MECHANISMS OF CELL PROLIFERATION AND DIFFERENTIATION

Development of 3D Cerebral Aggregates in the Brain Ventricles of Adult Mice

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Abstract—The cerebral organoids are three-dimensional cell cultures formed from brain-specific cell types arising from embryonic or pluripotent stem cells. Organoids provide an opportunity to study the early stages of brain development and diseases of the central nervous system. However, the modeling of organoids is associated with a number of unsolved problems. Organoid production techniques involve a complex cell culture process that requires special media, growth factors, and often the use of a bioreactor. Even under standardized conditions, structures of different morphology are formed: from disorganized cell aggregates to structured minibrains, which are selected for study. For natural reasons, organoids grown in vitro do not have a blood supply, which limits their development. We tried to obtain cerebral aggregates similar to organoids in an in vivo model, where vascular growth and tissue blood supply are provided, for which we transplanted a cell suspension from the mouse embryonic neocortex into the lateral ventricles of the brain of adult mice. Therefore, the medium for cultivation was the cerebrospinal fluid, and the lateral ventricles of the brain, where it circulates, served as a bioreactor. The results showed that the neocortex from E14.5 is a suitable source of stem/progenitor cells that self-assemble into three-dimensional aggregates and vascularized in vivo. The aggregates consisted of a central layer of mature neurons, the marginal zone free of cells and a glia limitans, which resembled cerebral organoids. Thus, the lateral ventricles of the adult mouse brain can be used to obtain vascularized cell aggregates resembling cerebral organoids.

Keywords: cerebral organoids, neocortex, transplantation, GFP, immunohistochemistry, RT-PCR
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INTRODUCTION

The study of brain development in normal and pathological conditions is carried out mainly in laboratory rodents, but when it comes to the human brain, it turns into a very difficult task, since experiments in vivo are impossible to carry out. This barrier can be partially overcome by using immunodeficient animals for transplantation of human neural cells, which can develop in the brain of the recipient for a long time, which allows us to study their differentiation (Han et al., 2013; Mansour et al., 2018). A lot of work on mammalian brain development is carried out on adhesive cultures in vitro (Koo et al., 2019). However, 2D cultivation does not form the three-dimensional organization of nerve and glial cells and the specific spatial intercellular interactions characteristic of the developing brain (Pacitti et al., 2019). Not so long ago, the possibility of creating three-dimensional organoids was demonstrated in vitro (Watanabe et al., 2005; see review by Sukhinich and Aleksandrova, 2020). Cerebral organoids are self-organizing, three-dimensional cellular aggregates composed of brain-specific cell types arising from embryonic or pluripotent stem cells

(Qian et al., 2019). Organoids reproduce morphogenetic processes at early stages of development, including specific cell migration and cytoarchitectonics of the embryonic brain (Eiraku et al., 2011; Mariani et al., 2012; Todd et al., 2013). The organoid model has also proven successful in studying a number of diseases of the central nervous system, including, for example, microcephaly (Lancaster et al., 2013) and even Alzheimer's disease (Gonzalez et al., 2018). However, despite the wide interest in the modeling of organoids, the technology of their reproduction is associated with a number of unresolved problems. First, this is a complex, multistage process of cell cultivation, as a result of which only some organoids acquire a characteristic histotypic organization, and, second, is the absence of vascularization in organoids and some types of cells specific to the brain, which limits its growth. An interesting approach can be the cultivation of organoids in the "natural microenvironment," namely, in the cavities of the ventricles of the brain of experimental animals in vivo. In this model, the need for special nutrient media can be avoided, since they are replaced by cerebrospinal fluid, which is a biologically active

Table 1. Primers used for real-time RT-PCR

Gene	Sequence, 5'–3'
GAPDH	Fv: TGCACCACCAACTGCTTAGC Rv: GGCATGGACTGTGGTCATGAG
ACTB	Fv: CCCTGAACCCTAAGGCCAAC Rv: ATGGCTACGTACATGGCTGG
DCX	Fv: CCCCATGTGTGTGAGGTGTT Rv: GCTCTTTGGCTGCCCAGTAT
GFAP	Fv: TGAATCGCTGGAGGAGGAGA Rv: GCCACTGCCTCGTATTGAGT
NES	Fv: GGCTGTATTCCCCTCCATCG Rv: CCAGTTGGTAACAATGCCATGT
NEUN	Fv: GGATTCGGGTTCGTAACTTTC Rv: GGCGTGACCATCTTCTTGTT
PAX6	Fv: ACCCAAGAGCAGATTGAGGC Rv: TTGGCTGCTAGTCTTTCCCG
SOX2	Fv: TGCGCGCTCTGCACAT Rv: GAAGCGTGTACTTATCCTTCTTCA
SYP	Fv: CCAATCAGATGTAATCTGGTCAGT Rv: AGGCCTTCTCTTGAGCTCTT
TUBB3	Fv: CAGCGGCAACTATGTAGGGG Rv: CCAGGTTCCAAGTCCACCAG

medium (Rahimi and Woehrer, 2017) and has an important regulating effect on progenitor cells during neurogenesis of the cerebral cortex (Lehtinen et al., 2011); it is also possible to use already committed cell types. The aim of this study was to study the ability to self-organize into three-dimensional aggregates similar to organoids of transplanted stem and progenitor cells of the embryonic neocortex during their development in the cerebrospinal fluid of the ventricles of the adult mouse.

MATERIALS AND METHODS

Animals

Transgenic heterozygous C57BL/6-Tg (ACTB-EGFP) 10sb/J mice (Jackson Laboratories, Bar Harbor, ME) and C57Bl/6 mice were used to obtain dated gestation and transplantation. The morning of finding the vaginal plug was recorded as E0.5. At the time of development E14.5 ($n = 4$) and E19.5 ($n = 4$), tissue of the frontal part of the neocortex was isolated from the embryos of transgenic EGFP mice.

RT-PCR

RNA isolation was performed according to the Qiagen commercial RNeasy Kit (Qiagen, #74004) protocol using the Qiagen DNase Kit (Qiagen, #79254) to remove genomic DNA residues. Lysis of neocortex

samples was performed without prior homogenization of the organ. To obtain the first strand of cDNA on the template of isolated RNA, we used a Evrogen MMLV RT commercial kit (Evrogen, #SK021). Due to the different location of amplification targets on the transcript, we used a mixture of random decanucleotide primers and oligo (dT) primers in a 1 : 1 ratio (primers included in the MMLV RT kit). For one reaction, 1 μg of RNA was taken. For real-time RT-PCR, primers were selected using the NCBIPrimer-BLAST online resource (Table 1). Mostly, primers were selected for sequences lying at the junction of exons to avoid amplification of genomic DNA. Real-time PCR was performed using a CFX96 Touch thermal cycler (Bio-Rad). For RT-PCR, a ready-made qPCRmix-HSSYBR mixture from Evrogen was used (Evrogen, #PK147L). Expression was assessed using the 2ΔCt method, where a culture of immortalized mouse fibroblasts 3T3-NIH was used as a control sample. The results were normalized according to the expression of the housekeeping genes GAPDH and ACTB. The 2ΔCt values were used to calculate the change in the expression level of the studied genes: $R = 2^{-\Delta Ct}$, for the convenience of plotting the data obtained were presented in the $\log_{10}(R)$ format.

Transplantation

When preparing a cell suspension for transplantation, neocortex tissue obtained from E14.5 mice (the

number of embryos, $n = 4$), dissociated using Akutase solution (Sigma-Aldrich, United States) and washed in Hanks solution (Gibco). The volume of each graft was 1.5 µL and contained 300000 cells. Adult mice C57Bl/6 ($n = 9$) were anesthetized with isoflurane. The operating surface was shaved, the skin between the ears was cut, and the bones of the skull were thinned. A suspension of the frontal neocortex was injected stereotaxically with a Hamilton syringe. Injections were performed in the following coordinates relative to Bregma: +0.3 mm (anterio-posterior), 1 mm (lateral), 1.5 mm (dorso-ventral). Immunosuppression was not performed.

Preparation of Slides

On days 5, 30, and 90 after transplantation, transcardial perfusion was performed using Phosphate buffered saline (PBS) and 4% paraformaldehyde. Each brain was removed from the skull and incubated overnight in the same fixative. The brain was then washed in PBS and transferred to 30% sucrose in PBS. Coronal sections were obtained using a cryostat (Leica CM1900), the thickness of the sections was 40 µm. The same protocol was used for the brain of E14.5 mouse embryos. Sections were stored in cryoprotectant solution at −20°C.

Immunohistochemistry

Slides with sections were incubated for 1 h at room temperature in a blocking solution: a mixture of 5% normal goat serum (Sigma-Aldrich), 0.3% Triton X-100 (Triton), and 0.01 M PBS (pH 7.4). After that, they were incubated overnight at 4°C in a mixture of a blocking solution and primary antibodies. The following antibodies were used: rabbit anti-NeuN antibodies (Abcam, ab104225, 1 : 500), rabbit antibodies against GFAP (Abcam, ab7260, 1 : 800), chicken antibodies against GFP (MolecularProbes, A-11122, 1 : 500), anti-Sox2 rabbit antibodies (Abcam, ab92494, 1 : 500), rabbit antibodies against DCX (Abcam, ab18723, 1 : 800), rabbit antibodies against Pax6 (Abcam, ab195045, 1 : 500), rabbit antibodies against beta-III tubulin (Abcam, ab18207, 1 : 500), rabbit antibodies against Synaptophysin (Abcam, ab32127, 1 : 600). The sections were then washed and incubated for 2 h in a mixture of 0.3% Triton x-100 (Triton), 0.01 M PBS (pH 7.4), and the following secondary antibodies: goat antibodies against IgY chicken (Abcam, ab150169, AlexaFluor488, 1 : 600) and goat anti-rabbit IgG (Abcam, ab150080, AlexaFluor 594, 1 : 600). For vascular imaging, sections were stained with lectin (Lycopersicon Esculentum) labeled DyLight® 594 fluorochrome (Vector Laboratories, DL-1177-1). The sections were then washed in PBS and the nuclei were stained with DAPI solution (2 µg/mL, Sigma, D9542). Histological images were obtained using a BZ-9000E fluorescence microscope (Keyence, Japan).

Statistical Analysis

Real-time RT-PCR for each sample was performed in four technical repetitions. To assess the differences between the expression patterns in E14.5 and E19.5 cells, the Wilcoxon−Mann−Whitney test was used.

RESULTS

Characterization of Cells of the Embryonic Neocortex

To select the most suitable material for transplantation, the neocortex of embryos at two developmental stages, E14.5 and E19.5, was studied. The method of quantitative RT-PCR in real time showed that there is a statistically significant decrease in the markers of stem cells Sox2 and a tendency towards a decrease in Nes (nestin) at the stage E19.5. At the same time, there is a statistically significant increase in the expression of NeuN and Syp (synaptophysin) compared to E14.5, which indicates the processes of differentiation into neurons. The most important indicator is the change in the expression of mRNA of the astrocyte gene, GFAP, which was not expressed at all at E14.5 in the neurogenic phase of differentiation, while it shows a high level at E19.5 in the gliogenic phase (Fig. 1). It should be noted that stable expression of a marker of undifferentiated neurons is observed at both periods: Dcx (doublecortin) and Tubb3 (beta-III tubulin). To confirm the poorly differentiated status of the E14.5 neocortex, an immunohistochemical study (IHC) was performed using neuronal markers (Fig. 2). The results of IHC analysis showed that cells with neural stem cell (NSC) markers Pax6 and Sox2 are strictly distributed in the V-SVZ (ventricular-subventricular zone) and are absent in other layers of the cortex. Migrating neuroblasts expressing DCX occupy the intermediate area of the cerebral cortex. In the area of the cortical plate, neuroblasts expressing the beta-III tubulin (marker of early neurons') are located. Outside the V-SVZ, all neurons express the synaptic contact marker synaptophysin, since synaptic vesicles are already present in growing axons and their endings, despite the fact that stable synapses do not yet exist at this stage of development. The nuclear marker of mature neurons NeuN is expressed in cells at a very low level, which indicates a small number of differentiated neurons. Thus, the low level of differentiation of cells of the embryonic neocortex E14.5, as well as the absence of astrocytes in it, makes it a more suitable material for transplantation as compared to the neocortex of the E19.5 stage.

Embryonic Neocortex Cells Form Three-Dimensional Aggregates after Transplantation into the Brain Ventricle of Mice

At the time of transplantation, the cells in the suspension were staggered, located singly, or formed small aggregates. After transplantation of a suspension
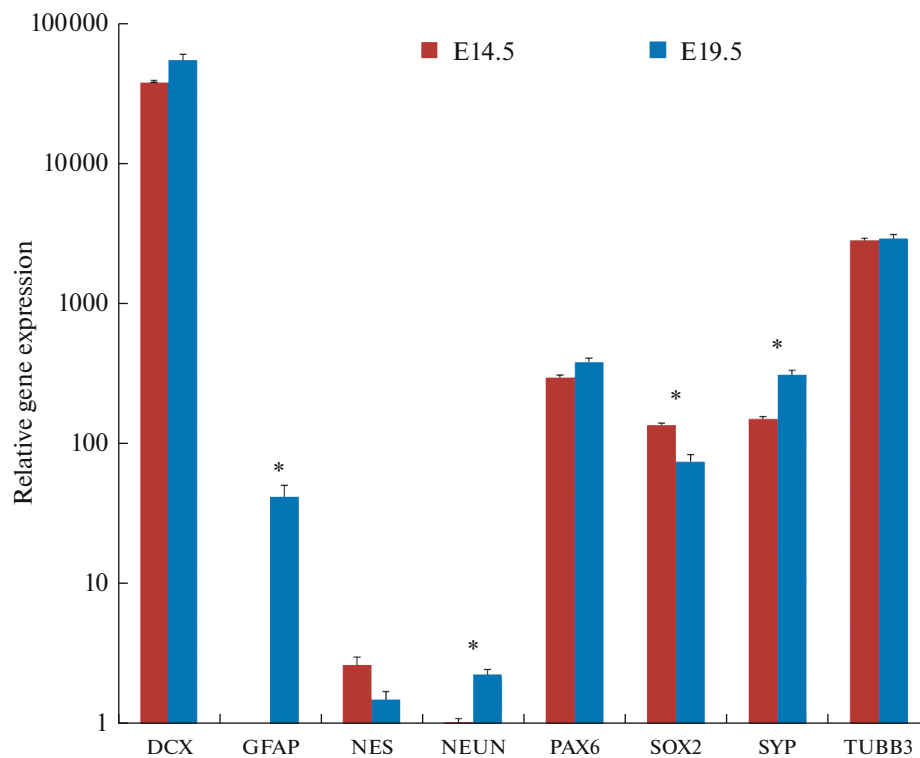
Fig. 1. Comparison of the expression of neural markers in brain cells of mouse embryos at stages E14.5 and E19.5. At stage E19.5, cells become more mature and gliogenesis begins. Data is displayed in $\text{Log}_{10}(\text{R})$ format, where $R = 2^{-2\Delta Ct}$. * $p < 0.05$ by Wilcoxon−Mann−Whitney test, the difference in expression is statistically significant.

of the characterized cells of the mouse embryonic neocortex (stage E14.5) into the brain ventricle of adult mice, morphological examination was carried out 5, 30, and 90 days after the operation. After 5 days, the presence of a graft in the brain ventricle of adult mice was shown (Fig. 3). However, at such an early stage, the grafts were still poorly integrated into the tissue, which led to the "loss" of a number of grafts when working with free-floating sections. Nevertheless, it was possible to show that the transplanted cells are located precisely in the lateral ventricle of the brain, they are not "washed out" by the cerebrospinal fluid, and already form a volumetric graft in which the cells are located chaotically. IHC analysis revealed that the graft cells have a positive reaction to DCX at this time, which indicates the processes of cell migration within the cell aggregate.

By the 30th postoperative day, the grafts significantly increased in size, and migrating DCX$^+$ cells were no longer detected. Despite individual differences in size, the grafts already had a clear cytoarchitectonics. In the central part of the graft, mature neurons positive for the NeuN marker were grouped (Fig. 4), while a marginal zone without NeuN$^+$ neurons, similar to the first layer of the cerebral cortex, was identified along the periphery. At this time of the study, GFAP-positive astrocytes were evenly distributed over

the graft (Fig. 5). Vascular ingrowth into the graft was revealed morphologically.

On day 90, the grafts were well developed. No neuroblasts positive for DCX$^+$ were found in them, which indicated the completion of cell migration and differentiation processes. Differentiated NeuN$^+$ neurons were distributed in the central part of the graft. They were located less densely in the center and denser in the periphery of the central region. According to the size of cell nuclei, it can be assumed that large neurons located on the periphery of the central region are neurons of the V−VI layer of the cortex, which are formed in the first place. On the periphery of the graft, a zone without NeuN$^+$ cells was identified, corresponding to the first layer of the cortex (Fig. 6). The synaptophysin staining for the synaptic contact marker was fairly uniform over the graft tissue (Fig. 7). Sox2$^+$ cells were also evenly distributed over the graft (Fig. 8). The distribution of astrocytes (GFAP$^+$) had a characteristic pattern: uniform distribution over the graft and the formation of a zone of the border glial membrane (glia limitans) at the border between the graft and the ventricle of the recipient's brain. No scar formation was detected at the site of contact between the graft and the recipient (Fig. 9). In addition to the structural organization, extensive vascularization of the graft by blood vessels from the side of the recipient's brain was
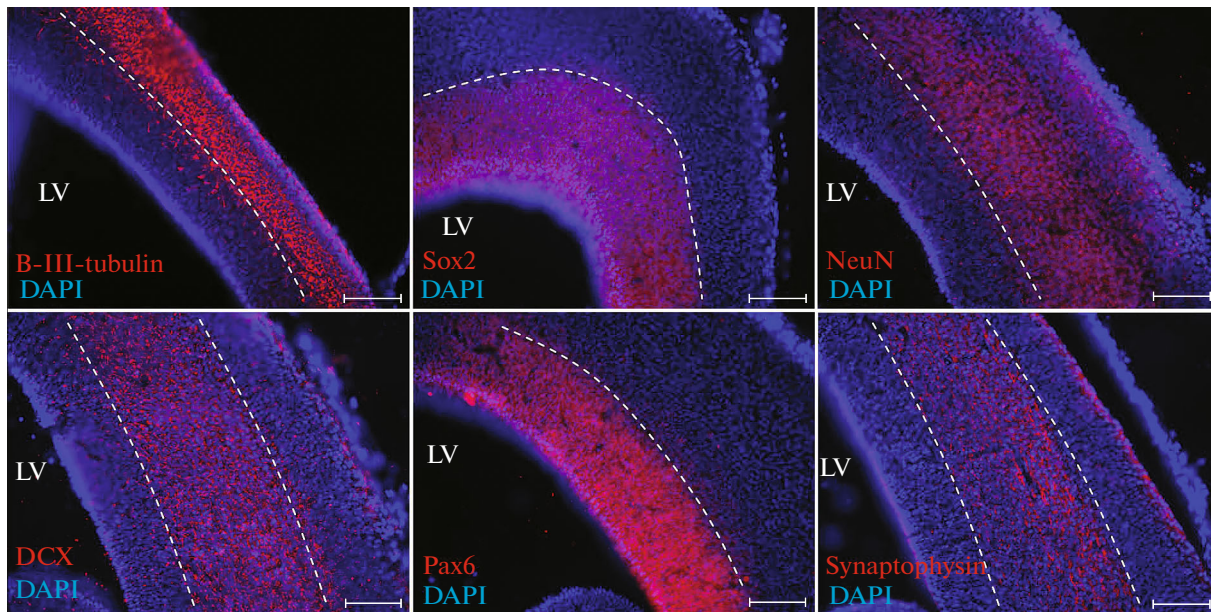
Fig. 2. Immunohistochemical study of the neocortex of a mouse embryo at stage E14.5. Immature neurons (beta-IIItubulin$^+$) are in the cortical plate. DCX$^+$ neuroblasts occupy the intermediate zone, synaptophysin is detected in their axons. Neural stem cells (Sox2$^+$, Pax6$^+$) are detected only in the ventricular and subventricular zones. Mature neurons (NeuN$^+$) are in the cortical plate. LV—lateral ventricle. Scale bar: 100 μm.
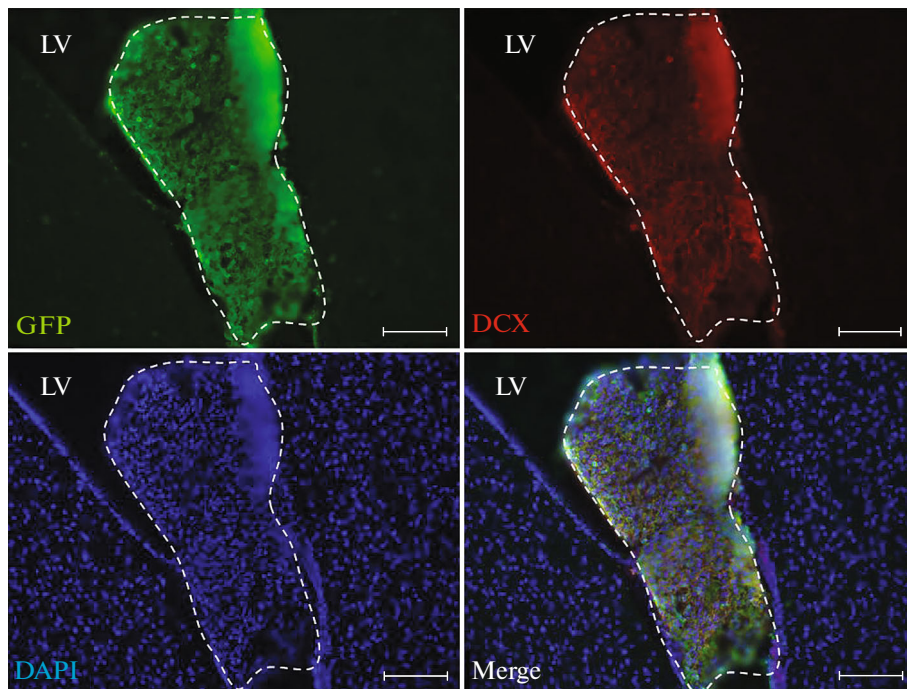

Fig. 3. Graft of cells of the embryonic neocortex of mice (E14.5) 5 days after transplantation into the ventricle of the mouse brain. Positive immunohistochemical reaction of the graft cells to the DCX neuroblast marker. The graft is highlighted with a dotted line. LV—lateral ventricle. Scale bar: 100 μm.

revealed (Fig. 10). At the same time, it was found that DCX$^+$ cells migrate along the vessels in the area of contact between the graft and the subventricular zone of the adult brain. It can be assumed that these are undifferentiated neurons arising from the recipient's own endogenous stem cells (Fig. 11). Cell migration
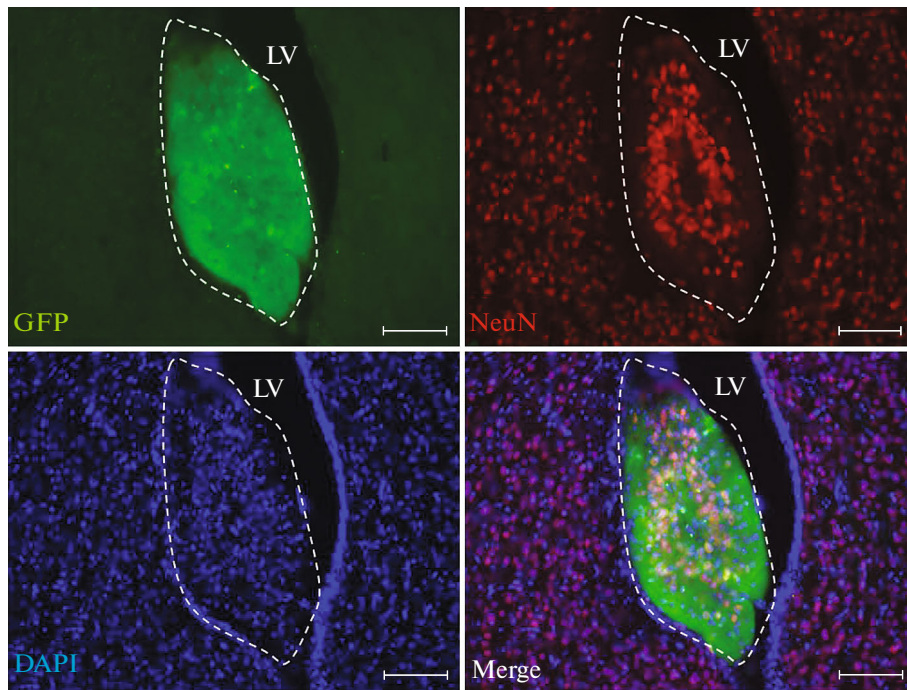
Fig. 4. Structure of the graft from the cells of the embryonic neocortex of mice (E14.5) in the ventricle of the recipient's brain (30 days). Positive immunohistochemical reaction of cells to the NeuN marker of mature neurons. There is a dense accumulation of cells in the central area; a marginal zone is formed at the periphery. The graft is highlighted with a dotted line. LV—lateral ventricle. Scale bar: 100 μm.

from the graft into the recipient's brain tissue was not detected at any time of observation. The presence of cytoarchitectonics and the absence of migration into the brain tissue of the recipient indicates that the resulting grafts are structures resembling organoids.

DISCUSSION

In this work, we tried to simulate adequate conditions for the growth of 3D cerebral aggregates using transplantation stem and progenitor cells of the embryonic neocortex of mice into the lateral ventricles of the brain of adult mice, where biologically active cerebrospinal fluid circulates and blood supply from the recipient's brain is possible (Lehtinen et al., 2011; Rahimi and Woehrer, 2017). It should be noted that the cavities of the lateral ventricles of the brain, due to their microenvironment, have been repeatedly used for transplantation of various types of neural and non-neural cells. Each scientific group posed a specific task in connection with the fact that either cells grow poorly in the parenchyma or because of the need for the products of cell synthesis directly into the cerebrospinal fluid (Freed et al., 1990; Zheng et al., 2006; Henzi et al., 2018; Barati et al., 2019). Our task was to develop a model for studying the self-organization and vascularization of neocortical cells isolated from the embryonic brain and cultured in the lateral ventricle of adult mice. For transplantation, we selected cells of the neocortex of GFP mice E14.5, which are at the

stage of neuronogenesis and, in contrast to uncommitted embryonic and induced pluripotent stem cells, cannot form teratomas (Gutierrez-Aranda et al., 2010). Using RT-PCR in real time, we determined the state of cell differentiation in the neocortex of embryos at stages E14.5 and E19.5. By mRNA expression of stem cell genes Pax6, Sox2, Nes; poorly differentiated neurons Dcx, Tubb3; differentiated neurons NeuN; astrocytes GFAP and synaptophysin Syp (begins to be expressed in undifferentiated neurons) have been shown to be preferable for transplantation of E14.5 cells. They have a high level of expression of stem cell markers, and there are no differentiated neurons and no glial cells. We confirmed the results of RT-PCR by immunohistochemical analysis of sections, where a distribution pattern of different types of cells over layers in the neocortex of E14.5 embryos was obtained, which generally corresponded to previous studies (Englund et al., 2005; Hori et al., 2014; Lodato and Arlotta, 2015; Güven et al., 2019). The next step in the development of technology for obtaining cerebral 3D aggregates in vivo was direct transplantation of cells of the embryonic neocortex of mice into the brain ventricle of adult mice. The isolated frontal sections of the E14.5 neocortex were used to prepare the suspension; during the treatment, the cells lost contacts and went around. As a result, the resulting suspension consisted of small aggregates and individual rounded cells without processes. We managed to find that a cell
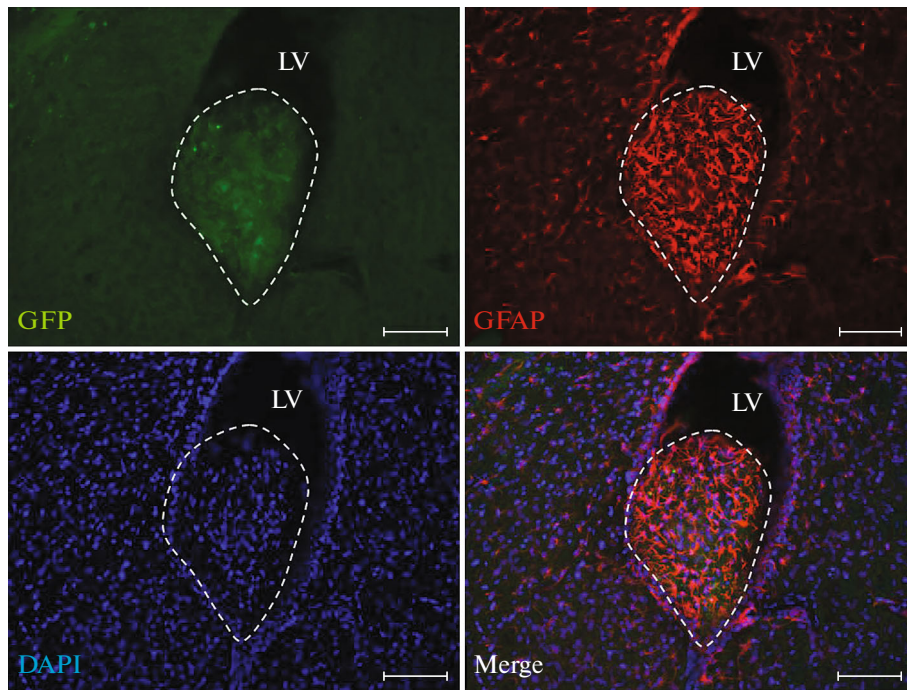
Fig. 5. Structure of a graft from mouse neocortex cells (E14.5) in the recipient's brain ventricle (30 days). GFAP$^+$ astrocytes are evenly distributed over the graft. The graft is highlighted with a dotted line. LV—lateral ventricle. Scale bar: 100 μm.
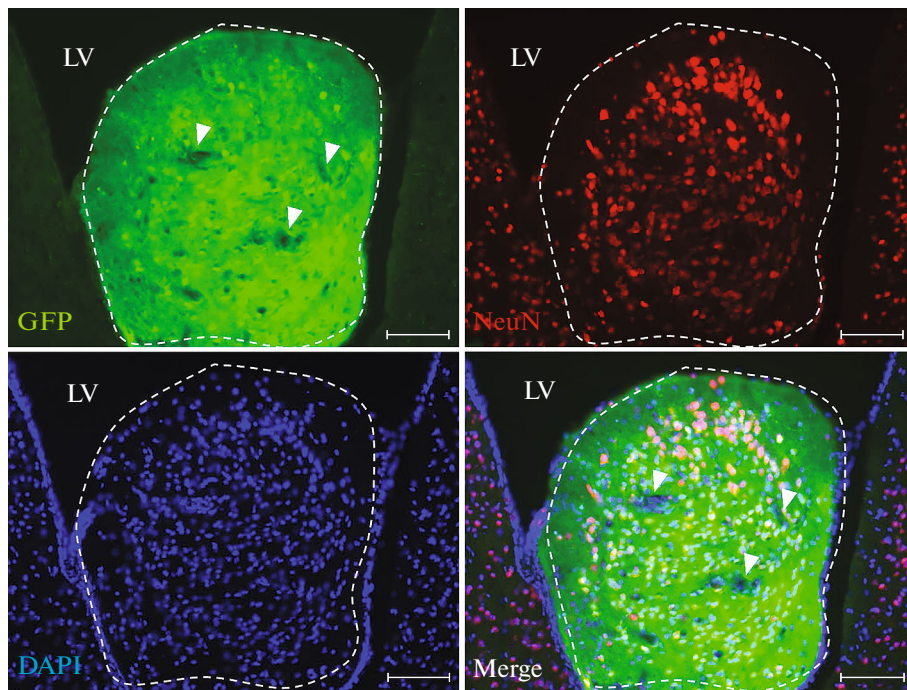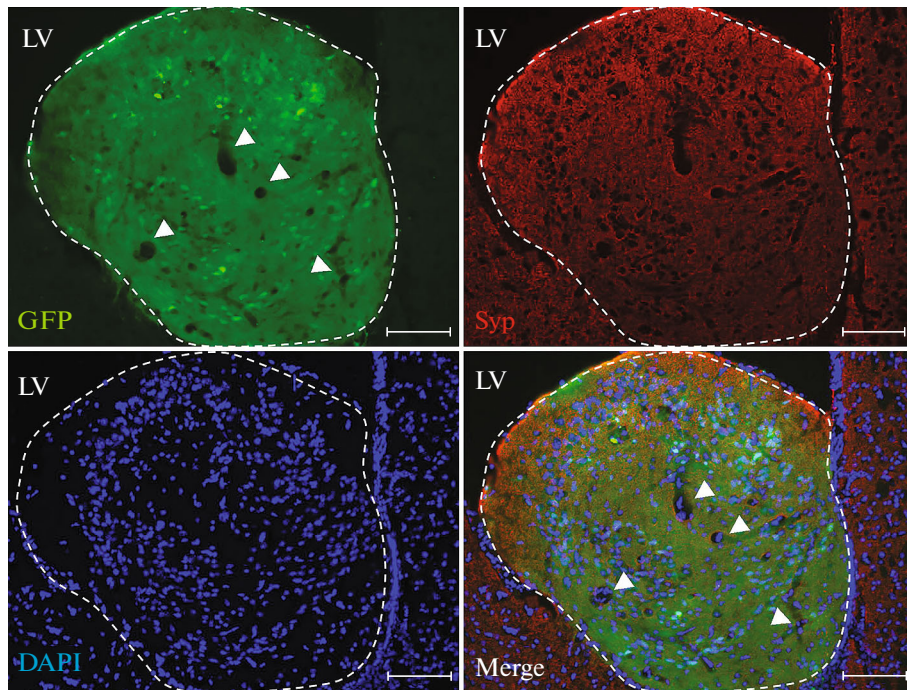

Fig. 6. Structure of the graft from the cells of the embryonic neocortex of mice (E14.5) 90 days after transplantation into the mouse brain ventricle. Positive immunohistochemical reaction of the graft cells to the NeuN marker of mature neurons. Neurons occupy the central region; a marginal zone is formed along the periphery. The vessels are visible in the graft (indicated with arrowheads). The graft is highlighted with a dotted line. LV—lateral ventricle. Scale bar: 100 μm.

Fig. 7. Graft from the cells of the embryonic neocortex of mice (E14.5) in the brain ventricle of the recipient 90 days after surgery. Positive immunohistochemical reaction of the graft cells to Synaptophysin (Syp). The vessels are clearly expressed in the tissue (indicated with arrowheads). The graft is highlighted with a dotted line. LV—lateral ventricle. Scale bar: 100 μm.
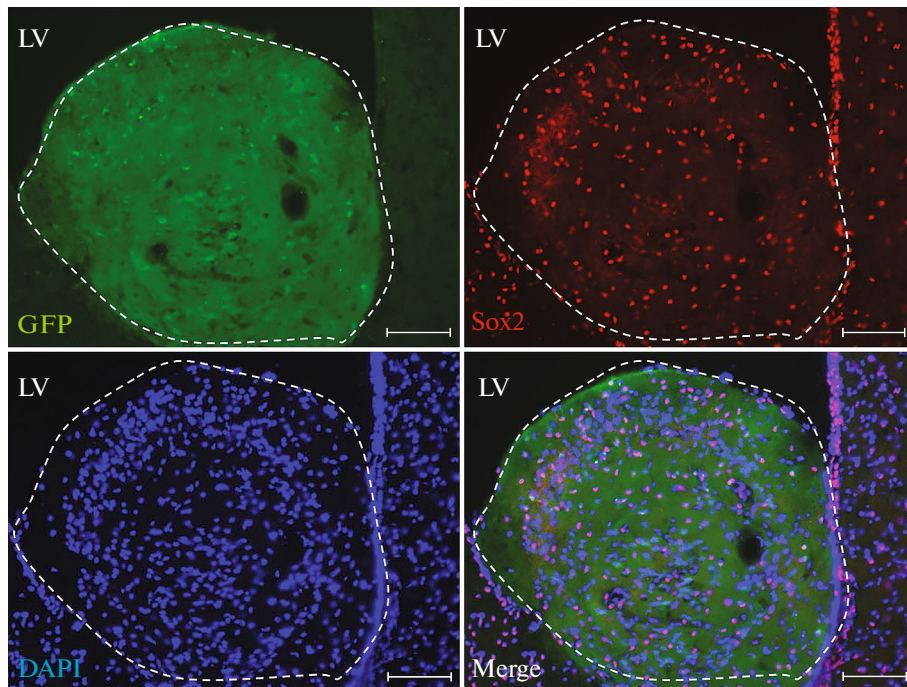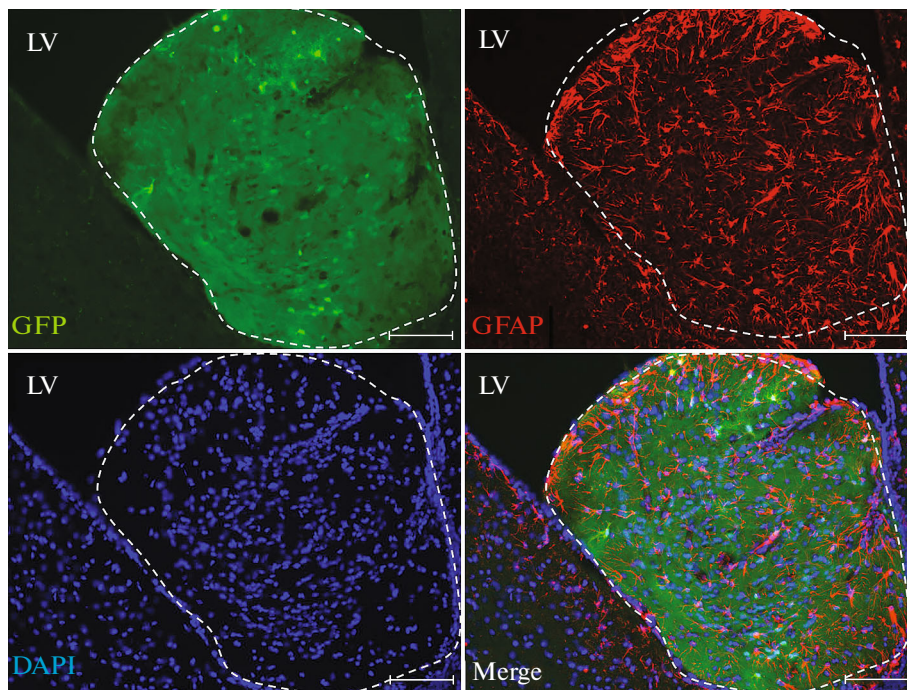

Fig. 8. Structure of the graft in the brain ventricle of the recipient 90 days after surgery. Positive immunohistochemical reaction of the graft cells to Sox2. The graft is highlighted with a dotted line. LV—lateral ventricle. Scale bar: 100 μm.

Fig. 9. Graft from the cells of the embryonic neocortex of mice (E14.5) 90 days after surgery in the recipient's brain ventricle. Positive immunohistochemical reaction of cells to the marker of astrocytes GFAP. At the graft border, cells form a border glial membrane (glia limitans). Glial scar does not form between the tissues of the graft and the recipient. The graft is highlighted with a dotted line. LV—lateral ventricle. Scale bar: 100 μm.
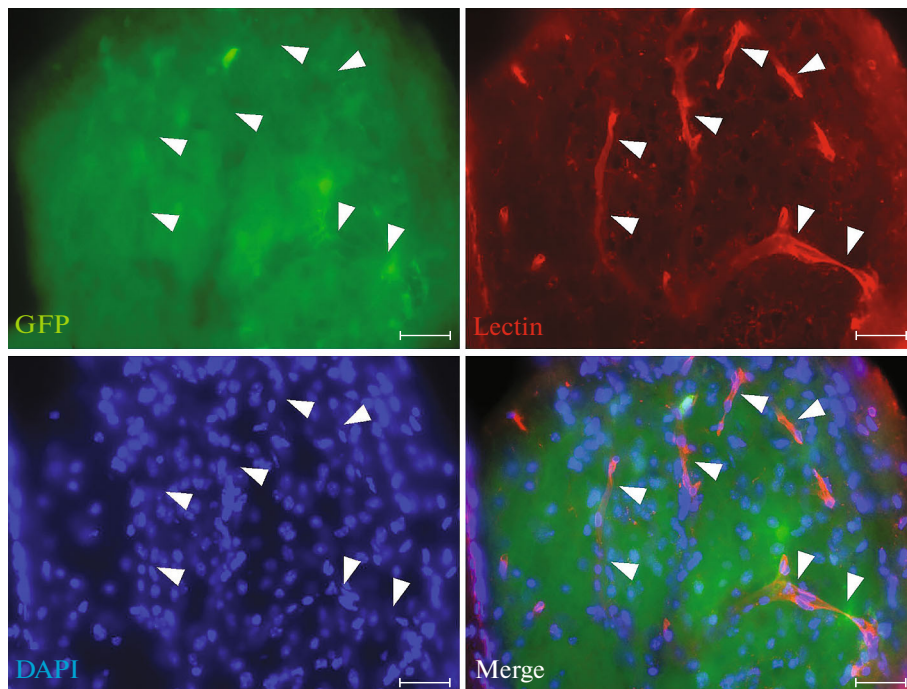

Fig. 10. Graft from the cells of the embryonic neocortex of mice (E14.5) in the ventricle of the recipient's brain 90 days after surgery. Graft vascularization detected with fluorescently labeled lectin. The vessels are marked with arrows. Scale bar: 40 μm.

aggregate is formed already 5 days after surgery in the cavity of the lateral ventricle in which migrating neuroblasts are detected. By day 30, the graft consisted of mature neurons and astrocytes. We found that a long period is required for the successful development of 3D cortical aggregates in the ventricles of the brain
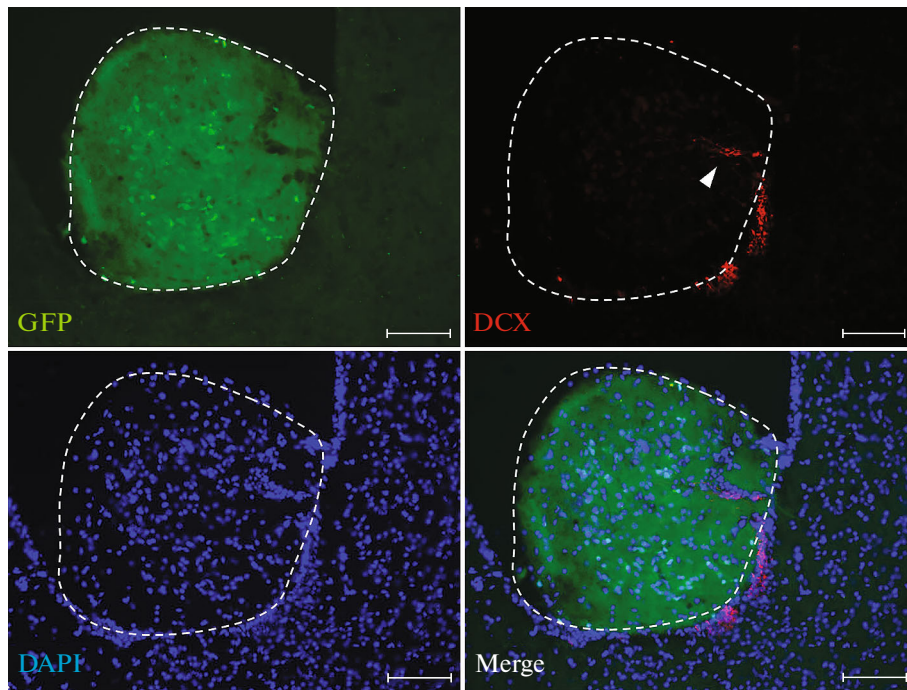
Fig. 11. Graft from the cells of the embryonic neocortex of mice (E14.5) in the ventricle of the recipient's brain 90 days after surgery. DCX$^+$ migration cells of the recipient's subventricular zone into the graft along the ingrowing vessels. The graft is highlighted with a dotted line. LV—lateral ventricle. Scale bar: 100 μm.

(90 days in our experiment). According to the literature, a long period of time is also required for the development of cerebral organoids in the in vitro system, approximately 60 days (Eiraku et al., 2008; Fan et al., 2019), and the growth of the graft ends after 60 days when stem cells are transplanted into the brain ventricle of young rats (Pothayee et al., 2018). In our experiment, it was found that the NeuN$^+$ marker of mature neurons is expressed in 3D cerebral graft cells by day 90. The cytoarchitectonics of the graft looked as follows, differentiated neurons were located only in the central part of the graft, a zone without cells was revealed at the periphery, which resembled the marginal zone or the first layer of the neocortex. Judging by the literature (Pothayee et al., 2018), this fact was not previously observed in grafts obtained from cortical NSCs. At the periphery of the graft, an area of the boundary membrane was formed, which, as in the normal brain, was formed by GFAP$^+$ astrocytes. The boundary membrane of the graft was in contact in many places with the ependymal wall of the ventricle; however, no glial scar between the tissues of the donor and the recipient appeared, which was also stated by other researchers (Pothayee et al., 2018). We observed, described by other authors, the penetration of DCX$^+$ cells of the recipient into the graft tissue (Pothayee et al., 2018) but did not reveal the migration of GFP cells from the graft into the recipient tissue, also described by these authors. Cells expressing Sox2 were detected in the grafts. According to the literature, Sox2

is usually considered a marker of neural stem cells that are distributed in different regions of the embryonic and adult brain. However, its expression has also been described in differentiated neurons in various regions of the nervous system (especially in the thalamus, striatum, and neocortex) (Ferri et al., 2004). In addition, there is evidence of Sox2 expression in individual astrocytes (Cahoy et al., 2008). Thus, the Sox2 expression found in some transplant cells cannot reliably indicate that stem cells are present in it but requires further study. In our study, the synaptophysin marker of synaptic vesicles was detected in the graft. According to Pothayee et al. (2018), the presence of mature synapses on cells is detected in such transplants using electron microscopy. In addition to the architectonic organization of 3D cerebral aggregates, we also found their vascularization from the side of the recipient brain. We morphologically identified vessels in 30-day old grafts, and vascularization begins on days 6—8 according to Pothayee et al. (2018). Interestingly, according to the literature, cerebral organoids obtained in vitro and transplanted into the parenchyma of the brain of rodents are also vascularized (Mansour et al., 2018). When making comparisons between our work and the work of Pothayee et al. (2018) and Mansour et al. (2018), it is necessary to pay attention to the difference in objectives and results. They studied the cooperation between the implanted cells and the recipient's brain, mutual fiber growth, cell migration, and vascularization. At the same time, we showed the possibility of
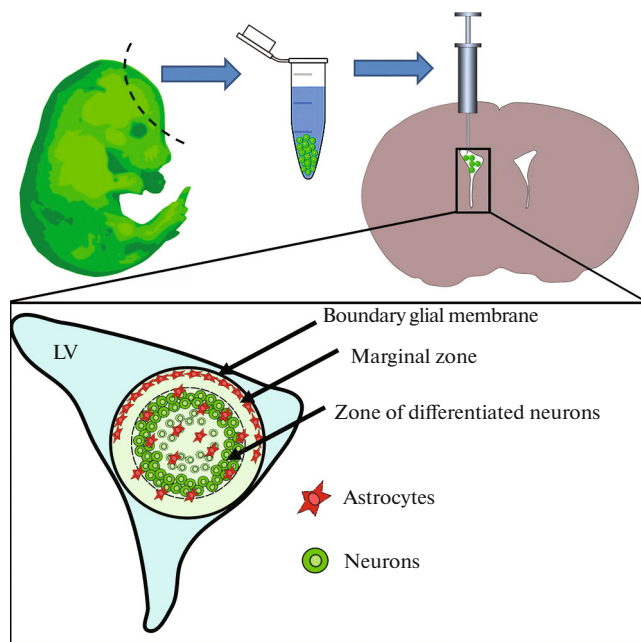
Fig. 12. Self-organization of suspended cells of the embryonic neocortex into 3D structures resembling cortical cerebral organoids after transplantation into the cerebral ventricle. LV—lateral ventricle.

self-organization of suspended cells of the neocortex into 3D structures resembling cortical cerebral organoids (Fig. 12). The use of markers for specific neurons in the layers of the neocortex will make it possible to more accurately determine their structure in the future. In addition, this model can be used to study the development of human cells when working with immunodeficient mice.

CONCLUSIONS

The study of cerebral organoids is of great importance for fundamental science and medicine. This paper presents an alternative experimental approach to the creation of 3D aggregates similar to cerebral organoids. We used the cultivation of neocortical stem and poorly differentiated cells in the cavities of the ventricle of the brain of experimental animals in vivo without special culture media that are replaced by cerebrospinal fluid. The formation of 3D aggregates with pronounced cytoarchitectonics occurs within 90 days. A characteristic feature of these structures is their high degree of vascularization, which distinguishes them from organoids created in vitro. Thus, the ventricles of the mouse brain can serve as an incubator for the development of vascularized, three-dimensional cerebral structures resembling organoids.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflict of interests.

Statement on the welfare of animals. All applicable international, national, and/or institutional guidelines for the use of animals in experiments and conditions for their care have been observed. No people in this study were used as research subjects.

AUTHOR CONTRIBUTIONS

K.K. Sukhinich, M.A. Shakirova, E.B. Dashinimaev, and M.A. Aleksandrova participated in the development of the experiment. K.K. Sukhinich and K.M. Shakirova conducted experiments. K.K. Sukhinich, K.M. Shakirova, and M.A. Aleksandrova participated in the preparation and writing of the article. K.K. Sukhinich, M.A. Shakirova, E.B. Dashinimaev, and M.A. Aleksandrova participated in editing the article. All authors participated in the discussion of the results.

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