Comparative Analysis of Neurogenesis and Cerebral Angiogenesis in the Hippocampal Neurogenic Niche in Animals with Two Experimental Models of Alzheimer's Disease

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Abstract

Introduction. *Various animal models are employed to uncover the mechanisms of Alzheimer's disease (AD) pathogenesis. Understanding brain damage pathogenesis in animal models of neurodegenerative diseases and identifying common patterns inherent to all relevant models is essential for adequate interpretation of findings, development of new models, as well as prevention and therapy strategies.*

The study *aimed to assess neurogenesis and remodeling of the microvasculature in the subgranular zone (SGZ) of the hippocampal dentate gyrus in mice with two AD models.*

Materials and methods. *The study employed two in vivo Alzheimer's disease models: 1) animals with intrahippocampal administration of amyloid-β protein fragment $A\beta_{25-35}$; 2) 5xFAD transgenic mice. Cognitive functions were evaluated using a passive avoidance test. On days 7 and 28 post-training, we assessed vascular network branching and density in the hippocampus using Evans Blue with subsequent software-based analysis of skeletonized images, analyzed proliferative activity of neuronal and endothelial cells, and their subpopulation composition using BrdU assay and multiparameter immunostaining of brain thin sections.*

Results. *Animals following intrahippocampal $A\beta_{25-35}$ administration demonstrated enhanced neurogenesis and neoangiogenesis over 28 days post-training, unlike 5xFAD mice which showed delayed and less pronounced proliferation of neuronal cells in the SGZ alongside transient increases in proliferating endothelial cells. Both AD models exhibited divergent changes in tip and stalk cell counts within the hippocampal SGZ, indicating non-productive neoangiogenesis confirmed by reduced vascular branching and density in the SGZ of animals from both models.*

Conclusion. *Cognitive deficits associated with experience-induced neurogenesis and cerebral angiogenesis mechanisms in the hippocampal neurogenic niche differ between AD models representing sporadic and familial variants, highlighting the need for fundamentally different approaches to pathogenetic therapy targeting non-productive angiogenesis and aberrant brain plasticity in various Alzheimer's type neurodegeneration scenarios.*

Keywords: *Alzheimer's disease models; neuroplasticity; neurogenesis; neurogenic niche; hippocampus; angiogenesis*

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Сравнительный анализ нейрогенеза и церебрального ангиогенеза в нейрогенной нише гиппокампа у животных с двумя моделями экспериментальной болезни Альцгеймера

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Аннотация

Введение. *Механизмы развития болезни Альцгеймера (БА) изучают с использованием разнообразных моделей на животных. Понимание особенностей патогенеза повреждения мозга у животных с разными моделями нейродегенерации и выявление общих закономерностей, присущих всем релевантным моделям, важно для корректной интерпретации полученных данных, разработки новых моделей и способов профилактики и терапии.*

Цель *исследования – оценить изменения нейрогенеза и ремоделирования микрососудов в субгранулярной зоне (СГЗ) гиппокампа головного мозга мышей с двумя моделями БА.*

Материалы и методы. *Для исследования были использованы две модели БА in vivo: 1) животные с интрагиппокампальным введением фрагмента β-амилоидного белка $A\beta_{25-35}$; 2) животные линии 5xFAD. Когнитивные функции оценивали с помощью теста условной реакции пассивного избегания. На 7-е и 28-е сутки после обучения выполняли оценку ветвления и плотности сосудистой сети в гиппокампе с помощью Evans Blue с последующим программным анализом скелетированных изображений, анализ пролиферативной активности нейрональных клеток, эндотелиальных клеток и их субпопуляционного состава – с помощью теста с BrdU и мультипараметрического иммуноокрашивания тонких срезов мозга.*

Результаты. *Животные после интрагиппокампального введения $A\beta_{25-35}$ демонстрировали усиленный нейрогенез и неоангиогенез в течение 28 сут после обучения, в отличие от животных с 5xFAD, у которых пролиферация клеток нейрональной природы в СГЗ носила замедленный и менее выраженный характер на фоне транзиторного увеличения количества пролиферирующих клеток эндотелия. У животных с разными моделями БА изменения количества tip- и stalk-клеток в СГЗ гиппокампа были разнонаправленными, что свидетельствует о несовершенном неоангиогенезе, подтверждаемом снижением ветвления и плотности сосудистой сети в СГЗ животных с обеими моделями БА.*

Заключение. *Формирование когнитивного дефицита на фоне различных по механизмам развития опыт-индуцированного нейрогенеза и церебрального ангиогенеза в нейрогенной нише гиппокампа у животных с моделями БА, характерными для спорадических и семейных вариантов, демонстрирует необходимость в разработке принципиально разных подходов к патогенетической терапии непродуктивного ангиогенеза и аберрантной пластичности мозга при разных вариантах развития нейродегенерации альцгеймеровского типа.*

Ключевые слова: *модели болезни Альцгеймера; нейропластичность; нейрогенез; нейрогенная ниша; гиппокамп; ангиогенез*

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Introduction

Neuroplasticity can be defined as the ability of the brain to respond to intrinsic or extrinsic stimuli by reorganizing its structure, function and connections [1]. This phenomenon plays a crucial role in developing and maintaining neural circuits and cognitive functions. While normal neuroplasticity is essential for brain functioning, its impairments underlie the development of neurodegenerative diseases. Alzheimer's disease (AD) is characterized by accelerated brain aging and aberrant plasticity, associated with progressive neuronal degeneration and the formation of amyloid plaques and neurofibrillary tangles in the brain. Despite extensive researchs, AD pathogenesis remains understudied, and several leading theories of its etiology – including amyloid, calcium, and other hypotheses – remain under debate [2].

Over recent decades, AD pathogenesis research has employed diverse animal models: administration of neurotoxic agents (colchicine, scopolamine, atropine, aluminum salts) or amyloid-β oligomers into brain tissue; transgenic animals carrying mutations in three or five genes encoding proteins associated with Alzheimer'stype neurodegeneration (amyloid precursor protein, presenilin, tau protein); and animals with induced dysmetabolic disorders characteristic of AD (insulin resistance, mitochondrial dysfunction) [3].

Each model has specific strengths and limitations, determining their varying suitability for preclinical and translational research, including pharmacotherapy development [4]. This raises questions about the applicability of specific models for assessing key neurodegeneration mechanisms and the comparability of findings [5].

For instance, 5xFAD mice exhibit significant neuronal loss at 9–12 months of age, while amyloid-β accumulation and neuroinflammation in brain tissue are observed as early as 1.5–2.0 months [6]. Conversely, intracerebral administration (ventricles, hippocampus) of various amyloid-β isoforms (1–40, 1–42, 25–35) induces rapid neuroinflammation (within 3 days), microvascular damage, and cognitive impairment – effects particularly pronounced in aged animals [7].

Suppression of neurogenesis in neurogenic niches is observed in triple transgenic (3xTg) animal model during early stages of postnatal ontogeny (1–2 months after birth) and prior to the onset of cognitive dysfunction [7]. In 5xFAD mice characterized by progressive spread of neuritic dystrophy, gliosis, and amyloid-β accumulation in brain tissue (starting from the *subiculum* and extending to the hippocampus and cortex) [8], neurogenesis suppression becomes evident by 2 months after birth. Paradoxically, some transgenic animals with other Alzheimer's disease (AD) models exhibit intensified neurogenesis at 2–3 months of postnatal ontogeny [9]. As we previously demonstrated, intrahippocampal amyloid-β administration increased Pax6 and Nestin expression in the hippocampal subgranular zone (SGZ) by day 9 [10], followed by a prolonged decline in neurogenesis efficiency [11] accompanied by progressive local neuroinflammation [12].

In this context, studying mechanisms of aberrant plasticity in AD should be grounded in understanding specific features of pathogenetic brain damage mechanisms across different animal neurodegeneration models. However, identifying common patterns inherent to all (or most) relevant models is particularly critical for accurate interpretation of findings.

Neurogenic niches in the brain, particularly the hippocampal SGZ, represent a valuable and informative focus for investigating neuroplasticity, including in progressive neurodegeneration. First, changes in hippocampal neurogenesis are directly linked to cognitive functions, such as through mechanisms regulating engram cell populations or the excitation/inhibition balance in the hippocampus [13]. Second, alterations in hippocampal neurogenesis are clearly detectable during training and memory formation [14]. Third, the hippocampus is one of the most affected brain regions in AD [15]. Fourth, long-term plasticity mechanisms driven by neurogenesis efficiency require support from changes in local microcirculation and remodeling of the microvascular bed in the hippocampus, such as through neoangiogenesis and microvascular regression [16]. Notably, in the hippocampal SGZ – unlike the subventricular zone (SVZ) – angiogenic activity is inherently high, with clusters of proliferating endothelial cells directly contacting neural stem cells and exhibiting high sensitivity to regulatory molecules in the local microenvironment [16]. Thus, alongside neurogenesis mechanisms underlying long-term experience-induced plasticity [17], assessing microcapillary bed remodeling in this neurogenic niche may provide critical insights into thebrain plasticity under normal conditions and during neurodegeneration.

The study **aimed** to compare neurogenesis and remodeling of the microvasculature in the SGZ of the hippocampal dentate gyrus in mice with two AD models: 1) animals intrahippocampal administration of amyloid-β and 5xFAD transgenic mice.

Materials and methods

The study design is shown in Fig. 1.

We employed two *in vivo* models of AD.

The first model involved animals with intrahippocampal administration of the amyloid-β protein fragment $A\beta_{25-35}$. Male C57BL/6 mice aged 6 months and weighing 30–35 g (n = 39) underwent stereotactic surgery. Zoletil 100 (Virbac Sante Animale) and Xyla (Interchemie Werken "de Adelaar" BV) were used for anesthesia. A standard Zoletil 100 solution (500 mg in 5 ml) diluted 1 : 4 with saline was administered intramuscularly at a dose of 1.5 mg active substance per 25 g of mouse weight. Xyla was diluted 1 : 2 with saline and administered intramuscularly at 0.6 mg per 25 g of mouse weight. Under anesthesia, bilateral craniotomies were performed using a manipulator (Stoelting) according to the coordinates from the Mouse Brain Atlas (AP – 2.0; ML – 1.9; DV – 1.3). Then, 2 μL of 1 mM oligomerized $A\beta_{25-35}$ (Sigma-Aldrich Co.) in 0.9% NaCl solution was injected into the hippocampal CA1 region The $A\beta_{25-35}$ solution was prepared according to the manufacturer's instructions: Aβ was dissolved in 1 mL of 0.9% NaCl and incubated at 37°C for 4 days. Sham-operated control group animals (SO; 6 months; n = 34) were injected with 0.9% NaCl in the same volume at matching coordinates.

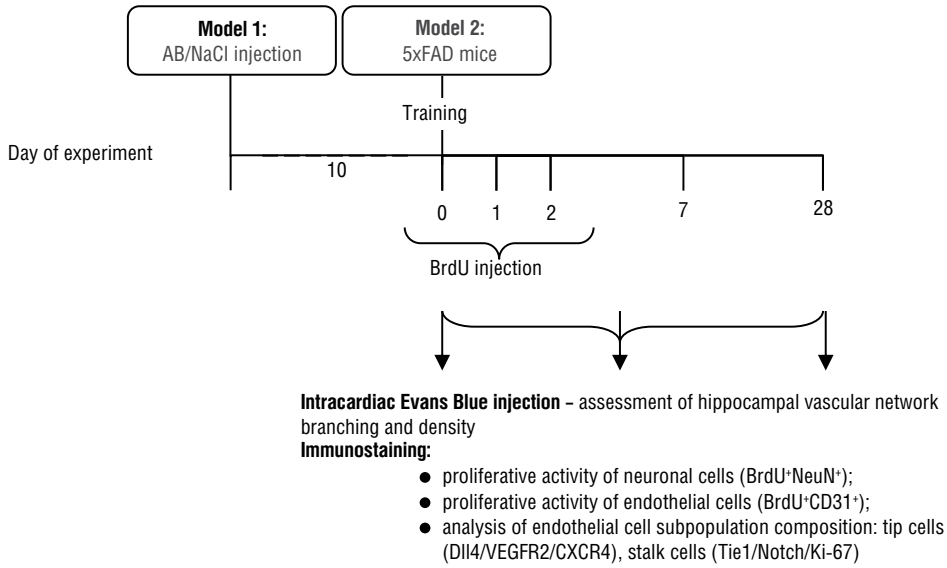

Fig. 1. General design of *in vivo* experiments using two AD models.
Model 1 – animals with intrahippocampal $A\beta_{25-35}$ injection (control – sham-operated animals injected with 0.9% NaCl solution); Model 2 – 5xFAD mice (control – intact C57BL/6 mice).

The second experimental *in vivo* AD model utilized 6-month-old male 5xFAD transgenic mice, strain B6SJL-Tg(APPSWFLon,PSEN1M146L,L286V)6799Vas (n = 21). The control group consisted of intact male C57BL/6 mice (6 months; n = 21).

All experiments complied with humane animal treatment principles and EU Directive 2010/63/EU on animal experimentation. Animals were housed in cages (⩽ 6 per cage) with ad libitum access to food and water under a 12-hour light/dark cycle. The studies were conducted in accordance with approval of the Local Ethical Committee (Protocol No. 5-3/22, June 1, 2022).

The Conditioned Reflex of Passive Avoidance test

Cognitive impairment was assessed using the Passive Avoidance (PA) test according to the standard protocol on day 1 after exposure to an inescapable aversive stimulus (0.2 mA electric current for 3 seconds through a metal floor grid upon entering the dark compartment), as well as on days 7 and 28 post-training. The time taken for the mouse to move from the illuminated compartment to the dark compartment was recorded.

Evaluation of neural and endothelial cell proliferation in the hippocampal neurogenic niche

Mice were intraperitoneally administered BrdU solution (50 mg/kg body weight) 1 hour after the training aversive stimulus, followed by a single BrdU injection every 24 hours for 2 days [18].

Immunostaining

At different time points (days 7 and 28 after PA training), 8 animals from each study group were euthanized by cervical dislocation. Brains were fixed in 4% paraformaldehyde (Wuhan Servicebio Co., Ltd), and 10 μm-thick cryosections were prepared using an FS800A cryostat (RWD). Sagittal sections were prewashed in phosphate-buffered saline (Rosmedbio) for 10 minutes, then in 0.1% Triton X-100 solution (Calbiochem Biochemicals) with 5% bovine serum albumin (BioFroxx) for 1 hour to block non-specific binding, followed by antibody staining. Antibodies were selected based on species specificity and applied according to manufacturer-recommended protocols: primary antibodies – anti-BrdU (Host–Mouse, 1 : 100, A1482, ABClonal); directly conjugated rabbit antibodies – anti-NeuN (1 : 100, FNab05669, FineTest); anti-CXCR4 (1 : 250, AF5279-F555, Affinity); anti-DLL4 (1 : 250, DF13221-F250, Affinity); anti-VEGFR2 (1 : 250, AF6281-F488, Affinity); anti-CD31 (1 : 250, AF6191-F555, Affinity); anti-TIE1 (1 : 250, AF4582-F555, Affinity); anti-Ki-67 (1 : 250, AF0198-F350, Affinity); anti-Notch (1 : 250, AF5307-F488, Affinity); secondary antibodies – anti-Rabbit (1:100, AS011, ABClonal); anti-Mouse (1 : 500, ab150116, Abcam). Sections were covered using fluorescence-preserving mounting medium "Fluoroshield Mounting with DAPI" (Sigma Aldrich).

For assessment of SGZ cell proliferation using BrdU, 26 μm-thick sagittal brain sections were prepared, and-$BrdU^+NeuN^+DAPI^+$- и $BrdU^+CD31^+DAPI^+$ cells were quantified. This method identifies progeny populations of proliferating NSCs/NPCs or endothelial cells activated on day 1 of the experiment (training) [18].

Digital images were acquired using an EVOS M7000 imaging system (Thermo Fisher Scientific) and processed in ImageJ using a plugin for fluorescent label quantification. Expression levels of these markers were characterized by the count of stained cells normalized per 100 $DAPI^+$ cells in the SGZ.

Evaluation of vascular branching and density in the hippocampus

One hour post-PA (day 10 of experiment), mice were intracardially administered 2% Evans Blue solution (6 μl/g body

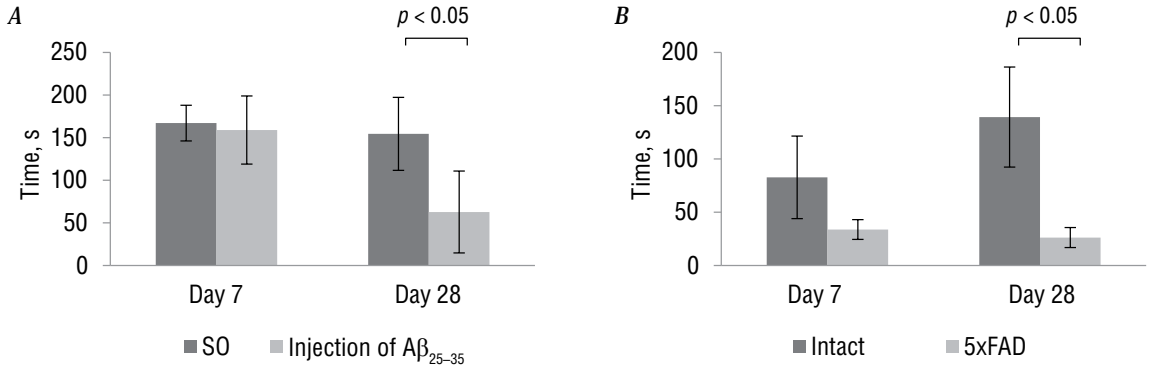

Fig. 2. Results of neurobehavioral PA testing in SO animals and animals with intrahippocampal $A\beta_{25–35}$ injection (*A*), intact animals and transgenic 5xFAD animals (*B*) on days 7 and 28 post-training, $p < 0.05$.

weight) following standard protocol [19]. After 5 minutes, animals were euthanized, and brains were extracted for angiogenesis analysis in thin sections. Microscopic images of sections were analyzed in ImageJ using the following protocol: images were binarized, background-cleared (Threshold), skeletonized (Skeletonize); skeletonized images were quantified using Analyze skeleton option (Shortest branch method) and Vessel Analysis plugin (Vascular Density option). Results were expressed as absolute values for vessel branch point counts in the region of interest, and as vessel length per unit area for vascular density assessment.

Statistical analysis

Statistical data processing utilized Statistica v. 13.3 software (StatSoft). Normality of data distribution was assessed usingthe Kolmogorov–Smirnov and Shapiro–Wilk test. The results were analyzed using the Mann–Whitney U test. The results were considered significant at $p < 0.05$. Data are presented as mean ± standard deviation.

Results

The mechanisms of brain plasticity are closely associated with neurogenesis and cerebral angiogenesis, demonstrating significant alterations during progressive neurodegeneration [19]. Plasticity related to brain development or acquired experience, along with adaptive plasticity inherent to the damaged brain, may influence angiogenesis and neurogenesis processes in different ways [20]. To assess neurogenesis and angiogenesis parameters in experience-induced plasticity within both AD models, we employed an aversive inescapable stimulus as a training stimulus. We found that animals in both models exhibited similar changes in cognitive dysfunction progression by day 28 following the first training session (Fig. 2), consistent with our prior findings using the amyloid-β intrahippocampal administration model [21]. Specifically, a sustained reduction in transition time to the dark compartment was observed in the PA test: $A\beta_{25–35}$-injected mice showed a decrease of 92 s, while 5xFAD mice demonstrated a 113 s reduction compared to control groups ($p = 0.0449$).

As we previously hypothesized [21], cognitive deficits by day 28 after the first training session may correspond to alterations in hippocampal neurogenesis processes, since the complete cycle from neural stem cell recruitment to the formation of a young neuron population takes approximately 4 weeks in mammals [22]. Therefore, we subsequently quantified proliferating neurnal cells whose mitotic entry was initiated during the first training session.

We found that the count of young $BrdU^+$ neurons in animals with intrahippocampal $A\beta_{25–35}$ administration was increased by 52% compared to controls ($p = 0.0449$) on day 7 after aversive stimulus presentation and showed further elevation by day 28 (Fig. 3). Concurrently, the count of $BrdU^+$ neuronal cells in 5xFAD line animals remained significantly lower than in both control groups and Aβ-injected animals at day 7, and subsequently increased without reaching values characteristic of neurodegeneration induced by intrahippocampal amyloid-β administration (Fig. 3). Transgenic animals showed no evidence of enhanced neurogenesis in the SGZ (Fig. 3) on day 7 post-cognitive stimulus presentation, while amplified proliferation of neuronal cells was detected only by day 28 (3.75-fold increase; $p = 0.0450$), potentially due to a mismatch between neurogenic demand and impaired plasticity mechanisms.

Overall, post-training animals with intrahippocampal $A\beta_{25–35}$ administration exhibited enhanced neurogenesis, unlike 5xFAD animals demonstrating delayed and less pronounced neuronal proliferation — likely attributable to neurodegeneration initiated during embryogenesis and depletion of neural stem/neuronal progenitor cell pools.

Cognitive reserve depends on adequate neurovascular coupling, microcirculation in active brain regions, while hippocampal vascularization significantly modulates cognitive performance [23, 24]. We therefore proceeded to analyze changes in local angiogenesis within the brain neurogenic niche. Similar to the assessment of $BrdU^+$ neuronal cells, we analyzed the count of endothelial cells entering mitosis in response to a training (aversive inescapable) stimulus.

We found that the count of proliferating $BrdU^+/CD31^+$ endothelial cells in the SGZ of the brain in animals with intrahippocampal $A\beta_{25–35}$ administration was initially 4.4 times higher than controls ($p = 0.0445$) and remained 95% greater than SO animals by day 28 (Fig. 4), consistent with neurogenic event dynamics. In 5xFAD transgenic animals, angiogenesis

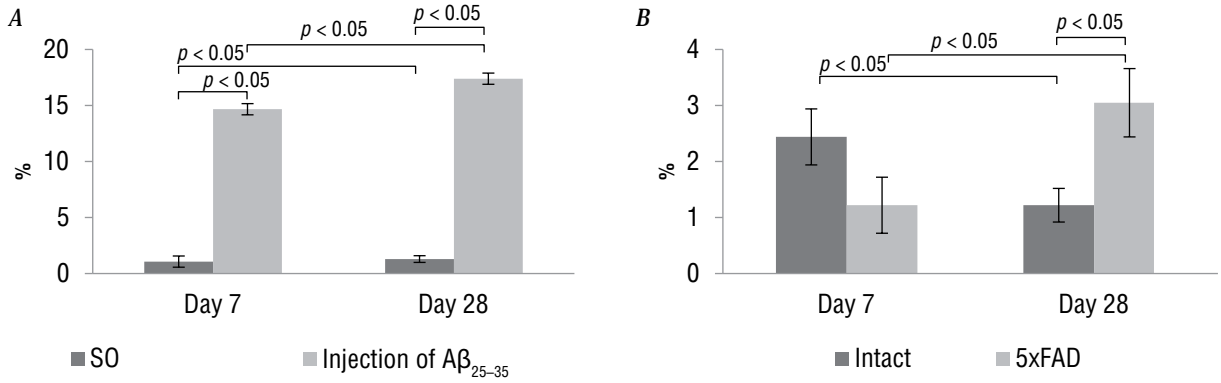

Fig. 3. Changes in the count of BrdU$^+$ neurons (NeuN$^+$) in the SGZ of SO animals and animals with intrahippocampal Aβ$_{25–35}$ injection (*A*), intact and transgenic 5xFAD animals (*B*) on days 7 and 28 post-training.
Data are normalized to 100 DAPI$^+$-cells and are presented as mean and standard deviation, $p < 0.05$.

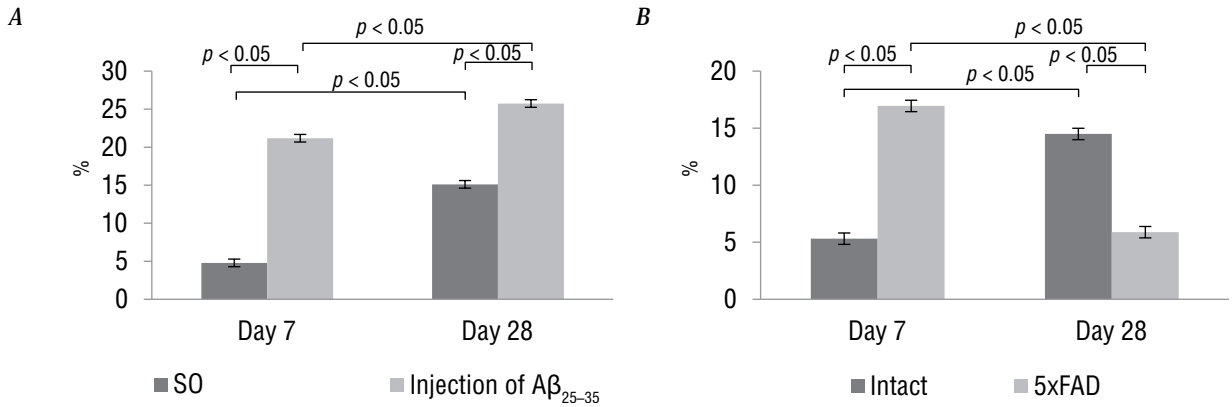

Fig. 4. Changes in the count of BrdU$^+$-endothelial cells (CD31+) in the SGZ of SO and animals with intrahippocampal injection of Aβ$_{25–35}$ (*A*), intact and transgenic 5xFAD animals (*B*) on days 7 and 28 post-training.
Data are normalized to 100 DAPI$^+$-cells and are presented as mean and standard deviation, $p < 0.05$.

in the SGZ showed transient enhancement (BrdU$^+$ endothelial cell count nearly tripled by day 7 post-training). However, by day 28 — corresponding to cognitive dysfunction manifestations — we observed a significant two-fold reduction in proliferating endothelial cells ($p = 0.0445$; Fig. 4).

Thus, in SO animals and those with intrahippocampal β-amyloid injection into the SGZ, a significant increase in the count of proliferating cells of neuronal and endothelial origin — whose mitotic entry was initiated on day 1 of training — was observed post-training. In 5xFAD line animals, the count of BrdU$^+$ neuronal cells increased by day 28, while the count of BrdU$^+$ endothelial cells significantly decreased by this time, indicating a mismatch between the demand for experience-induced angiogenesis and neurogenesis during prolonged progression of Alzheimer's type neurodegeneration.

Stimulation of angiogenesis and remodeling of the microvasculature are accompanied by changes in endothelial subpopulations, with the emergence of phenotypically and metabolically distinct tip and stalk cells that respectively mediate migration and new capillary wall formation [25]. To investigate abnormal angiogenesis features in two AD models, we analyzed the presence of the following endothelial cell types in the SGZ of experimental animals: CXCR4/Dll4/VEGFR2 tip cells and Tie1/Notch/Ki-67 stalk cells, consistent with their documented expression profiles as participants in neoangiogenesis [26].

The SO animals showed no significant changes in endothelial tip and stalk cell counts in the SGZ during post-training follow-up, whereas animals with intrahippocampal β-amyloid injection demonstrated a significant increase in tip-cell count by day 28 (Fig. 5). In intact animals, endothelial tip and stalk cell count increased by day 28, with the stalk cell profile correlating with BrdU$^+$CD31$^+$ cell changes (as stalk cells, unlike tip cells, retain proliferative capacity) [27]. 5xFAD mice exhibited no increase in tip-cell count between days 7 and 28 of follow-up, along with reduced stalk cell counts and significantly lower total endothelial cells of both activated phenotypes compared to controls (Fig. 5).

Thus, animals with different models of AD demonstrate multidirectional changes in the count of tip and stalk cells in the hippocampal SGZ, collectively indicating impaired neoangiogenesis. This conclusion is supported by our observed reduction in vascular network branching and density in the brains of animals with both models of Alzheimer's type neurodegeneration compared to intact controls on days 7 and 28 post-training (Figs. 6, 7).

Discussion

We analyzed the features of proliferative activity in neuronal and endothelial cells within the SGZ, as well as the characteristics of neoangiogenesis and remodeling of the microvasculature in this neurogenic niche of the brain in animals with two models of AD: an intrahippocampal amyloid-β administration model (simulating sporadic AD cases) and a transgenic 5xFAD mouse model characterized by multiple mutations associated with familial AD forms in humans [28].

We found that the development of cognitive dysfunction during the PA test used as an animal training model in 5xFAD mice coincides chronologically with our previous observations in mice receiving intrahippocampal $A\beta_{25–35}$ injections [29]: both experimental groups demonstrated cognitive deficits by day 28 post-initial training in the PA test, aligning with the 4-week cycle of induced neurogenesis in the hippocampal SGZ [22].

However, in both control groups, the implemented training protocol did not induce significant changes in the number of proliferating neuronal cells in the SGZ, whereas experimental modeling of Alzheimer's type neurodegeneration revealed intensified cell proliferation reaching peak levels by day 28. This observation aligns with our and others' experimental evidence that AD may involve not only suppressed neurogenesis in neurogenic niches but also its paradoxical stimulation, typically associated with pro-inflammatory microenvironment formation and amyloid-β oligomer accumulation. These effects have been documented in transgenic animals showing age-dependent multidirectional changes [10, 11, 30–32] and in AD patients [33].

On the other hand, these data indicate that proliferative activity in the SGZ of 5xFAD animals is *a priori* reduced compared to animals with intrahippocampal amyloid-β administration. This suggests that the pool of neural stem cells and neuronal progenitor cells in animals with a familial AD model is significantly impaired, limiting their ability to mount an effective recruitment response during experience-induced neurogenesis stimulation. We propose that this may be attributed to a prolonged period of aberrant neurogenesis starting from the embryonic developmental stage in animals with genetic AD models [34].

One potential reason for the lack of significant neurogenesis intensification during training in 5xFAD animals could be impairments in the local vascular scaffold that maintains the neural stem cell and neuronal progenitor cell pool, as well as their mobilization, proliferation, and differentiation in neurogenic niches [15]. Under conditions of increased neurogenesis demand (e.g., during learning and memory consolidation), suppression of cerebral angiogenesis leads to impaired learning and memory [35]. Indeed, we observed

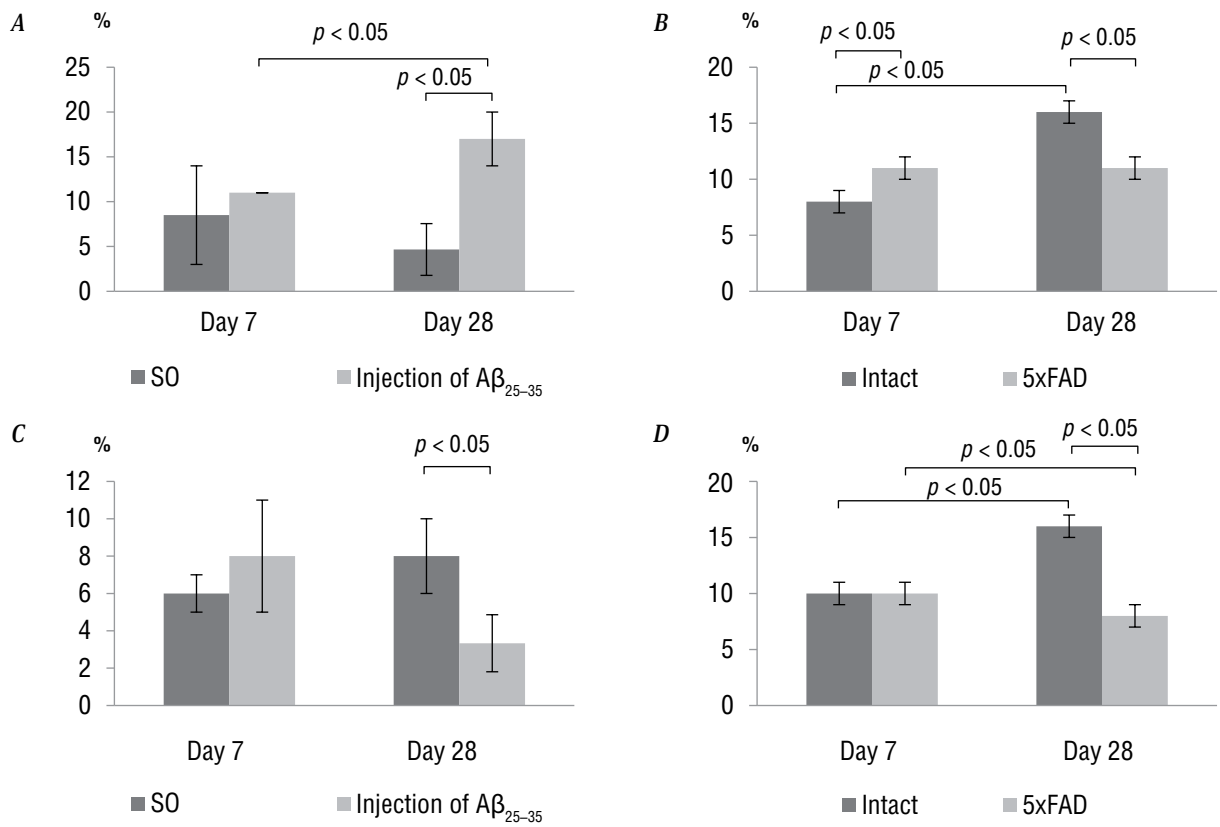

Fig. 5. Changes in the number of CXCR4/DLL4/VEGFR2-immunopositive (tip-) and TIE1/Ki-67/NOTCH-immunopositive (stalk-) endothelial cells in the SGZ of SO animals and animals with intrahippocampal injection of $A\beta_{25–35}$ (*A, C*), intact and transgenic 5xFAD animals (*B, D*) on days 7 and 28 post-training.
Data are normalized to 100 DAPI-positive cells and are presented as mean and standard deviation, $p < 0.05$.

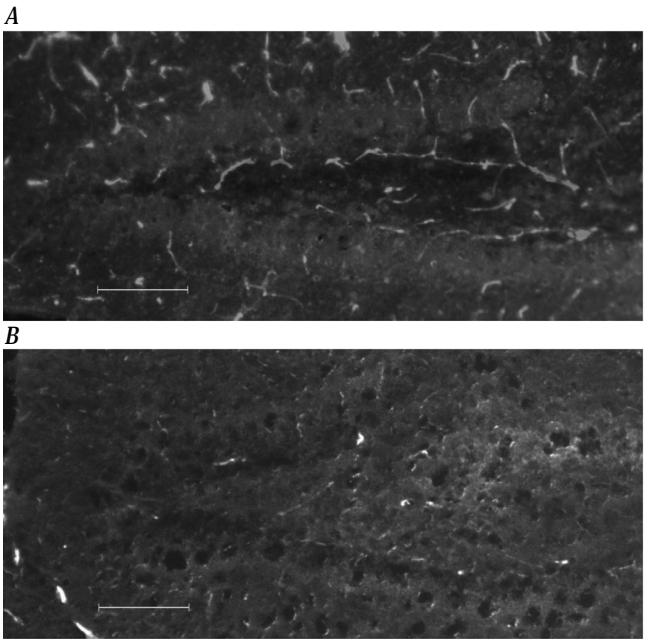

Fig. 6. The vasculature in the SGZ of the hippocampus on day 28 post-training.
A: the SGZ vasculature of an AO animal. *B*: the SGZ vasculature in an animal with intrahippocampal injection of $A\beta_{25-35}$. Scale bar – 65 μm.

reduced proliferative activity of endothelial cells and suppression of phenotypic conversion into tip- and stalk-like phenotypes in 5xFAD animals.

Control group animals demonstrated substantial intensification of neoangiogenesis (increased $BrdU^+CD31^+$ cell count) by days 7 and 28 after the first training session. A similar, and even more pronounced changes in $BrdU^+CD31^+$ endothelial cell counts was observed in animals following intrahippocampal amyloid-β administration. However, 5xFAD transgenic animals initially showed increased counts of endothelial cells entering mitosis after training, followed by a significant reduction in their number in the SGZ, which we associate with intensified cell death (apoptosis) and/or enhanced microvascular regression due to long-term neurodegeneration in transgenic animals, unlike animals primarily exhibiting acute amyloid-β toxicity after intracerebral administration.

Indeed, animals with genetic AD models exhibit all key features of long-term and progressive cerebral amyloid angiopathy with cerebral capillary bed damage, corresponding to AD pathogenesis in humans [36], including the formation of aberrant microvessels with compromised blood-brain barrier integrity [37].

It is noteworthy that in animals with intrahippocampal $A\beta_{25-35}$ administration, proangiogenic activity in the SGZ is high, but

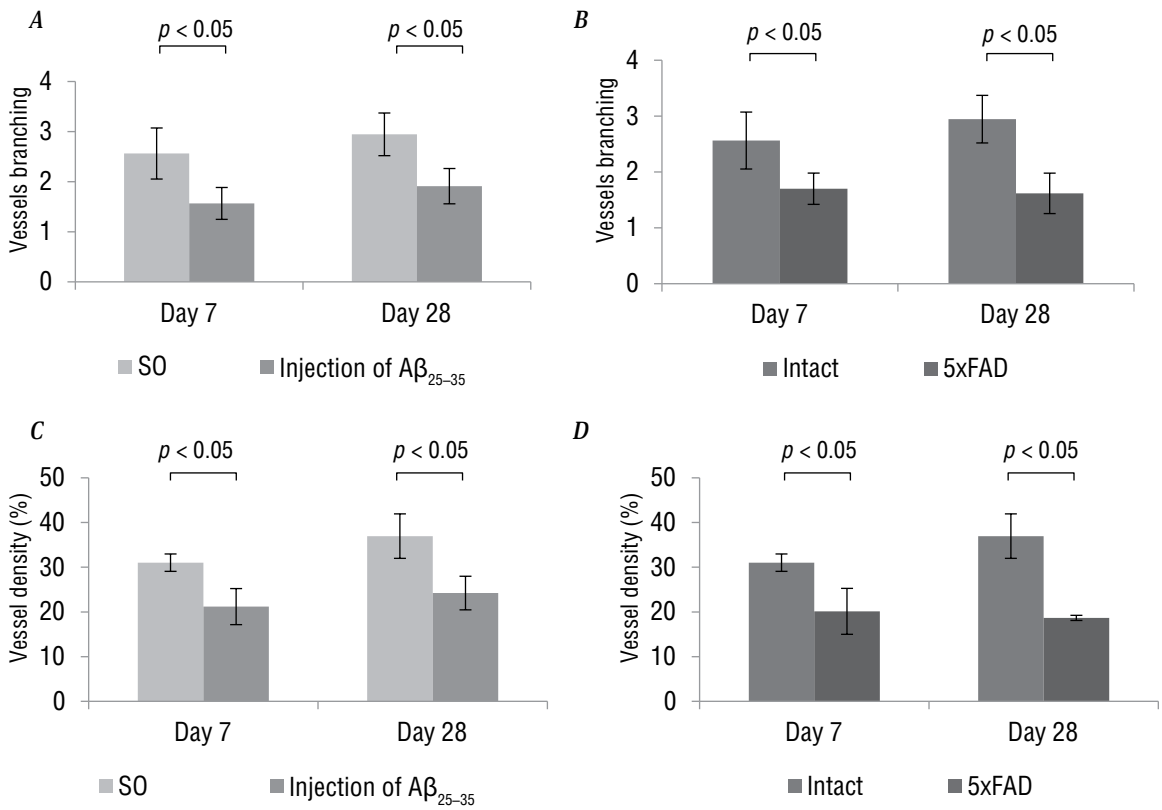

Fig. 7. Branching and density of the vasculature in the SGZ of SO animals and animals with intrahippocampal injection of $A\beta_{25-35}$ (*A, C*), intact and transgenic 5xFAD animals (*B, D*) on days 7 and 28 post-training, $p < 0.05$.

the mechanism of lateral inhibition is likely impaired (the number of tip cells exceeds that of stalk cells throughout the follow-up period). In contrast, transgenic animals exhibit a different dominant mechanism of angiogenesis impairment: proliferative activity of endothelial cells rapidly diminishes (likely due to apoptosis or stimulation of microvessel regression), accompanied by a lack of significant conversion of endothelial cells to the tip phenotype.

The newly obtained data generally align with key changes we previously documented in animals with Alzheimer's type neurodegeneration induced by intrahippocampal amyloid-β administration: imbalanced expression of LC3B, ZO1, VEGFR2, VEGFR3, CD146, ICAM2, Dll4, and Tie2 in the SVZ [38]; impaired mitochondrial dynamics and autophagy of endothelial cells in the SGZ and entorhinal cortex [39]; aberrant maturation of endothelial cells in the SGZ and SVZ, manifested by altered proportions of $CLDN5^+$ cells among total $CD31^+$ cells [40]; dysregulation of tip-, stalk-, and phalanx-type endothelial cells in the prefrontal cortex [41]; increased microvascular permeability and disrupted branching in the CA1, CA2, and CA3 hippocampal regions of 5xFAD mice [42]. Furthermore, in animals with intrahippocampal amyloid-β administration, we previously identified imbalanced neurogenesis and angiogenesis induction at the presymptomatic stage in the SGZ and SVZ [10], as well as differential dynamics of Arg3.1/Arc expression in postmitotic young neurons of the SGZ and SVZ alongside suppressed proliferative activity in the SGZ and elevated activity in the SVZ [29].

Thus, under physiological conditions, training stimulates proliferative activity of endothelial cells in the hippocampal SGZ; however, in the absence of significant neurogenesis changes, this is not associated with phenotypic alterations in SGZ microvascular endothelial cells. In animals subjected to the toxic effects of intrahippocampally administered $A\beta_{25-35}$ oligomers, induction of neurogenic and angiogenic events occurs in the SGZ, but the lateral inhibition mechanism regulating stalk-to-tip cell conversion is disrupted, leading to impaired microarchitecture of the forming hippocampal capillary network. In animals with a genetically determined AD model, the "demand" for enhanced neoangiogenesis during intensified post-training neurogenesis remains unfulfilled. This is accompanied by a lack of significant endothelial subpopulation changes and results in an aberrant microvascular network in the hippocampus.

Conclusion

Non-productive angiogenesis and hypervascularization, extensively studied in recent years within the context of AD pathogenesis [43, 44], manifest through fundamentally distinct mechanisms in the SGZ of animals with two different Alzheimer's type neurodegeneration models. Therefore, although cognitive deficits develop by day 28 post-cognitive "challenge" (training in the PA test) in both AD models regardless of the dominant plasticity impairment mechanism, the identified features of aberrant neurogenesis and angiogenesis implementation in the hippocampus must be considered when developing new therapeutic strategies for sporadic and familial AD.

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