The Use of Neural Transplantation for Suppression of Seizure Activity in Genetically Epilepsy-Prone Rats

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The possibility of correcting seizure activity with neural transplantation was studied in Wistar rats with audiogenic seizures and in Krushinskii—Molodkina rats with high level of audiogenic seizures. In Wistar rats seizures were absent during 24 weeks after combined bilateral transplantation of striatal and cerebellar tissue from newborn rats into the parietal cortex. The same transplantation performed in Krushinskii--Molodkina rats increased the latency of audiogenic seizures. In some rats the intensity of seizures decreased, but they did not completely disappeared. Suppression of seizure activity in Krushinskii—Molodkina rats was observed after transplantation of striatal and cerebellar tissue simultaneously into the parietal cortex and inferior colliculi.

Key Words: *transplantation; epilepsy; audiogenic seizures*

Neural transplantation is widely used in experimental studies and in clinical practice for compensation of disturbed brain functions [6,11]. However, the potency of neural transplantation to suppress seizure activity and correct behavioral functions in epilepsy has come under the scrutiny of science only recently.

Experimental seizures in animals can be evoked by chemical and electrical stimulation of the brain. There are several models of convulsive and non-convulsive genetic epilepsy. Different epilepsy subtypes are explained by imbalance of excitatory and inhibitory neurotransmitters. Convulsive epilepsy is mainly related to deficiency in the inhibitory transmitters (GABA, taurine, glycin, *etc.*)

In many papers on neural transplantation, the embryonic tissue isolated from various brain subdivisions and characterized by different transmitter functions was used for inhibition of seizure activity [13]. Inhibitory effect of neural transplants on chemically or electrically evoked seizures was observed mainly after transplantation of tissue isolated from noradrenergic or GABAergic cerebral structures [13], although this effect was weak or transient. Audiogenic seizures (AS), a form of genetic convulsive epilepsy [13], were also resistant to this treatment. In our studies on Wistar rats, transplantation of cultured embryonic cerebellar tissue into the amygdala damaged by kainic acid decreased audiogenic sensitivity, while transplantation of embryonic striatal tissue increased the density of benzodiazepine receptors [3]. The role of the cerebellum and striatum in suppression of seizure activity was described elsewhere [8]. These structures produce inhibitory transmitters (GABA and taurine in the cerebellum and GABA and glycin in the striatum) [15]. Our aim was to decrease seizure activity by transplantation of tissue isolated from two inhibitory structures: cerebellum and striatum.

It was hypothesized that the forebrain and brain stem are involved in the realization of generalized seizures [10]. An important role is also played by the cerebral cortex, in which pronounced electrophysiological and neurochemical changes were observed during seizures [8,14]. Among brainstem structures, the basic role in the generation of AS is played by inferior colliculi (IC) [12]. In this connection, the cerebral

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cortex and IC were chosen as the acceptor region for transplantation.

MATERIALS AND METHODS

The study was carried out on 59 male Wistar rats (initial weight 200 g) selected by enhanced audiogenic sensitivity (AS-Wistar) and on 34 male Krushinskii—Molodkina (KM) rats (initial weight 250 g) with pronounced AS. The rats were kept 3-5 per cage at natural illumination and food and water *ad libitum*. The tests were performed in a 40×30×30-cm box. AS were provoked by a complex audio signal, which had many peaks in the frequency range of 13-85 kHz (maximum within 20-40 kHz, mean intensity 50-60 dB, amplitude of peaks 80-90 dB) [6]. The duration of acoustic stimulation was 1.5 min. Intensity of provoked AS was scored [4]: no reaction (0), pronounced motor excitation, wild running, and jumping (1), motor excitation followed by clonic seizures in supine position (2), continuation of seizures in the lateral position (3), and culmination of the previous phases with tonic seizures (4).

Stability of audiogenic reactions was determined before surgery in repeated tests (3 times with a 1-3-week intervals).

The donor material was isolated from the brain of 1-3-day-old newborns of healthy parents by dissecting the chosen structure and cutting it to 0.1-0.3-mm^3 fragments. The donor tissue was injected via a glass needle (0.4-0.6 mm in diameter) connected to a syringe and filled with physiological saline. During surgery the recipient rats were narcotized with nembutal (30-40 mg/kg) and fixed in a stereotaxic apparatus.

Striatal and cerebellar tissue (SCT) and cerebral cortex were used for transplantation in different experimental series. The tissue (0.5 mm^3 in a 10-μl isotonic NaCl) was injected bilaterally into the parietal cortex and/or IC. Transplantation of cortical tissue served as the control for other experiments. Injection of 10 μl of isotonic NaCl (0.9%) according to the protocol used in transplantation served as the negative control. The tests were performed 1, 3, 10, 16, 20, and 24 weeks postoperation.

After the end of the experiments, the rats were decapitated under deep nembutal narcosis. The brain was isolated and fixed in 10% formaldehyde on phosphate buffer (pH 7.2-7.4). Frontal serial sections of the brain (30 μ) were prepared on a freezing microtome and stained with thionin and crezyl violet by the method of Nissl. Graft take was studied using donor tissue stained with fluorescent dye bisBENZIMIDE (Hoechst). In this case, the rats were sacrificed 2 months after transplantation. Morphological state of the transplants and the recipient brain was analyzed under an MBS-9 and Axioplan (Opton) microscopes.

The results were analyzed statistically using STATGRAFICS software. The groups were compared according to the excitability level (χ^2 test) and the presence or absence of seizures (Fisher's exact test). The improvement in the state of recipients was evaluated by excitation level before and after transplantation using Mann—Whitney *U* test and the dynamics of this parameter was assessed by Wilcoxon's rank test.

RESULTS

Audiogenic sensitivity in AS-Wistar rats is considerably lower than in KM rats [6]. In a large group of Wistar rats (n=370), acoustic stimulation induced moderate AS in 40% animals. In 10% AS rats subjected to triple testing performed in repeated tests with 1-3-week intervals, the seizure response was unstable. These rats were sorted out.

AS-Wistar rats developed two-wave seizure [4,6]: the first and second waves were observed 15-20 sec and 40-50 sec after acoustic stimulation, respectively. In 60% AS Wistar rats the intensity of seizures was 1 point, in 35% it was 2-3 points (clonic phase), and in 5% it reached 4 points (tonic phase).

In control rats, injection of physiological saline into cerebral cortex did not significantly change audiogenic sensitivity (n=15, p>0.05 in all tests, Fig. 1, *a*). A decrease in the intensity of seizures was observed for 3 weeks after combined transplantation of SCT into parietal cortex ($p<0.01$ compared to control) followed by pronounced suppression of seizures in the following period ($p<0.001$, Fig. 1, *b*).

Acoustic stimulation of KM rats before neural transplantation provoked severe AS, developed according to a classical scheme: the reaction started 2-5 sec after stimulation with a short, but severe motor excitation followed by clonic and tonic seizures (3-4 points). Transplantation of SCT into the cerebral cortex of KM rats did not change significantly seizure intensity, although in 4 of 8 rats this parameter decreased by 1-2 points, and in all rats seizure latency increased to 20 sec and more. Seizure in these rats did not disappear. This group of rats was described in details previously [2]. Transplantation of SCT into IC prevented seizures in 4 of 7 rats, although this effect was weak and unstable. Suppression of AS was observed in 5 of 38 tests (13%) and in 13% cases AS intensity decreased by 1-2 points. The best effect was observed after transplantation of SCT simultaneously into the parietal cortex and IC. AS were absent in 40% tests performed in all 8 rats. In 19% cases AS intensity decreased by 1-2 points. The rats with eliminated AS can be subdivided into 3 categories: 1) stable elimination of seizures (3 rats, up to 83% tests), 2) unstable effect (2 rats, up to 43% tests), 3) only a sin-

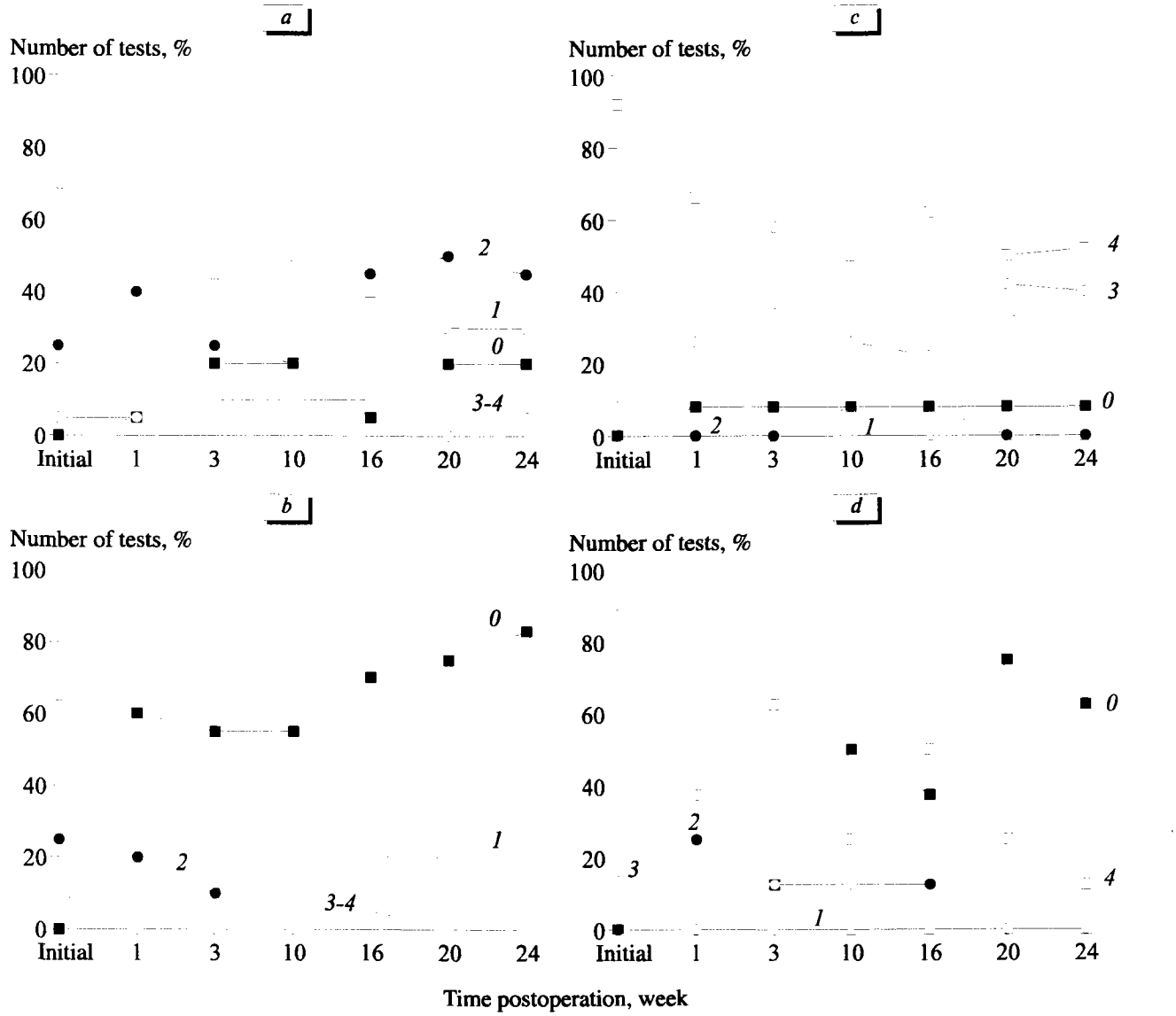

Fig. 1. Effect of transplantation on intensity of audiogenic seizures in Wistar (*a*, *b*) and Krushinskii—Molodkina rats (*c*, *d*). *a* and *c*) control, *b* and *d*) transplantation of striatal and cerebellar tissue into the cerebral cortex and inferior colliculi, respectively. Audiogenic sensitivity is scored in points: no reaction (*0*), running (*1*), clonic seizures (*2* and *3*), and tonic seizures (*4*).

gle-pass elimination of seizures (3 rats). No significant changes in the latency and intensity of AS was observed in the control KM rats receiving the corresponding volume of isotonic NaCl or cortical tissue.

Morphological control was carried out 6 months after neural transplantation. Only in one AS-Wistar rat pronounced tissue necrosis developed at the site of transplantation and no transplants were found. In other AS-Wistar rats and in KM rats transplants were found in one or both hemispheres (Fig. 2). In KM rats (subgroups 2 and 3), partial necrosis of the transplants was frequently observed. In some cases, the transplant was found in the cerebral ventricles. Hydrocephalus was observed in 2 rats. This probably explains instabile and transient suppressive effect of neural transplantation in these rats.

Thus, the best results were obtained in Wistar rats with transplantation of SCT into the parietal cortex and in KM rats after transplantation of SCT simultaneously into the parietal cortex and IC. The order and nature of AS phases indicate that during the first phase excitation simultaneously involves both hemispheres, while the final tonic phase and concurrent cardiac and respiratory changes attest to extensive generalization of paroxysmal activity to the inferior subdivisions including the brainstem structures. Seizures in Wistar rats were usually limited by the first phase of severe motor excitation, while the tonic phase was absent. In this case, transplantation into the parietal cortex successfully eliminated seizures. In KM rats with high audiogenic sensitivity exhibiting generalized clonic-tonic seizures, this transplantation scheme was less

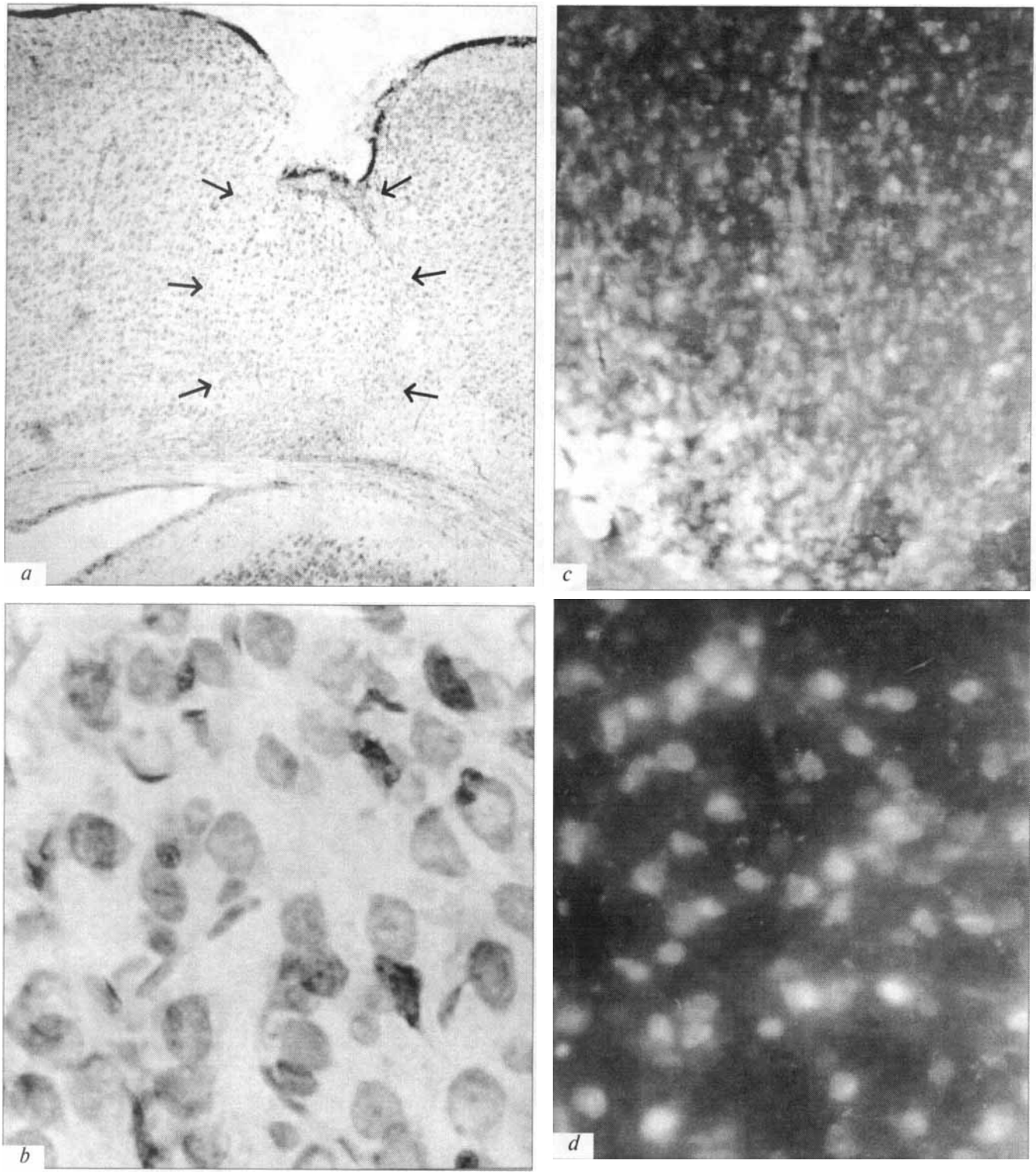

Fig. 2. Transplant of striatal and cerebellar tissue in the cerebral cortex. *a*) general view, ×25. The transplant boundary is shown by arrows; *b*) Nissl-stained cells in the transplant, ×400; *c* and *d*) cells stained with fluorescent dye bisBENZIMIDE in the transplant, ×80 and ×400, respectively.

effective. Clonic-tonic seizures were absent only after simultaneous transplantation into the cortex and brainstem, which means that severe seizures can be prevented only by complex influences at different structural levels of seizure development. The mechanism of therapeutic effect of transplantation is still unknown. Since seizures disappeared only a few days after transplantation, it can be suggested that the transplant ex-

erts some chemical effect on the brain. It is also possible that the transplant isolated from "inhibitory structures" restores the disturbed balance between the excitatory and inhibitory neurotransmitters and suppresses neuroimmune processes inducing the pathogenetic mechanism of the convulsive syndrome [5].

A possible way of transplant action is diffusion of neurotransmitters and trophic factors into the intercellular space [9]. This is also confirmed by the data on a positive effect of transplantation into the cerebral ventricles [7], and by appearance of generalized biochemical changes after neural transplantation [1].

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