Threshold of Seizure Activity of the Brain and Higher Nervous Activity in the Postresuscitation Period for Intracerebral Allotransplantation of Fetal Neocortex Tissue

V. V. Semchenko, S. I. Ereniev, and K. K. Makovetskii

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 121, № 2, pp. 234-237, February, 1996
Original article submitted June 16, 1995

The threshold of seizure activity of the brain, long-term memory, and learning ability are studied in Wistar rats for bilateral transplantation of fetal nervous tissue in area CA1 of the hippocampus. The grafts are performed on the 2nd, 7th, 14th and 30th days after clinical death caused by asphyxia. A neurotransplantation performed on the 2nd day of the postresuscitation period is found to prevent seizure activity, whereas that performed on the 7th-14th days results in a sharp decline or cessation of spontaneous and induced epileptiform convulsive seizures, prolonged preservation of the long-term memory trace, an improvement of learning ability, and a lessening of defensive and phobic behavior in a large proportion of the animals.

Key Words: *seizure activity; higher nervous activity; postresuscitation period; fetal nervous tissue transplantation*

The homeostatic control mechanisms regulating the seizure readiness of the brain are known to fail after clinical death (CD), resulting in spontaneous convulsive seizures and enhanced sensitivity to the epileptogenic effect of different stimuli, as well as a lowering of the threshold of brain seizure activity (TBSA) and disturbances of long-term memory [9,11]. Other sequelae in the postresuscitation period are disturbances of the cerebral microcirculation and metabolic processes, a reduction in the density of neurons and synapses, reorganization of the synaptoarchitectonics, and a shrinking of the receptive area of neurons in the neocortex, hippocampus, amygdaloid complex, and ventrolateral nucleus of the thalamus [8,9]. It is known that transplantation of fetal nervous tissue leads to a TBSA increase, and improvement of higher nervous activity values in rats of the Krushinsky-Molodkina line with a genetically determined high brain seizure readiness and focal lesions of the hippocampus and amygdaloid complex [4,5]. However, the influence of neurotransplantation on TBSA and higher nervous activity for diffuse lesions of the brain in the postresuscitation period after CD has been analyzed insufficiently.

The aim of this investigation was to study the effect of intracerebral allotransplantation of fetal neocortex tissue on the TBSA and higher nervous activity on rats after CD.

MATERIALS AND METHODS

CD was modeled on animals under ether anesthesia by interruption of the systemic blood flow as a result of 6-min mechanical asphyxia [12]. The animals were revived with closed chest massage and artificial pulmonary ventilation.

TBSA was determined using dosed sound stimuli (86 dB) in a transparent soundproof chamber 1 day after the procedure and then every 3rd day for 180

Department of Histology and Embryology, Omsk State Medical Academy (Presented by V. A. Negovskii, Member of the Russian Academy of Medical Sciences)

0007-4888/96/0002-0217$15.00

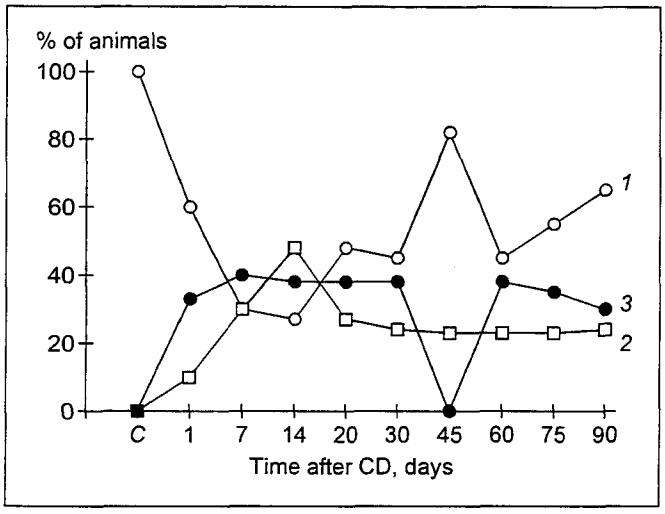

Fig. 1. Time course of sensitivity to epileptogenic effect of an audiogenic sound stimulus at different times of the postresuscitation period in mature Wistar rats. Here and in Fig. 2: *1*) 0 points, no motor reaction; *2*) 1 point, motor excitation; *3*) 3-4 points, convulsive seizures. *C* - controls, intact animals. *CD* - clinical death.

days [6]. The intensity of the audiogenic motor reaction was evaluated in points. The number of waves of motor excitation, the latency of the reaction, the duration of the tonic and clonic phases of convulsions, and of postparoxysmal motor excitation or inhibition, and disturbances of the frequency and rhythm of respiration and heart rate were considered.

The functional state of the central nervous system was followed up using a set of behavioral tests characterizing the cognitive function of the brain (learning and memory). Disturbances of brain integrative activity were studied by a modification of the conditioned passive avoidance response (CPAR) [5] using a method [1] with unavoidable punishment. CPAR was elaborated 6 days after resuscitation and neurotransplantation. The initial time that animals stayed in the illuminated compartment of the chamber was fixed at 3 min once a day for 4 days before CPAR formation. CPAR was considered to have been elaborated when an animal remained in the safe compartment for 80% (144 sec) or more of the total time (180 sec). If memory traces were absent during the first 3 days after CPAR formation, the reflex was again elaborated. The number of times reflex formation had to be repeated was taken into account. The durability of the reflex and the time course of its loss over 60 days were examined during the experiment. The acquisition of the habit was verified daily for a period of 15 days, then every 5 days to the 30th day and then every 15 days to the 60th day after conditioning. Vertical and horizontal motor activity, number and duration of groomings, number of standstills, and number of defecations and urinations were considered in the course of test time.

Neurotransplantation was performed by a routine procedure [4,5]. Wistar rat fetuses of 15-17 days of development were donors. Female rats with a precisely dated term of pregnancy were used. Fetuses were removed under sterile conditions under chloral hydrate anesthesia, after which they were decapitated, the skull was opened, and the brain was excised. Sensorimotor cortex fragments without the dura mater were minced with a razor blade. Two or three pieces (2-3 mm^3) with 0.5 ml of physiological saline were taken into a glass needle and bilaterally injected through a trephination aperture into area CA1 (*PA*=-3.75; *L*=2.4; *H*=2.75 mm) of the hippocampus [15] using stereotactic apparatus. As a preliminary procedure we mapped out the track with a glass needle according to the given coordinates and arrested bleeding. The interval between fetal sampling and graft injection was 15-20 min. After the transplantation the animals were injected i.m. with bicillin-3 (10,000 U).

Neurotransplantation was performed on the 2nd, 7th, 14th, and 30th days after CD. Besides intact animals, sham-operated animals were used as controls (bilateral puncture with a glass needle according to the given coordinates), as well as animals not subjected to CD with transplantation of fetal neocortex tissue, liver, and skeletal muscles and animals after CD with transplantation of liver and skeletal muscles on the 7th day of the postresuscitation period. The values of the parameters in these animals did not reliably differ from those of control animals.

The experiment was performed on 180 mature male Wistar rats held under standard vivarium condi-

TABLE 1. Learning Ability of Mature Wistar Rats for Bilateral Allotransplantation of Fetal Nervous Tissue in Area CA1 of Hippocampus at Different Times during the Postresuscitation Period

Intervention, day		Number of repeats needed for CPAR formation	Animals requiring reconditioning, %
CD	3.2±0.2	100.0	
CD+neurotransplantation,	2nd1.5±0.4*	80.0	
	7th	1.7±0.3*	70.0
	14th	1.6±0.4*	80.0
	30th	2.6±0.5	80.0
Intact animals	0	0.0	

Note. CD - clinical death. *Differences from intact animals and animals after CD is reliable ($p<0.05$).

tions and initially insensitive to the epileptogenic effect of the dosed sound stimulus. Data with a morphologically verified stereotactic effect and engraftment of the transplant were analyzed. The graft "took" in 80-90% of cases, depending on the time of transplantation after CD. The results were processed statistically using the Student *t* test and the reliability of differences between the frequencies of the effects in experimental and control groups was determined [2].

RESULTS

Threshold of brain seizure activity. The state of CD considerably lowered TBSA in 58.7% of the rats. Some (23.1%) animals demonstrated spontaneous convulsive seizures in the first week after CD. The sensitivity to the epileptogenic effect of the sound stimulus showed a wavelike rise with a peak on the 1st, and 28th-30th days after CD (Fig. 1) and there were audiogenic convulsive seizures up to the end of the experiment. Spontaneous convulsive seizures were not recorded in 12.5% of animals, but audiogenic convulsive seizures appeared one day after CD and during the first week of the resuscitation period were recorded in response to each sound stimulus. Then they tapered off and from the 40th-45th days were absent. No spontaneous or sound-induced convulsive seizures were noted in 23.1% of cases. These animals demonstrated a partial TBSA drop, expressed as audiogenic motor excitation which was not transformed into a convulsive seizure. Bilateral intracerebral allotransplantation of fetal neocortex tissue, performed at different times during the postresuscitation period, produced an unidirectional effect on TBSA. The variation of TBSA for neurotransplantation was wavelike. No audiogenic seizures appeared during 28 days after CD for neurotransplantation on the 2nd day of the postresuscitation period, and then TBSA fell and remained low until the 40th-45th day. After that TBSA rose once again and convulsive seizures were not recorded up to the end of the experiment. No audiogenic convulsive seizures were observed during 22 days after CD for neurotransplantation on the 7th (Fig. 2) and 14th days of the postresuscitation period, TBSA dropped by the 30th day, and there were no convulsive seizures from the 40th-45th day. Audiogenic seizures were observed in some animals up to the end of the experiment in the case of neurotransplantation on the 30th day of the postresuscitation period. In comparison with animals subjected to CD without a neurotransplant, in animals which did receive a transplant and reacted to the sound stimulus the audiogenic motor reaction was significantly decreased, the latency of motor excitation increased, the duration of the tonic and clonic phases of audiogenic convulsive seizures decreased, and the

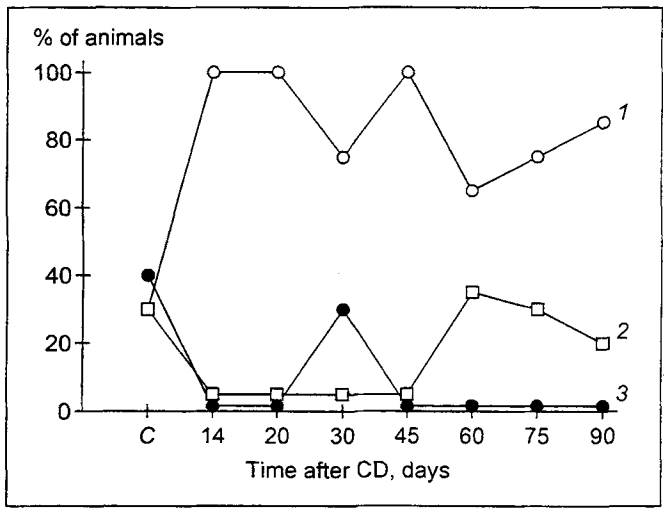

Fig. 2. Time course of sensitivity to epileptogenic effect of an audiogenic sound stimulus at different times of the postresuscitation period in mature Wistar rats for bilateral allotransplantation of fetal neocortex tissue in area CA1 of hippocampus on the 7th day after CD caused by mechanical asphyxia. *C* - control, 7th day after CD.

recovery time for the frequency and rhythm of heart rate and respiratory movements reduced, as was the period of postseizure excitation and inhibition.

Long-term memory. Memory traces remained in 60% of intact rats up to the 60th day after CPAR formation. CD caused a marked activation of defensive and phobic behavior, which was expressed in prolonged standstills (10-180 sec), a reduction of orienting-exploratory activity, a decreased number of explorations of the dark compartment of the chamber and of rearings, and an increased frequency of defecations and urinations. This led to a significant increase of the time spent in the illuminated compartment of the chamber in all observation periods in comparison with the control group, which made it difficult to estimate memory trace preservation in the postresuscitation period by the CPAR method and necessitated the use of other methods of evaluating cognitive function after CD. The duration of the stay in the illuminated compartment of the chamber 15 days after CPAR formation did not differ from the control value for neurotransplantation at all times after CD. Orienting-exploratory activity increased and defensive and phobic behavior decreased.

Learning ability. CPAR was formed from the 1st test in all intact rats (Table 1). After CD CPAR was formed after 2-5 tests (mean number 3.2±0.2). The response was elaborated after the 1st test in 20% of cases for neurotransplantation on the 2nd day after CD, in 30% of cases for neurotransplantation on the 7th day, and in 20% of animals for neurotransplantation on the 14th and 30th days. Thus, the neurotransplantation facilitated CPAR formation and increased learning ability in 20-30% of animals depending

on the time during the postresuscitation period when the procedure was performed.

It is noteworthy that the neurotransplantation affected animal lethality in the postresuscitation period. Among the resuscitated animals without a neurotransplant, 16% died of inflammatory disease of the respiratory organs and lymphadenitis by the end of the 3rd week after resuscitation and 50% by the end of the 7th week. No lethal outcomes were recorded in animals which received a neurotransplant on the 7th, 14th, or 30th day of the postresuscitation period. Neurotransplantation performed on the 2nd day after CD did not lower the lethality in the postresuscitation period.

Thus, bilateral allotransplantation of fetal neocortex tissue in area CA1 of the hippocampus performed at different times after resuscitation has a significant effect on the threshold of seizure activity of the recipient brain. The level of seizure activity fell off markedly in all animals which received the transplant early during the postresuscitation period (2nd, 7th, and 14th days), and heightened seizure activity was preserved to the 180th day of the postresuscitation period in some animals which received the graft later (on the 30th day after resuscitation). Some animals demonstrated a temporary increase of seizure activity on the 23rd-29th day after neurotransplantation, followed by a TBSA rise from the 40th-45th day. These processes reflected the integration of the maturing graft and the brain of the recipient as well as individual features of adaptation of the recipient brain to the graft. An improvement of long-term memory and learning ability in some animals and a lessening of defensive and phobic displays in the postresuscitation period were also observed. These were due to a reduction of destructive and dystrophic changes of neurons and glial cells, stimulation of compensatory-recovery processes in the nervous tissue, reconstruction of damaged interneuronal connections, and restoration of the structural integrity and functioning of the recipient brain [7].

Such an effect of the neurotransplant is related to its neurotrophic and neurotransmitter influence, the prevention of dystrophy and destruction of neurons, stimulation of the recovery of dendrites of reactively altered neurons of the sensorimotor cortex and hippocampus, recovery of the blood-brain barrier, decrease of lipid peroxidation in the brain tissue, establishment of interneuronal connections with the recipient brain, and normalization of the immune reactions in the postischemic period [3,7,10,13,14].

Neurotransplantation performed on the 2nd day of the postresuscitation period was found to prevent seizures, whereas when it was performed on the 7th-14th days it resulted in a sharp reduction or cessation of spontaneous and induced epileptiform convulsive seizures, complicating the course of the postresuscitation period.

The differences in the effect of neurotransplantations performed at different times of the postresuscitation period on the threshold of seizure activity and higher nervous activity are evidently due to peculiarities of the structural and functional state of the recipient brain at these times.

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