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Threshold of the Sensitivity to a Epileptiform Stimulus for Intracerebral Allotransplantation of Embryonal Nervous Tissue of Various Ergicity

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A lowering of the threshold of neuron spastic activity and a rise in the brain spastic readiness under various extra- and intracerebral influences on man and animals [10] have been found to be accompanied by brain atrophy as well as a reduction of the numerical density of neurons and synapses in the cerebral cortex, hippocampus, amygdaloid complex, and other brain structures [4,15] which are arbitrarity grouped together as multilevel "epileptic system" [9]. In the epileptic brain considerable alterations in transmitter exchange have been found [7]. The necessity of a search for fundamentally new methods of therapy for this disease is due to the complexity of the pathogenetic mechanisms of brain epileptization.

Increasing the numerical density of neurons of different ergicity in the brain structures with a pheno- and genotypically-caused low threshold of spastic activity for the purpose of reconstructing interneuronal bonds, decreasing the afferent impulse deficit, correcting transmitter exchange, as well as raising the threshold of neuron excitability by means of embryonal nervous tissue grafts is one of the approaches which have obviated destructive intervention in the brain [2,3,12,14].

The lack of fundamental investigations concerning the influence of embryonal nervous tissue (ENT) on the level of brain spastic activity complicates the effective use of the neurotransplantation method with respect to epilepsy. There is no comparative analysis of the effect of ENT of various ergicity on the threshold of brain spastic activity (TBSA). The purpose of the present work was to study the influence of intracerebral allotransplantation of embryonal neocortex, hippocampus, septum, cerebellum, and substantia nigra tissue in animals with pheno- and genotypically-caused low TBSA.

MATERIAL AND METHODS

Two hundred two rats weighting 190-210 g were used in the experiments. Wistar rats with the hippocampus, dentate gyrus, and amygdaloid complex destroyed partially by injection of a relatively large volume (0.1 ml) of physiological solution, as well as albino rats with stable TBSA reduction and epileptiform spastic fits resulting from a 10-min clinical death due to mechanical asphyxia and compression of the vascular bundle near the base of heart [5] were used in the capacity of animals with a phenotypically caused low TBSA. Rats of the Krushinskii-Molodkina (KM) line were used in the capacity of animals with a genotypically-caused low TBSA.

Rat embryos of the same lines of 17-19 days of embryonic development were ENT donors. Embryonal brain tissue fragments (3-4 mm^3) without the dura mater in 0.01 ml of physiological solution were injected stereotactically [13] through the trephination aperture with a glass needle at the CA_1 (AP 4.75; L 2.4; H 2.75) and CA_4 (AP 6.0; L 3.5; H 7.0 mm) sites of the left and right hippocampus areas and adjoining sites of the dentate gyrus, as well as in a basolateral section of the right and left amygdaloid complexes (AP 3.25; L 4.5; H 8.0).

The sampling and the extraction of brain structures of different ergicity [11] (neocortex, hippocampus - polyergic, septum - mainly cholinergic, cerebellum - mainly GABA-ergic, substantia nigra - dopaminergic neurons) and the graft were performed under sterile conditions under chloral hydrate anesthesia. The graft was injected 15-20 min after the embryo sampling. Morphological verification of the precision of the stereotactical manipulations and of the "taking" of the graft were performed. Besides intact animals, pseudooperated animals were used as controls (uni-

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and bilateral puncture of parts of the hippocampus, amygdaloid complex, and adjacent parts of the dentate gyrus with a glass needle and the injection of 0.01 ml physiological solution, unilateral destruction of these sites, and the transplantation of embryonal liver and muscle tissues to these areas).

TBSA dynamics during 120-140 days was determined using dosed threshold (86 dB) acoustic epileptiform stimuli after Krushinskii [6] from the second day after transplantation. The expression of a response motor reaction to a sound stimulus was evaluated in points; the time of the latent period, as well as the duration of the tonic and clonic phases of convulsive fits and postparoxygmal motor excitation, disturbances of the frequency and rhythm of respiration and heart rate, and the number of waves of motor excitation were taken into account.

The data were processed statistically using the Student t-test, and the difference between the frequency of audiogenic convulsive fits of rats with and without ENT grafts was determined [1].

RESULTS

No changes in TBSA of the Wistar rats (groups I-III) were found for the unilateral puncture, unilateral destruction, and bilateral puncture of the hippocampus and dentate gyrus (see Table 1). Bilateral destruction of the hippocampus and dentate gyrus sites (group IV) resulted in TBSA reduction in 87.5% of animals and to the appearance of epileptiform convulsive fits. Transplantation of embryonal hippocampus tissue under these conditions was found to prevent TBSA reduction in 75% of animals. Infrequent motor excitation without transformation into convulsive fits was recorded in the remaining cases. In rats of the K-M line transplantation of embryonal hippocampus tissue (group VI) was found to be accompanied by the absolute cessation of convulsive fits in half the animals; 37.5% of animals demonstrated a far more infrequent and less pronounced response to a sound stimulus.

Transplantation of embryonal septum tissue to destroyed hippocampus sites was shown to prevent TBSA reduction in 91% of the Wistar rats (group VII). In addition, 56.6% of rats of the K-M line (group VIII) demonstrated the absolute cessation of audiogenic convulsive fits, and 22.2% of animals showed a 3-5-fold decrease in the frequency and expression of fits.

Transplantation of embryonal cerebellum tissue into the destroyed hippocampus and dentate gyrus was found to prevent TBSA reduction as well as the appearance of audiogenic convulsive fits in Wistar rats (group IX). Eighty percent of group X (Wistar rats with partial TBSA reduction resulting in motor excitation without transformation into convulsive fits) were found to cease to react to the threshold sound stimulus under the influence of embryonal cerebellum tissue transplantation. Transplantation of embryonal cerebellum tissue in rats of the K-M line (group XI) led to a discontinuation of audiogenic convulsive fits in 90% of animals; in addition, audiogenic motor excitation ceased in 60% of animals.

The unilateral puncture of basolateral sites of the amygdaloid complexes in Wistar rats (group XII) led to TBSA reduction to motor excitation and noncontinuous convulsive fits in 66.7% of animals. No TBSA change due to the unilateral destruction of these brain sites (group XIII) was found.

Temporary TBSA reduction due to the bilateral puncture of these structures (group XIV) was registered

Table 1. Threshold of Sensitivity of Albino Rats to Epileptiform Stimulus for Intracerebral Allotransplantation of Tissue Fragments of Embryonal Hippocampus, Septum, Cerebellum, Substantia Nigra, and Neocortex.

Group	Number of animals	Sensitivity to epileptiform stimulos				Group	Number of animals	Sensitivity to epileptiform stimulos			
		before treatment		after treatment				before treatment		after treatment	
		motor excitation	convulsive fits	motor excitation	convulsive fits			motor excitation	convulsive fits	motor excitation	convulsive fits
I	6	0	0	0	0	XIV	6	0	0	0	2
II	6	0	0	0	1	XV	6	0	0	0	0
III	6	0	0	0	1	XVI	8	0	0	0	0
IV	8	0	0	0	7*	XVII	5	5	0	1*	0
V	12	0	0	0	3*	XVIII	8	0	8	2	2*
VI	8	0	8	0	4*	XIX	23	0	0	10	3
VII	11	0	0	0	1*	XX	6	2	2	2	1
VIII	9	0	9	0	4*	XXI	6	2	1	1	0
IX	10	0	0	0	0*	XXII	5	1	0	0*	0
X	10	10	0	2*	0*	XXIII	6	6	0	0*	0
XI	10	0	10	4	1*	XXIV	9	0	0	4*	1
XII	6	0	0	0	4*	XXV	6	0	3	3	0*
XIII	6	0	0	0	0						

Note: Asterisk: differences from control are reliable ($p<0.05$).

in 33.3% of cases. Bilateral destruction of these structures (group XV) caused no changes in TBSA.

In no cases was TBSA reduction detected due to the transplantation of embryonal substantia nigra tissue in rats with destroyed sites of the amygdaloid complex (group XVI). Eighty percent of Wistar rats with a partial TBSA reduction (group XVII) ceased to react to a sound epileptogenic stimulus after the transplantation. In addition, 75% of rats of the K-M line (group XVIII) showed absolute cessation of audiogenic convulsive fits and 50% also showed cessation of audiogenic motor excitation.

On the first day after clinical death resulted from mechanical asphyxia (group XIX) 13% of animals demonstrated TBSA reduction to convulsive fits and 43.5% showed TBSA reduction to motor excitation. Transplantation of embryonal neocortex tissue in CA_1 and CA_4 right and left hippocampus sites 2 days after reanimation (group XX) caused no TBSA increase. Transplantation 7 and 14 days after reanimation (group XXI,XXII) was shown to prevent TBSA reduction in all cases. Transplantation on the second day after reanimation (group XXIII) resulted in TBSA increase in rats with partial TBSA reduction before clinical death.

After clinical death resulted from the compression of the vascular bundle at the base of the heart (group XXIV), 11.1% of animals showed TBSA reduction to convulsive fits and 44.4% to motor excitation. Transplantation of embryonal neocortex tissue 7 days after clinical death (group XXV) resulted in TBSA increase in all cases.

Therefore, transplantation of embryonal nervous tissue has proven to effect threshold of spastic activity of the brain. The TBSA increase observed in animals with a phenotypically-caused low TBSA 4-6 days after transplantation is assumed to be associated with changes in metabolism in the recipient brain under the influence of neurotrophic factors secreted by the graft, which enchance the regeneration processes in the recipient brain, as well as with the reduction of reactive gliosis, acceleration of wound closing, and, eventually with the reconstruction by the graft of damaged intraneuronal bonds [8].

When transplanted into the preliminarily destroyed hippocampus, dentate gyrus, and amygdaloid complex, grafts containing mainly monoergic neurons, in particular, grafts of the septum (cholinergic), cerebellum (GABA-ergic), or substantia nigra (dopaminergic), were found to cause a larger TBSA increase as compared to grafts with a primarily polyergic neuron content (hippocampus).

In the case of clinical death followed by diffuse injury to the brain, transplantation of embryonal neocortex with polyergic nerve cells content was found to be the most effective with respect to TBSA increase.

In rats of the K-M line the later TBSA increase (39-44 days after transplantation) is due to the more complicated epileptogenesis in the case of genotypically-caused TBSA as well as to the formation by this time of connections between the graft and the recipient brain, the connection of transmitter exchange, the recovery of structural integrity, and the reduction of the functional deficit of recipient brain.

On the basis of the investigations carried out, the development of the functional-reconstructive method using embryonal nervous tissue of different ergicity has proven to be promising for the correction of pheno- and genotypically-caused high spastic activity of the brain.

LITERATURE CITED

1. A.I. Venchikov and V.A. Venchikov, *Main Principles of the Statistical Treatment of the Results of Observations in Physiology.* [in Russian] Moscow (1974).
2. R.I. Genne, S. I. Ereniev, and A.E. Nikel, *3rd Int.Symp. "Functional Neurosurgery".* [in Russian] Abstract. Tbilisi (1990) p. 74-75.
3. S.I.Ereniev, *Disturbances of the Regulatory Mechanism in Extremel and Terminal States.*[in Russian] Omsk (1991) p.22-32.
4. S.I.Ereniev, R.I.Genne, V.V.Semchenko, *et al., Zh.Neuropat.i.Psikh.*,**90,** No. 10, 45-48 (1990).
5. V.G.Korpachev, S.P.Lysenkov, and L.Z.Tel, *Pat.Fiziol.*, No. 3, 78-80 (1982).
6. L.V.Kryshinskii, *Formation of Animal Behaviour in the Norm and Pathology.*, [in Russian] Moscow (1960).
7. V.K.Pozdeev, *Transmitter Processes and Epilepsy.*, [in Russian] Leningrad (1983).
8. L.V.Polezaev and M.A.Aleksandrova, *Transplantation of Brain Tissue in the Norm and Pathology.*, [in Russian] Moscow (1986).
9. Yu.N.Savchenko and R.I.Genne, *Zh.Nevropatol.i Psikh.*, **81**, No. 7, 859-865 (1981).
10. V.V.Semchenko, S.I.Ereniev, S.S.Stepanov, *et al., Arkh.Anat.Gistol.Embriol.*, **99** No. 7, 40-45 (1990).
11. D.Eccles, *The Inhibitory Pathways of the Central Nervous System.*, Liverpool Univ.(1982).
12. S.I.Ereniev, V.V.Semchenko, G.V.Nikel, *et.al., Soviet-Indian Symp.on Neurotransplantation and Developmental Neurobiology, July 1-5, 1991:Abstracts.*, Puschino (1991) p. 6-7.
13. G.Paxinos and Ch.Watson, *The Rat Brain in Stereotactic Coordinates.* Acad Press., Toronto (1982).
14. Yu.N. Savchenko, R.I.Genne, S.I.Ereniev, *et al., 9th European Congress of Neurosurgery: Abstracts.* Moscow (1991) p. 339.
15. V.V.Semchenko, S.I.Ereniev, S.S.Stepanov, *et al., Constituent Congress of International Society for Pathophysiology, Moscow, May 28 - June 1, 1991;* Abstracts. Kuopio, Finland (1991) p. 334.