Alzheimer’s disease (AD) is characterized by the accumulation of amyloid-β (Aβ) which ultimately forms plaques. These Aβ deposits can be induced in APP transgenic mouse models by prion-like seeding. It has been widely accepted that anosmia and hyposmia occur during the early stages of AD, even before cognitive deficits are present. In order to determine the impact of seed-induced Aβ deposits on olfaction, we performed intracerebral injections of seed-competent brain homogenate into the olfactory bulb of young pre-depositing APP transgenic mice. Remarkably, we observed a dramatic olfactory impairment in those mice. Furthermore, the number of newborn neurons as well as the activity of cells in the mitral cell layer was decreased. Notably, exposure to an enriched environment reduced Aβ seeding, vivified neurogenesis and most importantly reversed olfactory deficits. Based on our findings, we conclude that altered neuronal function as a result of induced Aβ pathology might contribute to olfactory dysfunction in AD.

**INTRODUCTION**

Alzheimer’s disease (AD) is a neurodegenerative disease that is characterized by cognitive decline and memory impairment. In addition, olfactory dysfunction is an early symptom that has been proposed as a possible biomarker to assess the onset and progression of AD [1]. Studies of olfaction in AD have shown a number of deficits such as impaired odor identification, detection, recognition, sensitivity and discrimination [2, 3]. It is also well established that patients with AD exhibit olfactory deficits and impaired odor identification much earlier than during normal aging or the onset of dementia [4-6]. Yet, the mechanism behind this dysfunction remains poorly understood. In line with these functional impairments, the two neuropathological hallmarks of AD, senile plaques and neurofibrillary tangles, are encountered in the olfactory bulb of AD patients and in mouse models of AD, suggesting that it is one of the first sites undergoing pathological changes [1, 7–12]. Interestingly, in AD patients Aβ deposits can be found especially in the olfactory glomerular layer [13] and the anterior olfactory nucleus [14].

In general, the aggregation of Aβ is considered an essential early trigger in AD pathogenesis that leads to neurofibrillary tangles, neuronal dysfunction and dementia [15, 16]. Ample evidence from in vivo seeding model studies in the hippocampus suggests that Aβ aggregation can be initiated by prion-like seeding [17–21]. Interestingly, such seed-induced Aβ deposits were recently shown to impair memory and to diminish adult neurogenesis [22].

Importantly, the olfactory bulb is constantly supplied with newly generated neurons from the subventricular zone (SVZ) of the lateral ventricles which is one of the two neurogenic niches in the adult mammalian brain [23–26]. From the SVZ, the progenitor cells migrate through the rostral migratory stream (RMS) to the olfactory bulb, where neuroblasts mature into olfactory bulb interneurons. Those newborn interneurons are required for odor detection, discrimination, olfactory memory and responses (e.g., avoidance) [27–29]. Newborn granule and periglomerular cells are continuously added to the olfactory system to modulate the activity of mitral and tufted cells, which in turn project to other brain areas including the piriform cortex, entorhinal cortex and amygdala [30–33].

Despite compelling evidence that seed-induced Aβ deposits in the hippocampus elicit neuronal functional deficits and behavioral phenotypes [22], it remains unknown whether seed-induced Aβ deposits in the olfactory bulb have an impact on the cellular level and on olfaction. Addressing these questions is however fundamental to advance our understanding of the very early disease stages and of disease progression.

**METHODS**

**Animals**

We used heterozygous 5xFAD transgenic mice coexpressing human APPK670N/M671L (Sw) and PS1M146L-I716V under the control of the neuron-specific Thy-1 promoter [34] and heterozygous APP23

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single hemisphere was immunostained. Areas such as the olfactory bulb, mitral cell layer, piriform cortex and the SVZ were defined based on the mouse brain atlas [38]. Total Aβ load was determined by calculating the % areal fraction occupied by Aβ positive staining in the olfactory bulb using the imaging software ImageJ (National Institutes of Health freeware). 5–6 animals per group and 6 sections per animal were analyzed. The sections represented always the same layers in each animal, starting from Bregma 5.0 to Bregma 3.7.

Cell number was quantified by counting the number of positive labeled cells in the area of interest of the animals. 5–7 animals per group and 3–4 sections per animal were analyzed. The 25 μm thick serial coronal sections represented always the same layers in each animal, starting from Bregma 5.0 to Bregma 3.7. The sections for the piriform cortex were chosen between Bregma 2.3 and 1.6.

Cell counting was done in the olfactory bulb and the area of the olfactory bulb was measured with the ImageJ software. Cell counts were performed within a defined volume based on the region of interest and the thickness of the section (25 μm). All analyses were conducted in a blinded manner.

**Environmental enrichment**

8 weeks after intracerebral injections into the olfactory bulb transgenic mice and their non-transgenic siblings were housed in an enriched environment (EE) or in standard conditions (SH) for 4 weeks. Mice were housed in groups of 4 mice. The enriched environment consisted of larger cages (40 x 60 cm) that contained 1 running wheel, tunnel systems, small plastic houses and extra nesting material. The animals had free access to the running wheel. At the end of the experiment at the age of 20 weeks all mice were sacrificed.

**Electrophysiology**

Mice were deeply anesthetized with an i.p. injection of ketamine/xylazine mixture (100 mg/10 mg per kg body weight) and placed in a stereotaxic frame. A craniotomy was performed over the olfactory bulb, and a custom-made head plate was cemented to the skull. A 4-channel 4-shank silicon probe (Cambridge Neurotech) was inserted into the brain until the mitral cell layer was detected by prominent multi-unit activity. Continuous recordings were sampled at 30 kHz with an openEphys acquisition system. For each shank with an electrode in the mitral cell layer power spectral density was obtained offline by fast-Fourier transform using Welch’s method in 1-s windows.

**Olfaction test**

For all olfaction tests mice were used 12 weeks after intracerebral injections into the olfactory bulb. All experiments were done in the morning.

**Buried food test**

The buried food (cookie) test was based on the time it took the mice to find a hidden buried cookie in the bedding, as described previously [39]. In brief, mice were exposed to the cookie two days before the test. The next day, mice were fasted 12 h before the test and habituated to the testing room for 1 h. Then mice were placed into a clean cage (41 cm length x 26 cm width x 18 cm height). The test began by placing the mouse in a clean cage containing 3 cm deep bedding. Following 10 min of habituation, a cookie was placed 0.5 cm below the bedding. The timer was started and the latency to find the cookie was recorded. The mouse was considered to have uncovered the cookie when it started to eat the cookie. If the mouse did not find the cookie within 15 min, the trial was ended and the mouse was excluded from the experiment.

The following animal numbers were used: for the two WT groups n = 11, SxFAD un.injected n = 13, SxFAD + WT n = 15 and SxFAD + SxFAD n = 15.

**Habituation/Dishabituation test**

The capability of mice to detect and differentiate various odors (non-social odors) was examined with the olfactory habituation/dishabituation test. The test was done according to established protocols, with minor changes [39]. The main aim of this test is to measure an animal’s tendency to investigate novel smells. This phenomenon can be assessed through presenting the mice with a sequence of different odors. Habituation is defined by a decrease in time spent sniffing the same odor. Dishabituation is represented by a reinstatement of olfactory investigation when a novel odor is presented. Prior
to testing, mice were allowed to acclimate for 30 min to the test room and a clean test cage with new bedding. Non-social odors (Carl Roth) were prepared on the same day of the test, which included: (1) distilled water; (2) solution with orange extract; (3) solution with cinnamon extract, (4) solution with coconut and (5) solution with pine extract. The solutions were prepared by adding 10\(\mu\)l of the test extract to 990\(\mu\)l of mineral oil (Sigma Aldrich, 1:1000 dilution). Stimuli were presented in the following order: water × 3, orange × 3, water, cinnamon × 3, water, coconut × 3, water and pine × 3. A trial period of 60 s was given for each stimulus presented, and thus the time spent sniffing the tip for each stimulus was recorded in seconds using a silent stopwatch. The odors were presented on a piece of whatman paper (1 × 1 cm). There was a 60 s break between each stimulus.
The following animal numbers were used: for the two WT groups \( n = 10 \), 5xFAD uninjected \( n = 12 \), 5xFAD + WT \( n = 15 \) and 5xFAD + 5xFAD \( n = 17 \).

**Olfactory avoidance test**

An olfactory avoidance test was performed as described previously [40]. The mice were habituated to the testing room for 1 h. Then mice were placed into a clean cage (33 cm length \( \times \) 20 cm width \( \times \) 12.5 cm height). The test started by placing the mouse in a clean cage containing 3 cm deep bedding. The test cage was divided into two equal areas. Following 10 minutes of habituation to the cage, a cotton swab scented with nTMT (2,4,5-Trimethylthiazole, Sigma Aldrich, 1:100 diluted in water) was placed in one half of the test cage. Avoidance time was measured during a 60 s test time. "Avoidance time" was defined as the time spent in the area without a cotton swab scented with nTMT. Avoidance behavior was represented by an avoidance index as follows: avoidance index \( = (P-50)/50 \), where \( P \) is the percentage of avoidance time during a 60 s test period.

The following animal numbers were used: WT \( n = 8 \), WT + 5xFAD and 5xFAD + WT \( n = 7 \), 5xFAD uninjected \( n = 9 \) and 5xFAD + 5xFAD \( n = 10 \).

**Immunoblot analysis of the olfactory bulb**

Mouse olfactory bulb tissue was dissected on ice and homogenized in 10x volume RIPA buffer. After passing the sample 10 times through a syringe needle and incubation at 4 °C for 30 min, the samples were centrifuged at 20 °C until the supernatant was stored at \(-20 \) °C until use. Protein concentration was determined by Pierce BCA Protein Assay Kit (Thermo Fisher Scientific).

Brain homogenates from the olfactory bulb were subjected to SDS-PAGE using 10% SDS or 4–12% NuPAGE Bis-Tris gels (using NuPAGE 4xLDS sample buffer, NuPAGE 10x sample reducing agent and NuPAGE MES SDS running buffer (Invitrogen)).

Proteins were transferred onto a nitrocellulose or PVDF membrane (0.2 µm pore size; Protran, Whatman and immunoblotted with antibodies specific to BDNF (rabbit, 1:2000, Santa Cruz, sc-456), CCL2 (rabbit, 1:1000, Novus Biologicals NB2P-41209), DCX (rabbit, 1:3000, abcam, ab18723), TNFa (goat, 1:1000, Invitrogen, PA5-46945), β-actin-HRP (mouse, 1:5000, abcam, ab20272), α-tubulin (chicken, 1:1000, abcam, ab98994), anti-chicken IgG HRP-linked Antibody (1:5000, Santa Cruz, sc-2947), anti-goat IgG HRP-linked Antibody (1:3000, Santa Cruz, sc-2033), and anti-rabbit IgG HRP-linked Antibody (1:5000, abcam, ab16264). Proteins were visualized using Clarity Western ECL Substrate (Biorad) or Supersignal West Femto Maximum Sensitivity Substrate (Thermo Fisher Scientific) and ChemiDoc MP Imaging System (Biorad).

Gene expression analysis. Olfactory bulbs were homogenized in extraction buffer (Pico Pure Kit, Life Technologies). Afterwards RNA was isolated with the Arcturus Pico Pure RNA Isolation Kit (Life Technologies) according to the manufacturer’s protocol. Reverse transcription and real-time PCR analysis were performed using the high capacity RNA-to-cDNA Kit and Gene Expression Master Mix reagents (Applied Biosystems) according to the manufacturer’s recommendations. qPCRs were analyzed with a LightCycler 480 (Roche). For gene expression analysis, we used the following TaqMan Gene Expression Assays: Actb (Mm01205647_g1), Ccl2 (Mm00441242_m1) and Tnfa (Mm00443258_m1).

**RESULTS**

**Aβ seeding in the olfactory bulb of 5xFAD mice is mainly located in the subependymal layer and the anterior commissure**

As exogenous induction of Aβ deposition is a time-dependent process [20, 22, 41] we performed intracerebral injections of Aβ containing brain homogenate into the olfactory bulb of 2-month-old pre-depositing 5xFAD mice and analyzed the mice at different time points (4, 8, 12 and 16 weeks post-injection) (Fig. 1a). First signs of seed-induced Aβ deposits appeared 8 weeks post-injection (p.i.), increasing with time at 12 and 16 weeks p.i. (Fig. 1b, c). For our further experiments we decided to use 12 weeks p.i. because un.injected control 5xFAD mice at this age (5 months) are still devoid of any endogenous Aβ plaques and rather start developing Aβ plaques later at the age of 6 months in the granular cell layer of the olfactory bulb (Supplementary Fig. 1). Only 5xFAD mice injected with Aβ-containing brain homogenate developed numerous seed-induced Aβ plaques in the subependymal layer and the anterior commissure (aco), the central region of the olfactory bulb, while no seeded Aβ deposits were found in all other groups tested (WT or control injected animals) (Fig. 1d, e). The seeding pattern is mostly diffuse and negative for Thiazine red (Fig. 1f). In order to generalize these findings, we confirmed our results in APP23 transgenic mice [35] by performing injections into the olfactory bulb of 6-month-old APP23 mice and incubating them either for 4 or 6 months. In line with our previous results, massive seed-induced Aβ depositions were found in the subependymal layer of APP23 mice very similar to seeded 5xFAD mice (Supplementary Fig. 2a, b) suggesting that Aβ seeding in the olfactory bulb is a general phenomenon and not restricted to specific APP-transgenic mouse model.

**Aβ seeding induces olfactory deficits in young 5xFAD mice**

Inspired by our previous study where we reported memory deficits due to Aβ seeding in the hippocampus [22], we proceeded to analyze olfactory functionality and performed olfactory tests in order to directly determine the effect of seed-induced Aβ deposits on olfactory performance. Therefore, we injected 2-month-old 5xFAD mice with Aβ-containing brain homogenate followed by...
different olfaction tests after an incubation time of 12 weeks (Fig. 2a). Remarkably, seeded 5xFAD mice required significantly more time to find the buried food when compared to all other groups (Fig. 2b). Moreover, during olfactory avoidance tests, seeded 5xFAD mice spent significantly more time in the avoidance area (Fig. 2c), again indicative of olfactory deficits. 5xFAD mice injected with WT homogenate did not show any differences in olfactory behavior compared to the other control groups, indicating that the injection itself has no effect on olfaction. Since it has been reported that AD patients also show difficulties in discriminating different odors \(42, 43\), we finally conducted a habituation/dishabituation test. Indeed, 5xFAD mice injected with Aβ-containing brain homogenate had profound problems differentiating between familiar and unfamiliar odors (Fig. 2d), while all control groups were able to discriminate between different odors, corroborating the results obtained with the buried food and avoidance tests. Thus, Aβ seeding seems to exert a direct influence on the olfactory behavior of mice.

**Aβ seeding alters adult neurogenesis and neuronal activity in the olfactory bulb**

Based on the finding that induced Aβ deposits are primarily located in the subependymal layer and the aco where neuroblasts reach the olfactory bulb to become interneurons, we used DCX, a well-established marker for neurogenesis expressed by immature newborn neurons, and found significantly less DCX positive neurons in seeded 5xFAD mice relative to controls (Fig. 3a, b). Likewise, immunoblotting confirmed lower DCX levels in the olfactory bulb of seeded mice (Fig. 3c). Immunofluorescence of the proliferation marker Ki67 and Sox2 revealed a decreased amount of proliferating neural precursor cells in the SVZ (Supplementary Fig. 3a, b) and ependymal layer (Supplementary Fig. 3c, d). Again, this lower amount of Sox2-expressing neural stem cells was confined to the ependymal layer and coincided with the seeding formation in 5xFAD mice (Supplementary Fig. 3c, d). Interestingly, the number of Calretinin positive granule cells was as well significantly reduced in seeded 5xFAD mice (Supplementary Fig. 3e, f). However, this drop in newborn neuron numbers was not reflected by the proliferative activity since the total number of BrdU positive cells in the whole granule cell layer remained unchanged in all experimental groups (Fig. 3d). Nevertheless, the number of BrdU positive cells in the principal type of projecting neurons, the mitral cells, was substantially decreased in those seeded mice (Supplementary Fig. 4a–c), putting the mitral cell layer center stage. Therefore, we intended to study in detail whether Aβ seeding had an effect on the mitral cell layer visualized by NeuroTrace staining (green fluorescent Nissl stain) and observed indeed a reduction in the number of mitral cells in

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**Fig. 2: Seed-induced Aβ deposits result in olfactory deficits.** a Scheme of Aβ seeding experiments in combination with olfactory tests of WT and 5xFAD mice. b Quantification of the buried food test. Shown is the time the mice needed to find a hidden cookie. Data are presented as mean ± s.e.m. Significant differences were determined by the one-way ANOVA, followed by Tukey's multiple comparison test \(F(4,59) = 26, p < 0.0001\). WT uninjected and WT + 5xFAD \(n = 11\), 5xFAD uninjected \(n = 13\), 5xFAD + WT \(n = 15\) and 5xFAD + 5xFAD \(n = 15\). c Quantification of the olfactory avoidance test. Data are presented as mean ± s.e.m. Significant differences were determined by the one-way ANOVA, followed by Tukey’s multiple comparison test \(F(4, 36) = 9.561, p = 0.0003\); \(p = 0.0145\). WT uninjected \(n = 8\), WT + 5xFAD and 5xFAD + WT \(n = 7\), 5xFAD uninjected \(n = 9\) and 5xFAD + 5xFAD \(n = 10\). d Quantification of the habituation/dishabituation test. Shown is the time the mice explored the scents. Data are presented as mean ± s.e.m. Significant differences were determined by the two-way ANOVA (\(p < 0.0001\)). WT uninjected and WT + 5xFAD \(n = 10\), 5xFAD uninjected \(n = 12\), 5xFAD + WT \(n = 15\) and 5xFAD + 5xFAD \(n = 17\).
the seeded mice (Fig. 3e, f left), whereas the number of tufted cells in the external plexiform layer did not change (Fig. 3f right). It is well known that fast gamma oscillations rely on the dendrodendritic interaction between excitatory mitral cells and inhibitory granule cells [44–46]. Thus, to assess whether the OB was functionally impaired in seeded mice, we conducted local field potential recordings from anesthetized mice. Spectral analysis of electrodes located in the mitral cell layer revealed a significant reduction in the power of spontaneous gamma oscillations, suggesting indeed impaired activity of mitral cell-granule cell...
Fig. 3 Aβ seeding reduces adult neurogenesis, neuronal function and activity. a Fluorescence microscopy of Aβ seeding (6E10, red), DCX (green) and DAPI (blue). Shown are representative images of olfactory bulbs from mice sacrificed at the age of 5 months (12 weeks p.i.). Mice were WT or 5xFAD mice uninjected, injected with WT or 5xFAD brain homogenate. Scale bar represents 50 μm. b Quantification of DCX-positive cells in the olfactory bulb of WT and 5xFAD mice uninjected, injected with WT or 5xFAD homogenate. Scale bar represents 50 μm. c Immunoblotting confirmed higher DCX levels in the olfactory bulb of seeded 5xFAD mice housed in EE (Fig. 4d).

Enriched environment prevents Aβ seeding in the olfactory bulb and reverses olfactory deficits

There is a wide range of studies reporting beneficial effects of an environmental enrichment (EE) and voluntary running on adult hippocampal neurogenesis, Aβ plaque pathology and behavior [22, 49–51]. However, information on the effect of EE on the olfactory bulb are rare and inconsistent [52–54]. In order to determine the effect of EE on Aβ seeding in the olfactory bulb, we injected 5xFAD mice with transgenic homogenate and exposed them 8 weeks p.i. for another 4 weeks either to EE or SH (Fig. 4a). Remarkably, the seeding capacity of mice housed in an EE was dramatically reduced (Fig. 4b) whereas the number of DCX positive cells on the contrary was significantly increased in the EE group (Fig. 4c). Immunoblotting confirmed higher DCX levels in the olfactory bulb of seeded 5xFAD mice housed in EE (Fig. 4d).

Furthermore, elevated levels of BDNF in the EE group imply a stimulation of adult neurogenesis via neurotrophic factors in this context (Fig. 4d). Strikingly, olfactory deficits induced by Aβ seeding could be rescued by housing the mice in an EE as detected by the buried food and the avoidance test (Fig. 4e).

DISCUSSION

Several recent studies have implicated olfactory loss in neurodegenerative diseases such as Parkinson’s and Alzheimer’s disease [4–6], but this relation has remained ambiguous. Our study presents converging evidence for detrimental effects of seed-induced Aβ deposits on olfaction. Aβ seeding, which resembles early stages of Aβ plaque formation exerted a direct negative influence on neuronal development, neuronal activity and function. Moreover, by modulating Aβ seeding via enriched housing conditions these neuronal malfunctions were restored finally leading again to improved olfaction. Our findings thus provide insight into an Aβ driven mechanism for the loss of olfaction in a mouse model of AD. The seeding model of AD

loops (Fig. 3g). This impairment was observed only in seeded mice suggesting that it is not caused by the injection itself because the control injected mice showed normal gamma oscillation. In order to further investigate odor-induced neuronal activity, we focused on cFos expression as a reporter for activated neurons [47, 48] in brain regions known to be involved in olfaction. As a first step we determined the number of cFos positive labeled cells in the mitral cell layer. Consistent with our electrophysiological results, we found significantly lower numbers of cFos-positive cells in seeded mice when compared to controls (Fig. 3h, i). This finding raised the question if Aβ seeding would also have an impact on those brain areas, which receive direct projections from the olfactory bulb such as e.g., the piriform cortex, which is involved in olfaction as well although no seeding could be observed in this region. Notably, we obtained similar results when we quantified the number of cFos positive cells in the piriform cortex (Fig. 3j, k). Moreover, the performance in the olfactory avoidance test correlated with the amount of cFos positive cells insofar that with better test performance more cFos labeled cells were present in the brain (Fig. 3k, l). We thus conclude that seed-induced Aβ plaques disturb adult neurogenesis and neuronal activity, further supporting the concept of seeding-evoked diminished olfactory functionality.

Enriched environment prevents Aβ seeding in the olfactory bulb and reverses olfactory deficits

There is a wide range of studies reporting beneficial effects of an environmental enrichment (EE) and voluntary running on adult hippocampal neurogenesis, Aβ plaque pathology and behavior [22, 49–51]. However, information on the effect of EE on the olfactory bulb are rare and inconsistent [52–54]. In order to determine the effect of EE on Aβ seeding in the olfactory bulb, we injected 5xFAD mice with transgenic homogenate and exposed them 8 weeks p.i. for another 4 weeks either to EE or SH (Fig. 4a). Remarkably, the seeding capacity of mice housed in an EE was dramatically reduced (Fig. 4b) whereas the number of DCX positive cells on the contrary was significantly increased in the EE group (Fig. 4c). Immunoblotting confirmed higher DCX levels in the olfactory bulb of seeded 5xFAD mice housed in EE (Fig. 4d).
pathology in mice offers a unique tool for studying Aβ plaque formation in vivo at its very early stage and within a defined period of time. The biggest advantage for using seeding in the olfactory bulb is that we can study the direct effect of Aβ on olfaction without aging.

Only a few studies have addressed olfactory behavior in AD mouse models in the past, however with different outcomes. While one study reported intact odor discrimination but deficits in olfactory memory [55], others uncovered no apparent dysfunction in odor detection but rather detected pronounced odor habituation/dishabituation changes [56, 57]. Hence, we set out to investigate the formation of Aβ deposits in respect to olfactory performance in an in vivo seeding model. This model has the advantage to display Aβ plaque formation at its very early stage within a defined time period and that therefore the age of the newborn plaques is easily determinable due to the predictability of the model [58]. We first established Aβ seeding in the olfactory bulb in two different APP transgenic mouse models. Importantly, in both, the extent and the progression of Aβ seeding was similar with a considerable amount of Aβ seeding already 12 weeks p.i. in 5xFAD mice. Although at this age, seed-induced Aβ pathology was restricted to the injection site (subependymal layer and aco), neuritic plaques also become evident in the subependymal layer and granule cell layer across the bulb in uninjected control 5xFAD mice at 8 months of age [59]. Further studies need to investigate spreading to other brain regions.

We had previously shown that Aβ seeding in the hippocampus induced neuronal cell death leading to memory deficits [22]. In
agreement with this finding, we indeed discovered problems in olfaction and olfactory discrimination in seeded mice that reached a certain level of induced Aβ, providing evidence that Aβ might be the driver of olfactory deficits. Because Aβ seeding occurred predominantly in the subependymal layer and the aco of the olfactory bulb where adult born neurons from the SVZ arrive, mature to interneurons and integrate into the olfactory network [60–62], we hypothesized that adult neurogenesis is impaired in those mice as well. In fact, the number of DCX positive cells was dramatically decreased most likely due to fewer cells that are generated in the SVZ although the question still remains whether more dying cells on their way towards the olfactory bulb might account for this. Nevertheless, the overall number of BrdU/NeuN positive cells was similar, indicating that most of the cells reaching the olfactory bulb also integrate in the granule cell layer. Since Aβ seeding affected bulbar neurogenesis as well as some other neuronal populations in the olfactory bulb such as the mitral cells, we decided to analyze bulbar physiology with a focus on the mitral cell layer. Our finding of reduced spontaneous gamma oscillations is in line with the fact that gamma oscillations become altered when projection neurons or their circuitry are damaged [63]. Moreover, our result that shows the strong impairment of the mitral cell layer even without hardly any noticeable proximity to the seeding pattern points towards a disrupted network/connectivity between the different types of neurons involved in olfaction. It is important to note that the control injected animals did not show any deficits, which makes it clear that the injection alone did not cause the deficits. Furthermore, reduced cFos expression in mitral cells and piriform cortex correlated with the performance in the olfactory tests providing a mechanistic link. These data fit to our previous observations based on Aβ seeding in the hippocampus [22] and support the notion that seed-induced Aβ deposits may be a source of toxicity with functional relevance.

Enriched environment, physical activity and voluntary running have shown great promise for many different conditions [49, 50] and individual laboratories have reported ameliorated neuro-pathological AD phenotypes [22, 51, 64–67], but the potential beneficial effects for the olfactory bulb and olfaction in AD mouse models has been so far completely overlooked. Intriguingly, both our data on adult neurogenesis and the activity measure of mitral cells and neurons in the piriform cortex indicate a reversal to normal levels in mice exposed to EE. Reminiscent of our previous study performed in the hippocampus, we hypothesize that Aβ seeding was reduced possibly due to activated microglia under EE housing, finally leading to improved olfactory behavior. Collectively, our study elucidates a mechanism by which Aβ seeding initiates a pathological process that possibly culminates in olfactory dysfunction and highlights EE as a promising approach to ameliorate these early pathological effects with the potential to even restore olfactory function.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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AUTHOR CONTRIBUTIONS
SZ-W and MM-L conceived and designed the experiments. SZ-W contributed to all aspects of the experiments and data analysis. MF, DL, SK, Pd'E, AH, DE and CH assisted with the experimental work. J-FS performed the electrophysiology recordings and analysis. MB supervised the electrophysiology experiments. SZ-W, MP and MM-L discussed the results and wrote the manuscript; MM-L supervised the project. All authors edited the manuscript.

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