro* on hippocampus sections [5], because hippocampus is a structure facilitating epileptic process, while cerebellum and cerebellar nuclei are antiepileptic structures [4].

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Thus, cyclicity is a characteristic feature of seizure activity at the organism level. However, there are no data on circadian and seasonal oscillations of seizure activity in neurons of specific cerebral structures.

In the present study circadian and seasonal rhythms of organism and neuronal resistance to the effects of epileptogenic agents and the role of biorhythms in the interaction between epileptogenic and antiepileptic structures were investigated on experimental epileptogenic models both *in vivo* in the entire organism and *in vitro* on cerebral sections.

MATERIALS AND METHODS

Circadian rhythm of the resistance to convulsive effect of strychnine was studied on male mice weighing 18-23 g (n=80). The mice divided into 8 groups received single intramuscular injection of 25 mg/kg strychnine nitrate with a 3 h intervals (10 animals per point). The latency (time from strychnine injection to ES) and the number of animals died during the seizures in each group were evaluated.

Seasonal resistance to the epileptogenic action of strychnine was studied during 3 years on white male rats weighing 180-210 g (n=260) every 3 h during a day in autumn (October), winter (January), spring (April), and summer (June). Experiments were not carried during geomagnetic storms.

Similar to *in vivo* experiments, *in vitro* tests with mouse cerebellar sections (300-350 μ, n=288) were performed every 3 h during a day in the same months. IA of cerebellar neurons was recorded extracellularly 40-60 min after adaptation of cerebellar sections in Earl's solution (33-34°C) aerated with carbogen (95% O_2 and 5% CO_2). Penicillin was added to the perfusate in a dose of 7500 U/ml. The latency of neuronal response to penicillin was determined for cells located near the tip of the recording electrode. In high doses this drug acts as a convulsant and GABA antagonist increasing the amplitude of excitatory postsynaptic potential [6].

The results were analyzed statistically using Student's t test.

RESULTS

The same doses of strychnine administered in different daytimes produced different effect. Seizure latency from 0:00 to 6:00 in mice was about 6 min (350±36 sec at 0:00, 361±35 sec at 3:00, and 355±59 sec at 6:00). The latency was maximum at 9:00 (507±128 sec) and minimum at 12:00 (210±22 sec). The differences between minimum and maximum values are significant (p<0.05). Starting from 15:00, the latency increased again: it was 288±45 sec at 15:00, 270±43 sec at 18:00, and 290±35 sec at 21:00. Five (50%) and 10 (100%) animals died during ES at 9:00 and 12:00, respectively.

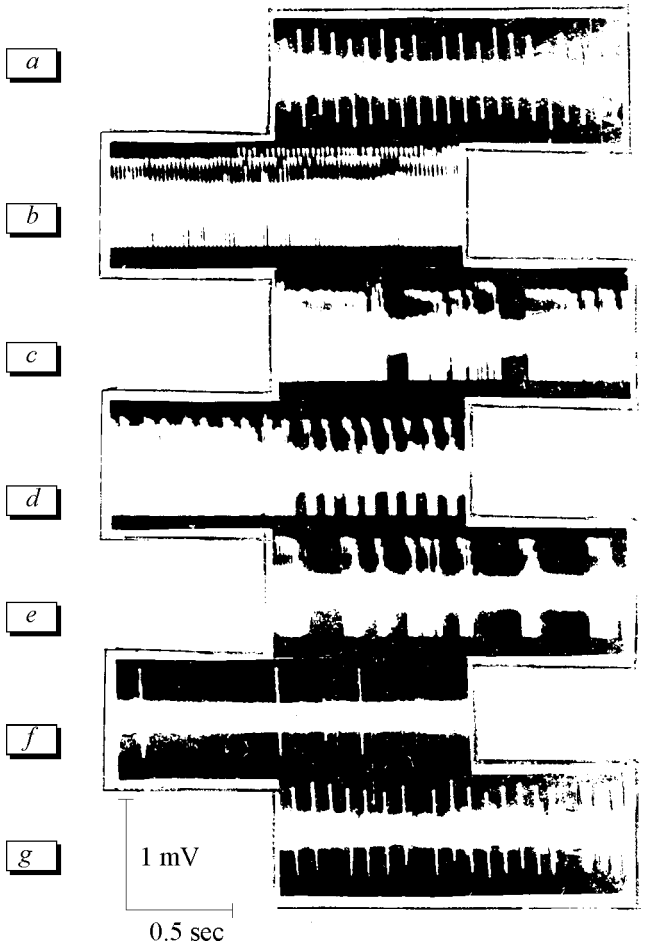

Fig. 1. Effect of penicillin on impulse activity of neurons in cerebellar section. *a*) initial impulse activity; *b-f*) impulse activity under the effect of penicillin; *g*) recovery of impulse activity after washout.

Therefore, in the night and morning time (0:00-9:00) the resistance to convulsant tended to increase: the latency was longer and the mortality lower (67.5%) than in the day and evening time (12:00-21:00), when latency became shorter and mortality was higher (90%).

TABLE 1. Seizure Resistance in Rats in Various Seasons (M±m)

Index	Autumn	Winter	Spring	Summer
Latency of seizure, sec	894±42	1086±48	1062±42	966±60
Latency of death during seizure, sec	1206±60	1252±60	1404±54	1344±72

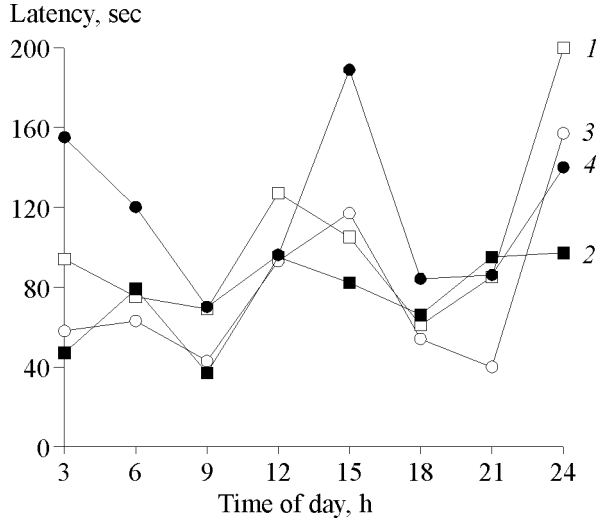

Fig. 2. Circadian and seasonal oscillations of latency of the first neuronal response in cerebellar section treated with penicillin in winter (*1*), spring (*2*), summer (*3*), and autumn (*4*).

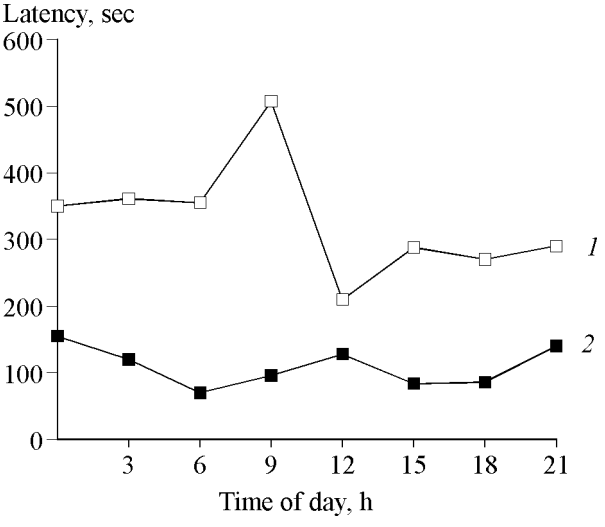

Fig. 3. Latencies of epileptic seizures in mice treated with strychnine (*1*) and latency of the first neuronal response to penicillin in cerebellar section (*2*) in different day times in autumn.

These data are consistent with those obtained by other authorities on rat model of limbic epilepsy [12], who showed that the peak of ES corresponded to the inactive period *i.e.* daytime (12:00-17:00, rats are night animals). Sixty-three and 60% ES occurred in the daytime in rats and humans, respectively [14]. The resistance to convulsant is characterized not only by circadian, but also seasonal periodicity. In autumn, convulsion resistance in rats peaked at 4:00 (latency 1208±126 sec), *i.e.* in the dark period, and was minimum at 13:00 (latency 708±102 sec); the differences between the maximum and minimum values were significant ($p<0.05$). In winter, convulsion resistance peaked at 13:00 (latency 1368±114 sec) and was minimum at 1:00 (latency 726±114 sec, $p<0.01$). In spring latency was maximum at 4:00 (1326±132 sec) and minimum at 1:00 (826±108 sec, $p<0.01$). In summer convulsion resistance was maximum at 22:00 (latency 1308±138 sec) and minimum at 4:00 (latency 600±78 sec, $p<0.05$). Table 1 shows the averaged latency for different seasons.

These data show that convulsion resistance is minimum in autumn, when seizures appear with shortest latency and rats die more quickly than in other seasons. By contrast, in winter convulsion resistance is maximum ($p<0.01$). Our results are consistent with published data that the convulsion thresholds in response to stimulation of limbic structures were maximum in winter (February) and minimum in autumn (September) [8]. Japanese researchers described seasonal variations of fever seizures in children, which had two peaks: in November-December and June-August [15].

Cerebral section is a neuronal model preserving interneuronal relationships characteristic of *in situ* neuronal population. Neurons possessing pacemaker properties and interconnecting both anatomically and functionally compose a system, whose state can be reflected by value and variation of IA recorded from individual neurons.

The mean latency of the first neuronal response penicillin application was minimum in spring (74.92± 3.24 sec), it increased in summer (78.31±6.42 sec) and winter (101.92±6.41 sec), and attained a maximum in autumn (117±5.48 sec). The convulsant first increased firing rate (by 7.50±0.37 times, $p<0.05$) then convulsion-like IA was appeared, and finally IA decreased by 89.60±1.82%. Washout restored initial IA after 114.20±4.87 sec (Fig. 1). In all seasons, low resistance to penicillin was observed in the early morning (6:00-9:00) and evening (16:00-21:00) hours (Fig. 2). In spring, summer, winter, and autumn the morning latencies were 37.60±1.39 sec, 43.40±0.60 sec, 69.40± 1.64 sec, and 70.00±1.95 sec, respectively. Low resistance to penicillin in the morning can be explained by low level of melatonin at this period. In rats, melatonin, a humoral regulator of biorhythm [10], exhibits anticonvulsivant activity [11]. High resistance to convulsant was observed in autumn (latency 188.80± 3.43 sec) and spring (94.80±1.99 sec) during daytime (9:00-15:00). By contrast, in summer and winter, high convulsion resistance was observed in the evening and night hours (21:00-24:00), the corresponding latencies were 157.60±1.39 sec and 200.00±1.97 sec ($p<0.05$).

Comparison of latencies of mouse seizures and seizure activity of neurons in cerebellar sections revealed reciprocal relationships between the examined indices for each season and daytime. The shorter ES latency in animals, the longer latency of neuronal seizure activity in cerebellar sections in the same period

(Fig. 3). Cerebellum is an antiepileptic structure, which prevents appearance of convulsive discharges in epileptogenic brain regions, first of all in the cortex [2]. If the epileptogenic neurons are controlled and inhibited by cerebellar neurons, the excitability of both neuronal populations in different time of day and various seasons as well as convulsion resistance should be in antiphase, which agrees with our findings. In the period of day, when cerebellar neurons are most active, they inhibit epileptogenic neurons in other brain regions. On the contrary, the inhibitory state of cerebellar neurons does not prevent the development of ES in the epileptogenic structures. These data demonstrate a possibility of efficient treatment of epilepsy by directed action on cerebellar neurons (with due account for the seasonal and circadian rhythms of their activity).

Therefore, individual cerebellar neurons and the system incorporating them have oscillatory properties, which are manifested even *in vitro*. Adaptive reactions to convulsive agents develop not only at the organism level, but also in neuronal populations. These cell populations are genetically programmed for specific functions in a certain biological period, but also for counteracting extreme environmental factors such as seasonal and circadian oscillations of solar activity, varying temperature, and the presence of chemicals, which change functional state of the nervous system.

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