Effects of subchronic dietary exposure to the engineered nanomaterials SiO₂ and CeO₂ in C57BL/6J and 5xFAD Alzheimer model mice

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Abstract

Background: There is an increasing concern about the neurotoxicity of engineered nanomaterials (NMs). To investigate the effects of subchronic oral exposures to SiO₂ and CeO₂ NMs on Alzheimer’s disease (AD)-like pathology, 5xFAD transgenic mice and their C57BL/6J littermates were fed ad libitum for 3 or 14 weeks with control food pellets, or pellets dosed with these respective NMs at 0.1% or 1% (w/w). Behaviour effects were evaluated by X-maze, string suspension, balance beam and open field tests. Brains were analysed for plaque load, beta-amyloid peptide levels, markers of oxidative stress and neuroinflammation.

Results: No marked behavioural impairments were observed in the mice exposed to SiO₂ or CeO₂ and neither treatment resulted in accelerated plaque formation, increased oxidative stress or inflammation. In contrast, the 5xFAD mice exposed to 1% CeO₂ for 14 weeks showed significantly lower hippocampal Aβ plaque load and improved locomotor activity compared to the corresponding controls.

Conclusions: The findings from the present study suggest that long-term oral exposure to SiO₂ or CeO₂ NMs has no neurotoxic and AD-promoting effects. The reduced plaque burden observed in the mice following dietary CeO₂ exposure warrants further investigation to establish the underlying mechanism, given the easy applicability of this administration method.

Keywords: Amorphous silica, Nanoceria, Subchronic oral exposure study, Neurobehavioral testing, Neurotoxicity, Alzheimer’s disease

Introduction

The development and steady introduction of new engineered nanomaterials (NMs) to the market has raised awareness about potential adverse health effects resulting from long-term exposures. The health risk concerns for NMs originated from inhalation toxicology studies that could substantiate the role of ultrafine particles in the epidemiological link between ambient air pollution exposure and cardiopulmonary diseases (reviewed by [1]). Likewise, the awareness about potential adverse effects of NMs on the central nervous system came from inhalation studies in more recent years. Neuroinflammatory, neurotoxicological and neurodegenerative effects observed by inhaled ultrafine particles and NMs in these toxicological studies provided experimental support to the growing number of epidemiological studies that showed associations between particulate air pollution exposure and neurological diseases [2, 3].

Specific concern has risen that long-term exposure to particulate air pollution could contribute to the pathogenesis of Alzheimer’s disease (AD) the most common
neurodegenerative disease in the world [3–5]. A major 
neuropathological hallmark of AD is the generation of 
hydrophobic Amyloid-β peptide (Aβ) containing plaques 
resulting from the sequential proteolysis of the amyloid 
precursor protein (APP) by β- and γ-secretase enzymes 
(reviewed in [6, 7]). Although the exact mechanisms of 
ininitiation and progression of AD are still incompletely 
understood, it has been suggested that specific NMs may 
be involved due to their ability to disrupt Aβ homeosta-
sis, resulting from reactive oxygen species generation 
(ROS) and oxidative stress, in similarity with other envi-
ronmental factors like specific neurotoxic metals and 
some pesticides [3, 8, 9].

With the growing evidence for a role of inhaled nano-
particles in AD, there is also an increasing debate regarding 
the neurotoxicity and potential AD-promoting effects of 
ingested NMs. Indeed, neurotoxic effects in mice have 
been reported following oral exposure to NMs composed 
of silver [10–12], zinc oxide [13, 14], titanium dioxide 
[15] and iron oxide [16]. However, to the best of our 
knowledge it has not yet been investigated if long-term 
oral exposure to NMs can promote the development and 
progression of AD. Therefore, the main goal of our 
study was to address if subchronic oral exposure to NMs 
can accelerate hallmarks of Alzheimer-like pathology in 
mice. For this purpose, we selected synthetic amorphous 
SiO2 (SAS) and CeO2 NMs (“nanoceria”), representing 
two of the most widely used and investigated types of 
nanoparticles.

SiO2 is extensively used in chemistry, agriculture and 
consumer products, including cosmetics [17, 18]. In the 
food sector, it finds application as an anti-caking agent in 
powdered food products and is listed in Europe as a food 
additive E551 [19]. CeO2 NMs are used as well in various 
commercial and industrial applications, e.g., as a catalyst, 
an ultraviolet-filter [20] and as a fuel additive to improve 
combustion [21]. They are also increasingly promoted in 
agricultural applications [22, 23]. Although CeO2 NMs 
are not used as a food additive, accumulation in agricul-
tural crops and trophic transfer have been reported [24, 
25]. Furthermore, as an additive to diesel and gasoline 
fuels, CeO2 NMs could be inhaled following their emis-
ion with the vehicle exhaust [21, 26] and subsequently 
reach the gastrointestinal tract following mucociliary 
clearance and swallowing as previously shown for other 
ultrafine particles [27, 28]. Finally, because of the coexist-
ence of Ce³⁺ and Ce⁴⁺ in nanosized CeO2 and its result-
ing unique redox-active properties, nanoceria has also 
received rapidly growing attention in biomedical and 
pharmaceutical applications [29, 30].

For our present investigations, the SiO2, and CeO2, NMs 
were incorporated into mouse feed pellets at 1% and 0.1% 
weight/weight (w/w) concentrations. The 1% dosing of 
the NMs in the pellets was selected on the basis of the 
amount of SiO2 that should not be exceeded in food 
applications, i.e. 2%, according to the US Food and Drug 
Administration [31]. While we selected the same doses 
for both types of NMs, it should be emphasized that for 
CeO2 the anticipated human exposures are likely much 
lower than for the food additive SiO2.

For the investigation of neurotoxicity and AD-like 
pathology, female heterozygous 5xFAD mice [32, 33] and 
their female nontransgenic C57BL/6J littermates were 
used. The 5xFAD mouse model is characterized by a 
steadily increasing amyloid deposition, starting from the 
age of 2 months and continuing to increase until at least 
after the age of 6 months [32]. Phenotype-dependent 
memory impairments and motor deficits can be observed 
in these mice from the age of 4–6 months [33]. Accord-
ingly, at an age of 9 weeks, the mice were fed ad libitum 
during 3 or 14 weeks with the various NM-dosed or con-

control pellets. In the 3rd and 14th week of exposure neu-
rotoxicity was assessed by a series of behavioural tests, 
while specific effects on AD-like pathology were evalu-
ated in the 5xFAD mice by evaluation of plaque load, 
Aβ-peptide levels and markers of oxidative stress and 
neuroinflammation. With the same mouse model, we 
previously demonstrated that inhalation exposure to die-
sel engine exhaust results in an accelerated formation of 
Aβ-plaques as well as motor function impairment [9]. In 
the present study, general toxicity beyond the brain was 
concurrently assessed by analysis of body weight gain as 
well as gross examinations, weight and histopathological 
analyses of specific organs.

Results

Body and organ weight changes

No effects on body weight gain were observed in the 
5xFAD mice or their C57BL/6J littermates during 3 weeks 
of feeding with the SiO2 or CeO2 dosed feed pellets (see 
Fig. 1 and Table 1). In the 5xFAD mice that were exposed 
for 14 weeks to 1% SiO2, a reduction in body weight gain 
was observed from weeks 5–7 as well as from weeks 9–13 
(Fig. 1D). In the corresponding non-transgenic mice, the 
subchronic exposure to SiO2 did not cause any significant 
reduction in body weight gain (Fig. 1C).

In the C57BL/6J mice that were exposed for 14 weeks 
to 1% CeO2 body weight gain and body weights at sac-
rifice were found to be significantly augmented in com-
parison to the corresponding control group (Table 1B). 
This effect was observed from exposure week 8 onwards 
(Fig. 1C).

We found no differences in the weights of liver, 
spleen, kidney, small intestine or colon of the 5xFAD 
and C57BL/6J mice after 3 weeks or 14 weeks exposure 
(Table 1). Treatment related effects on length and weight/
length ratios of small intestine and colon were also not seen, with one exception: A reduced colon length was observed in the 5xFAD mice after 3 weeks exposure to 1% SiO₂ (Table 2). However, colon weight and colon weight/length ratio were not significantly different in this group.

**Histopathology**

Histopathology was performed on liver and spleen, small and large intestine of C57BL/6J mice that were exposed for 14 weeks to evaluate potential treatment-related dose-dependent effects (Table 3). In the liver, increased glycogen was observed in 3 out of 6 mice exposed to 0.1% CeO₂ and periportal vacuolation in 3 out of 6 mice exposed to 1% CeO₂. In small and large intestine focal, minimal inflammatory infiltrates were seen, occasionally together with some focal irregular epithelial surfaces. These findings and all other findings seen in the

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**Fig. 1** Body weight gain (%) of C57BL/6J (A, C) and 5xFAD (B, D) mice during 3 weeks (A, B) or 14 weeks (C, D) exposure to CeO₂ or SiO₂ [0.1% or 1%] in feed pellets ad libitum. Statistical analysis was performed using ANOVA followed by Dunnett evaluation; *p < 0.05 and **p ≤ 0.01 versus mice exposed to control feed pellets. Number of animals per group: 3 weeks C67BL/6J control (n = 6); SiO₂ 0.1% (n = 6); SiO₂ 1% (n = 7); CeO₂ 0.1% (n = 6). CeO₂ 1% (n = 6); 5xFAD control (n = 10); SiO₂ 0.1% (n = 10); SiO₂ 1% (n = 9); CeO₂ 0.1% (n = 10). CeO₂ 1% (n = 10); 14 weeks C67BL/6J control (n = 5); SiO₂ 0.1% (n = 6); SiO₂ 1% (n = 6); CeO₂ 0.1% (n = 6). CeO₂ 1% (n = 6); 5xFAD control (n = 11); SiO₂ 0.1% (n = 9); SiO₂ 1% (n = 10); CeO₂ 0.1% (n = 10). CeO₂ 1% (n = 10)
### Table 1: Body and organ weights

|                  | Control C57BL/6J | C57BL/6J 5xFAD | SIO2 [0.1%] C57BL/6J | C57BL/6J 5xFAD | SIO2 [1%] C57BL/6J | C57BL/6J 5xFAD | CeO2 [0.1%] C57BL/6J | C57BL/6J 5xFAD | CeO2 [1%] C57BL/6J | C57BL/6J 5xFAD |
|------------------|-----------------|----------------|----------------------|----------------|------------------|----------------|---------------------|----------------|-------------------|----------------|
| **A**            |                 |                |                      |                |                  |                |                     |                |                   |                |
| Body weight, sacrifice (g) | 20.17±1.32   | 19.15±0.82     | 19.12±1.03           | 18.78±0.92     | 19.23±0.67      | 18.58±0.92     | 19.65±1.51         | 19.23±1.04     | 19.15±0.91      | 19.20±0.78     |
| Body weight gain (g)* | 2.47±0.66    | 2.21±0.33      | 1.80±0.47            | 2.03±0.61      | 1.83±0.35       | 1.79±1.03      | 2.17±0.52          | 2.46±1.17      | 2.15±0.42       | 2.38±0.47      |
| Liver/body weight (mg g⁻¹) | 45.28±8.89   | 44.71±5.55     | 43.52±4.78           | 47.36±5.57     | 42.64±6.70      | 45.27±8.54     | 47.04±2.06         | 44.50±4.74     | 44.96±3.04      | 44.08±7.27     |
| Spleen/body weight (mg g⁻¹) | 4.07±0.31    | 3.80±0.40      | 4.31±0.76            | 4.10±0.51      | 3.66±0.47       | 4.23±0.68      | 3.92±0.66          | 3.92±0.38      | 3.82±0.29       | 3.89±0.69      |
| Kidney/body weight (mg g⁻¹) | 10.20±3.01   | 8.99±3.61      | 10.18±3.22           | 8.60±3.25      | 9.92±2.62       | 8.60±3.41      | 10.74±3.82         | 8.60±2.98      | 9.64±2.83       | 9.22±3.21      |
| Colon/body weight (mg g⁻¹)  | 8.19±0.81    | 8.39±1.37      | 8.19±1.03            | 8.15±1.09      | 8.17±0.91       | 8.69±0.85      | 8.55±0.78          | 8.91±0.78      | 8.03±1.13       | 8.79±1.22      |
| Small int./body weight (mg g⁻¹) | 42.51±6.00 | 42.38±7.08     | 40.47±6.60           | 46.40±5.41     | 40.96±7.20      | 40.89±13.61    | 39.84±7.16         | 44.34±5.74     | 45.72±1.55      | 46.69±3.28     |
| **B**            |                 |                |                      |                |                  |                |                     |                |                   |                |
| Body weight, sacrifice (g) | 21.68±1.36   | 22.56±1.25     | 23.72±1.53           | 22.32±2.24     | 22.33±1.39      | 21.60±1.03     | 22.47±1.05         | 22.92±1.65     | 25.20±2.14      | 21.92±0.26     |
| Body weight gain (g)* | 3.98±0.80    | 4.96±0.60      | 5.10±1.19            | 4.72±1.07      | 4.28±1.28       | 3.88±0.67      | 3.73±0.69          | 5.45±1.64      | 6.72±1.59       | 4.47±0.77      |
| Liver/body weight (mg g⁻¹) | 42.59±2.92   | 42.20±4.57     | 39.62±2.99           | 42.97±1.97     | 43.67±3.97      | 43.47±161      | 42.37±5.91         | 43.80±2.96     | 36.77±3.20      | 43.83±3.51     |
| Spleen/body weight (mg g⁻¹) | 3.61±0.36    | 3.55±0.27      | 3.46±0.27            | 3.60±0.45      | 4.09±1.02       | 3.48±0.44      | 3.44±0.41          | 3.61±0.52      | 3.05±0.34       | 3.50±0.39      |
| Kidney/body weight (mg g⁻¹)  | 6.03±0.47    | 6.07±0.39      | 5.68±0.64            | 5.99±0.32      | 6.15±0.88       | 6.26±0.53      | 6.26±0.42          | 6.07±0.39      | 5.61±0.73       | 5.52±1.96      |
| Colon/body weight (mg g⁻¹)  | 8.08±1.05    | 8.25±0.84      | 7.77±0.86            | 8.36±0.67      | 9.13±1.09       | 8.38±0.58      | 8.68±1.01          | 8.50±0.46      | 7.55±0.41       | 8.84±0.83      |
| Small int./body weight (mg g⁻¹) | 36.80±1.84  | 36.39±3.50     | 36.83±5.11           | 38.14±3.78     | 35.88±3.88      | 36.89±3.59     | 37.05±4.18         | 36.19±4.20     | 31.29±2.33      | 36.65±3.92     |

Data represent mean ± SD of C57BL/6J and 5xFAD mice body and organ weights after 3 weeks (A) or 14 weeks (B) exposure to CeO2 or SIO2 [0.1% or 1%] in feed pellets ad libitum versus the body and organ weights of the corresponding mice that received control feed pellets. Statistical analysis was performed using ANOVA with Dunnett evaluation.

*Versus control feed pellets exposed C57BL/6J mice, p < 0.01

*Body weight gain at time interval between exposure start and sacrifice
### Table 2 Lengths and weight/length ratios of colon and small intestine

|                | Control C57BL/6J | SiO2 [0.1%] C57BL/6J | SiO2 [1%] C57BL/6J | CeO2 [0.1%] C57BL/6J | CeO2 [1%] C57BL/6J | Control 5xFAD | SiO2 [0.1%] 5xFAD | SiO2 [1%] 5xFAD | CeO2 [0.1%] 5xFAD | CeO2 [1%] 5xFAD |
|----------------|------------------|-----------------------|--------------------|----------------------|-------------------|----------------|------------------|----------------|------------------|----------------|
| **A** Colon length (cm) | 7.88 ± 0.66 | 7.77 ± 0.60 | 7.23 ± 0.65 | 7.41 ± 0.68 | 7.81 ± 0.53 | 6.97 ± 0.54 | 7.58 ± 0.56 | 8.00 ± 0.54 | 6.97 ± 0.89 | 7.56 ± 0.68 |
| C. weight/length ratio (g cm⁻¹) | 0.022 ± 0.002 | 0.021 ± 0.003 | 0.022 ± 0.002 | 0.021 ± 0.004 | 0.020 ± 0.003 | 0.023 ± 0.003 | 0.022 ± 0.003 | 0.022 ± 0.003 | 0.022 ± 0.003 | 0.022 ± 0.003 |
| Small intestine length (cm) | 31.30 ± 2.47 | 34.20 ± 2.55 | 31.48 ± 1.68 | 33.00 ± 2.66 | 32.34 ± 2.13 | 32.11 ± 2.52 | 31.95 ± 3.05 | 32.66 ± 2.83 | 31.55 ± 1.99 | 31.64 ± 2.43 |
| S.I. weight/length ratio (g cm⁻¹) | 0.028 ± 0.005 | 0.024 ± 0.005 | 0.025 ± 0.004 | 0.026 ± 0.003 | 0.024 ± 0.004 | 0.024 ± 0.008 | 0.024 ± 0.003 | 0.026 ± 0.003 | 0.028 ± 0.002 | 0.028 ± 0.003 |
| **B** Colon length (cm) | 8.60 ± 0.85 | 8.16 ± 0.73 | 8.32 ± 0.65 | 8.36 ± 0.55 | 8.55 ± 0.27 | 8.16 ± 0.62 | 8.55 ± 0.43 | 8.19 ± 0.85 | 7.80 ± 0.76 | 8.00 ± 0.51 |
| C. weight/length ratio (g cm⁻¹) | 0.021 ± 0.004 | 0.023 ± 0.003 | 0.022 ± 0.003 | 0.022 ± 0.002 | 0.024 ± 0.002 | 0.022 ± 0.002 | 0.023 ± 0.003 | 0.024 ± 0.004 | 0.025 ± 0.003 | 0.024 ± 0.003 |
| Small intestine length (cm) | 31.88 ± 2.65 | 31.19 ± 3.08 | 34.77 ± 1.53 | 31.79 ± 3.51 | 31.50 ± 3.12 | 32.27 ± 1.61 | 32.47 ± 1.83 | 33.55 ± 1.36 | 30.68 ± 1.64 | 32.22 ± 1.39 |
| S.I. weight/length ratio (g cm⁻¹) | 0.025 ± 0.003 | 0.027 ± 0.004 | 0.025 ± 0.001 | 0.027 ± 0.003 | 0.025 ± 0.003 | 0.025 ± 0.002 | 0.026 ± 0.003 | 0.025 ± 0.004 | 0.026 ± 0.003 | 0.025 ± 0.003 |

Data represent mean ± standard deviation of C57BL/6J and 5xFAD mice colon and small intestine length after 3 weeks (A) or 14 weeks (B) exposure to CeO2 or SiO2 (0.1% or 1%) in feed pellets ad libitum versus the body and organ weights of the corresponding mice that received control feed pellets. C = Colon; S.I. = Small intestine. Statistical analysis was performed using ANOVA followed by Dunnett evaluation.

*p < 0.05 versus 5xFAD mice exposed control feed pellets.
organ evaluated are not assessed to be treatment-related adverse effects.

** Behaviour 

Early memory deficits, followed by successive reduction of other cognitive functions are major characteristics of AD. A battery of behavioural tests was performed to assess for functional neurotoxic effects resulting from the oral exposures to the SiO2 and CeO2 NMs in the 5xFAD and C57BL/6J littermate mice, as well as to correlate their outcomes with Aβ neuropathology. Results of the behaviour studies are show in Fig. 2.

The X-maze test was used to assess for decreased spontaneous alternation behaviour as an indicator of impaired spatial working memory [33]. Spontaneous alternation is based on the natural behaviour of rodents to explore new environments and thus to rotate in the entries of the arms of the maze. We observed no significant treatment-related effects on spatial working memory in the 3-week and 14-week sub-studies for SiO2 and CeO2 (Fig. 2A, D). Total distance moved in the X-maze also did not differ between the treatment groups (Fig. 2B, E). The string suspension task was performed to evaluate the agility and grip strength of the mice [34] using a score rating system as described in detail in the methods section. For this test, also no significant differences were identified associated with the exposures to SiO2 or CeO2 although a trend toward impaired performance was observed in the CeO2 exposed C57BL/6J mice in the 14-week sub-study (Fig. 2F).

In addition to the aforementioned tests, in the 14-week sub-study the open field test [35] and the balance beam test were included. In the open field test a decreased proportion of time spent in the central versus the border regions of the arena has been proposed an indicator of increased anxiety. In this study, the WT mice that were exposed for the 14 weeks to 1% SiO2 as well as those that were exposed to 0.1% and 1% CeO2 spent significantly less time in the central region of the open field arena compared to the control mice (Fig. 2G). In the 5xFAD mice, these treatment-related differences in central residency times were not observed. However, the 5xFAD mice exposed to 1% CeO2 were found to be significantly more active and travelled a greater distance compared to the 5xFAD control mice (1.48 ± 0.45 m for control vs 2.13 ± 0.87 m, p = 0.030) indicative of increased locomotor activity (Fig. 2H). In the balance beam test, which was included as an independent indicator of motor coordination and balance [33, 36] the 14-week oral exposures to SiO2 and CeO2 revealed no significant differences, neither in the 5xFAD mice nor in the WT mice (Fig. 2I).

** Table 3 Liver and spleen, small and large intestine histopathology 

|                     | Control | 0.1% SiO2 | 1% SiO2 | 0.1% CeO2 | 1% CeO2 |
|---------------------|---------|-----------|---------|-----------|---------|
| Liver               |         |           |         |           |         |
| Focal inflammatory infiltrates | 1 (5)   | 1 (4); 2(2) | 1 (6)   | 1 (6)     | 1 (6)   |
| Focal necrosis      | 1 (2)   | 1 (1)     | 1 (2)   | 0 (6)     | 0 (6)   |
| Increased interstitial cells | 0 (5)   | 2 (1)     | 1 (1)   | 1 (1)     | 1 (1)   |
| Increased glycogen  | 0 (5)   | 0 (6)     | 0 (6)   | 2 (2); 3 (1) | 0 (6)   |
| Vacuolation         | 0 (5)   | 0 (6)     | 0 (6)   | 0 (6)     | 2 (3)   |
| Spleen              |         |           |         |           |         |
| Increased pigment   | 2 (1)   | 2 (3)     | 0 (6)   | 2 (2)     | 1 (1); 2 (1) |
| Congestion          | 0 (5)   | 2 (1)     | 0 (6)   | 0 (5)     | 0 (6)   |
| Increased extramedullary hematopoiesis | 0 (5) | 0 (6) | 3 (1) | 1 (1) | 2 (1) |
| Increased megakaryocytes | 0 (5) | 0 (6) | 2 (1) | 0 (5) | 0 (6) |

*One sample not evaluable due to embedding artefacts

Histopathology of C57BL/6J mice that were exposed for 14 weeks to CeO2 or SiO2 [0.1% or 1%] in feed pellets ad libitum. Shown is the grading of the lesion and the number of animals in brackets. The following grading has been used: 0 = no findings, 1 = minimal, 2 = slight, 3 = moderate, 4 = severe, 5 = massive

Small and large intestine

Minimal focal inflammatory infiltrates (intra-mucosal) in all specimens

In some cases, inflammatory infiltrates in adjacent tissue and pancreas with focal vacuolation (grade 2)

Clearly visible goblet cells in PAS stained slides (small intestine +++; large intestine ++++)

Partly mucus on surface. Gut associated lymphoid tissue (GALT) in almost all specimens detectable

*One sample not evaluable due to embedding artefacts
Plaque formation

Amyloid β-containing senile plaques are present before clinical symptoms of AD appear [37]. Therefore, parietal brain slices of 5xFAD mice were stained with an antibody against human Aβ42 to investigate the impact of the oral exposure to CeO₂ or SiO₂ on Aβ
Fig. 3 (See legend on previous page.)
plaque load in hippocampus and cortex of the 5xFAD mice. Results are shown in Fig. 3.

As observed in representative images, at younger age (i.e. 3-week exposure study) the 5xFAD mice display much less and smaller plaque formation (Fig. 3A) compared to the older animals (i.e. 14-week exposure study) (Fig. 3D). The relative extent of plaque formation detected in the control animals at the respective ages aligned well with the described accelerating phenotype of the 5xFAD model [32] and findings in previous studies in our lab [9, 38]. In the mice that were exposed for 3 weeks to the lower concentrations (i.e. 0.1%) of SiO2 and CeO2 tended to show some lower plaque levels, in cortex as well as hippocampus, in comparison to the control mice. However, these differences were not statistically significant. More importantly, the 14-week sub-study, plaque load tended to be decreased in dose-dependent fashion in the hippocampus of the CeO2 exposed mice. In the hippocampus as well as in the cortex, plaque load in the 1% CeO2 group was approximately half as abundant as in the control group, and statistically significant for hippocampus (ANOVA-Dunnett, $p < 0.01$) but not cortex ($p = 0.075$).

**Amyloid β levels**

To further evaluate effects of the oral exposures to the SiO2 and CeO2 NMs, cortex lysates were analysed for protein levels of Aβ40 and Aβ42 by ELISA (Fig. 4). In the tissues of the mice that were exposed for 3 weeks to the lower concentration of SiO2 and CeO2 NMs, protein levels of both Aβ40 and Aβ42 tended to be lowest, in alignment with the histopathological findings (see Fig. 3). The levels of Aβ40 in the 0.1% CeO2 group were significantly lower than the controls. Furthermore, the 1% CeO2 exposed mice revealed a significantly increased Aβ42/ Aβ40 ratio, which was mainly the result of the increased trend of Aβ42 levels in this group. In the 14-week sub-study, levels of Aβ40 as well as Aβ42 were lowest in the cortex tissues of the 1% CeO2 group. Although these differences were not significant, they aligned well with the Aβ plaque load findings (Fig. 3). Differences in Aβ42/ Aβ40 ratios were not observed at this time point.

![Fig. 4](image_url)

**Fig. 4** β-amyloid protein levels in 5xFAD transgenic mice exposed to different nanomaterials. Aβ40 (A, D) and Aβ42 (B, E) protein levels and Aβ42/ Aβ40 ratio (C, F) were determined by ELISA in cortex brain homogenates of 5xFAD mice after oral exposure to SiO2 and CeO2 nanomaterials [0.1% and 1%] for 3 weeks (A–C) or 14 weeks (D–F). Statistical analysis was performed using ANOVA with Dunnett post-hoc analysis; *$p < 0.01$ versus mice exposed to control feed pellets. $N = 6$ animals per group for 3 weeks study and $N = 7$ for 14 weeks study.
Oxidative stress

Oxidative stress resulting from a disruption of pro- and antioxidant balance has been proposed as a major mechanism of neurotoxicity of NMs [2, 39] has also been connected to β-amyloidogenesis and AD pathology [40–43]. To evaluate oxidative stress in the brains of the 5xFAD mice, we measured the levels of glutathione (GSH) [44]. In addition, we determined the ratio of reduced to oxidized glutathione (GSH/GSSG), as reduced ratios have been observed in AD [45, 46]. Results are shown in Fig. 5. The brain tissue levels of GSH were not affected following the subchronic oral exposures to SiO2 or CeO2. Also, no decreases in GSH/GSSG ratio were observed that would suggest increased oxidative stress in the brain by the nanomaterials. Interestingly, in the brains of the CeO2 exposed animals, rather a trend for a dose-dependent increase in GSH/GSSG was noted. However, this effect was not statistically significant. As an independent indicator of oxidative stress, we analysed the levels of the lipid peroxidation marker malondialdehyde (MDA) in selected brain tissue samples (Fig. 5). In alignment with the GSH findings, these results confirmed that neither SiO2 nor CeO2 cause sustained oxidative stress in the mouse brains.

Neuroinflammation

Neuroinflammation is a crucial pathological hallmark and mediator of neurodegenerative diseases including AD. We therefore evaluated the expression of ionized calcium-binding adapter molecule 1 (IBA-1) and glial fibrillary acidic protein (GFAP) in the brains of the 5xFAD mice after the subchronic exposures to SiO2 and CeO2. Increased IBA-1 expression is an indicator of activated microglia in the brain under conditions of inflammation [47] and therefore used as marker of neuroinflammation. The expression of GFAP is upregulated in most forms of reactive astrogliosis [48]. The results of the IBA-1 and GFAP analyses are shown in Fig. 6. As shown by representative immunohistochemical staining images from sections of paraffin-embedded brain hemispheres (Fig. 6A), no significant effects of the oral exposures to the NMs on IBA-1 expression were found in hippocampus (Fig. 6B) or cortex (Fig. 6C). For the cortex region this was also confirmed using Western blot analysis (Fig. 6D, E). Similarly, neither SiO2 nor CeO2 caused increased expression of GFAP. The expression of this astrocyte marker did not differ between the exposure groups as revealed by immunohistochemical analysis in hippocampus (Fig. 6F, G) and cortex (Fig. 6F, H) and independently by Western blot detection (Fig. 6I, J). Taken together, in alignment with the findings on Aβ plaque formation, Aβ peptide levels and oxidative stress markers, neither SiO2 nor CeO2 caused neuroinflammation upon long-term oral exposure.

Discussion

The present work was undertaken to address if long-term oral exposure to two of the most commonly used NMs, SiO2 and CeO2 can cause neurotoxicity and promote AD. The findings suggest that long-term oral exposure to these NMs has no adverse health impact on the central nervous system but, by contrast, support a potential anti-amyloidogenic role of CeO2 in Alzheimer’s disease.

With regard to SiO2, our findings are of main relevance in view of its use as a food additive. The amount of use of SiO2 in food applications is limited to 2% by the US Food and Drug Administration [31], while the European Food Safety Authority (EFSA), depending on the food category, authorizes the use of E551 quantum satis or mostly at a maximum permitted level (MPL) of 1%, with the exception of foods for infants and young children [19]. While EFSA concluded that there is no indication of a risk when used as a food additive, in a recent study in mice adverse effects were observed following 18 months
exposure via drinking water [49]. In the present study, mice were exposed to SiO$_2$ incorporated in the food. This differs from exposure via drinking water and the most commonly used administration by repeated gavage [12, 50]. Based on an estimated daily feed consumption of 4 g and average mouse body weight of 20 g, the ad libitum exposure to the dosed feed pellets (0.1 and 1% w/w) result in a daily intake of about 0.2 and 2 g kg$^{-1}$ body-weight (BW). Up to the highest 14-week cumulative dose, the dietary exposure to SiO$_2$ NMs did not cause accelerated plaque formation, oxidative stress, neuroinflammation, spatial working memory deficits, locomotor activity changes or motor coordination impairments. Solely, for the wildtype C57BL/6J mice an effect in the open field

Fig. 6  Ionized calcium-binding adapter molecule 1 (IBA-1) and glial fibrillary acidic protein (GFAP) pathology. Representative pictures of IBA-1 (A) and GFAP (F) (brown staining) in cortex and hippocampus of 5xFAD mice exposed orally to SiO$_2$ [1%] and CeO$_2$ [1%] nanomaterials. Analyses were performed using image analysis software (ZEN2011, Zeiss) at 200 x magnification. The output of the analyses represents the percentage of positive staining relative to the total area of the cortex or hippocampus and is defined as IBA-1 load for positive stained microglia and GFAP load for positive stained astrocytes in hippocampus (B, G) and cortex (C, H). Number of animals per group: GFAP staining control (n = 10); SiO$_2$ 1% (n = 8); CeO$_2$ 1% (n = 8); IBA-1 staining: control (n = 10); SiO$_2$ 1% (n = 3); CeO$_2$ 1% (n = 10). IBA-1 and GFAP were also visualized by western blot using FluorChem Imager (D, I). Total IBA-1 or GFAP was detected in cortex and normalized to the respective control sample (E, J). Animal numbers: control (n = 10); SiO$_2$ 1% (n = 9); CeO$_2$ 1% (n = 10)
test was observed for the 14-week 1% SiO₂ group. As this effect was not seen in 5xFAD mice and not accompanied with any further effects, it can be debated whether this reflects an adverse neurotoxic response. When applied in an unconditioned manner, the open field test has been suggested to reflect other effects than anxiety, like avoidance or natural preference responses [51].

The findings from our in vivo study in the 5xFAD model are in contrast to in vitro papers that suggest potential amyloidogenic effects of SiO₂ NMs [52, 53]. However, when investigating such direct effects of NMs on cells in vitro, it should be kept in mind that in vivo research has shown that the translocation and accumulation of SiO₂ into the brain following oral exposure is extremely low, if at all [54, 55]. Peripheral effects, including those in intestine, liver and spleen as major recognized target organs for ingested NMs, were also found to be mostly absent. However, a significantly diminished body weight gain was observed, exclusively, in the 5xFAD mice of the 1% SiO₂ group. This effect was first apparent at the 5th week of exposure but no longer present after week 14 as sacrifice. In association with this, a lower colon length was found in the 1% SiO₂ exposed 5xFAD group compared to the C57BL/6J mice. Indeed, in support of this hypothesis, differences in intestinal gene expression, trypsin levels, faecal microbiota composition and associated weight changes have been shown between 5xFAD mice and their wildtype littermates [56, 57].

Similar to SiO₂, the oral exposure to the other NM that we chose to investigate, CeO₂, did not result in adverse neurotoxic and AD-promoting outcomes. On the contrary, a marked anti-amyloidogenic effect was found in the 5xFAD mouse model. Unlike SiO₂, CeO₂ presently finds no intentional application in the food sector. However, its potential use in disease prevention, therapy and diagnostics has been promoted in several recent oral exposure studies in rodents, for instance, in models of non-alcoholic fatty liver disease [58] and colitis [59, 60].

To the best of our knowledge, this study is the first to demonstrate an inhibition of AD-like pathology following long-term oral exposure to CeO₂ NMs. Specifically, in the 14-weeks exposed mice, the Aβ plaque load was approximately 50% lower in both hippocampus and cortex of the 1% CeO₂ fed 5xFAD mice compared to the corresponding control group. In alignment with these pronounced immunohistopathology findings, cortical protein levels of Aβ40 and Aβ42 tended to be markedly lower in the 1% CeO₂ group as well, albeit not statistically significant. Since our study was a priori designed to address the potential adverse effects of long-term oral exposures to NMs, we can only speculate about underlying mechanisms of the observed beneficial effects of the CeO₂ feeding. While amyloid pathology in AD has been linked to oxidative stress and inflammation [61–63], the reduced plaque burden in the 1% CeO₂ exposed mice was not accompanied by significant changes in oxidative stress or neuroinflammation. Yet, it was interesting to observe the highest GSH/GSSG ratio in the brains of the 1% CeO₂ group. Lower GSH/GSSG ratios have been observed in AD [45, 46] and an increase might thus reflect a compensatory improved antioxidant status in the CeO₂ fed 5xFAD mice. Notably, the lower amyloid plaque burden was apparent after the 14-week cumulative exposure to CeO₂ despite the aggressive phenotype of the 5xFAD model. Using the same mouse model, we previously demonstrated a rapid acceleration of plaque formation following a 3-week inhalation exposure to diesel exhaust, representing a dominant contributor of nano-size air pollution particles in urban environments [9]. At 13-weeks exposure, the plaque promoting effect of the diesel exhaust was no longer present, most likely due to the strong age-dependent progressive nature of the 5xFAD model [9]. In our present study, a beneficial effect of the CeO₂ was not yet observed after 3 weeks, which could be due to the low absolute plaque load in cortex and hippocampus at this young age. Interestingly, however, at this time point a significant increase in Aβ42/Aβ40 ratio was detected in the 1% CeO₂ exposed 5xFAD mice, mainly as a result of the relatively higher levels of the more toxic and aggregation prone Aβ42 protein [64, 65]. Whether and how this seemingly contrasting finding at early exposure could relate to the lower formation of plaques at the later 14-week exposure needs further research. In another recent study, we investigated the neurotoxic and AD-promoting effects of CeO₂ NMs doped with varying amount of zirconium (ZrO₂) in a 4-week inhalation design in 5xFAD mice [38]. Here, unlike diesel exhaust, these CeO₂ containing NMs did not lead to an aggravated plaque formation following inhalation exposure and, unlike our current oral exposure study, also did not inhibit plaque formation in 5xFAD mice. While this may be explained by differences in levels and duration of exposure, it also demonstrates the likely importance of the route of exposure.

As a redox-sensitive nanomaterial, CeO₂ has been long recognized for its free radical scavenging properties and, therefore, is widely studied for its potential as an antioxidant and anti-inflammatory agent in the field of nanomedicine [66–68]. Several research groups have already investigated the neuroprotective properties of CeO₂ NMs and explored their potential therapeutic use in brain diseases. In a rat model of Parkinson’s disease, intrastraitral injection of CeO₂ NMs could attenuate neurobehavioral
impairments [69]. In a mouse model of multiple sclerosis, intravenous administration of citrate/EDTA-stabilized CeO2 ameliorated motor function deficits [70]. Interestingly, Kwon and co-workers [71] revealed therapeutic promise for triphenylphosphonium-conjugated CeO2 in AD by showing a suppression of reactive gliosis and mitochondria damage in 5xFAD mice upon stereotactic injection. However, in contrast to our findings, they did not observe a significant attenuation of plaque load in these mice.

While increasingly complex nanomedicine-based strategies are being proposed and developed for AD [72, 73], it was striking to observe the effects in our study (1) with pristine, non-stabilized/conjugated CeO2, and (2) by a mere dietary exposure instead of a forced intravenous injection. CeO2 NMs were shown to reduce ROS generation in PC12 cells [74]. As our study did not include a pharmacokinetic design, it is not known to what extent the CeO2 NMs may have reached and accumulated in the brain of the mice. Major progress in this specific research area has been achieved previously by Yokel and colleagues. Using CeO2 NMs of different primary size, they demonstrated that liver and spleen are major target organs in rat after a single intravenous administration, while only a small proportion of the dose enters the brain [75]. More recently, they demonstrated that translocation of CeO2 NMs from the lung to the rest of the body is less than 1% of the deposited dose and that translocation from the gastrointestinal tract is even lower [76]. However, they also observed that the organ burdens of the translocated fractions persisted for at least months, suggesting very slow clearance rates. Several other groups have confirmed the minimal to absent absorption of CeO2 NMs from the gastrointestinal tract of rats [77] and mice [78, 79]. Therefore, it should also be considered that the anti-amyloidogenic effect observed in our present study may be the result of peripheral effects of CeO2. Future studies on the neuroprotective effects of nanoceria should therefore also aim at the investigation of their effects on organs and tissues other than the brain.

In our study, indications of peripheral adverse effects of the CeO2 NMs were merely detected in the C57BL/6J mice. As a main finding, in these wildtype animals, a significant increase on body weight gain was observed during the 14 weeks with the 1% dosed pellets. Subsequent histopathology analysis revealed increased glycogen in 3 out of 6 animals of the 0.1% CeO2 group and periportal vacuolation in 3 out of 6 animals of the 1% CeO2 group. Changes in the weights of liver, spleen and kidney, as well as weights, lengths and weight/length ratios of colon and small intestine were absent. The observed histological findings in the livers of the CeO2 fed mice are likely features of increased glycogen storage and are therefore considered to be of no toxicologic significance. In contrast to our study, Yokel and co-workers recently found no increased liver vacuolization in C57BL/6 mice after a single intraperitoneal injection of CeO2 NMs, and even a decreased vacuolation in BALB/c mice [80]. In the behavioural studies, the only statistically significant effect observed in the C57BL/6J mice with CeO2 NMs at 0.1% and 1% was a diminished time spent in the centre of the open field arena. As already mentioned with regard to the comparable observations with SiO2, it can be debated whether the findings of this unconditioned test should be interpreted as an anxiety indicator or a mere change in the natural preference response [51].

As opposed to the C57BL/6 J mice, in the 5xFAD mice that were fed with 0.1% or 1% CeO2 NMs for up to 14 weeks no significant changes in body weights were found. Histopathology was not evaluated in these transgenic animals, but differences in organ weights, including length and weight/length ratios of small intestine and colon were not observed. This suggests that the beneficial plaque inhibiting effect occurred in the absence of any substantial peripheral toxicity. Moreover, behavioural changes were absent in all tests at both time points of investigation (i.e. week 3 and 14), except for the open field test. Here, the 1% CeO2 exposed 5xFAD mice at week 14 were found to be much more active compared to the corresponding 5xFAD controls. Interestingly, the distance covered in the open field test by the CeO2 fed 5xFAD animals was highly similar with that of the C57BL/6J controls (i.e. 2.13 ± 0.87 m versus 2.16 ± 0.84 m). Accordingly, it can be suggested that this activity change reflects an improved behaviour as a result of the inhibited plaque load following CeO2 exposure. Recently, the 5xFAD model has been proposed as a useful model to study motor dysfunction in AD [81]. Indeed, in line with our investigations, 5xFAD mice travel shorter distances in the open field test than WT mice with increasing age. Taken together, these initial findings in CeO2 exposed 5xFAD mice indicate a possible beneficial effect on AD-like pathology. However, before any further indication of a potential therapeutic or preventive use of orally administered CeO2 NMs should be given, designated pharmacokinetic and pharmacodynamic studies are needed, preferably using independent (rodent) AD models. This,
of course, then also requires an in-depth biocompatibility evaluation.

Conclusions
Our present study was designed to test the hypothesis that long-term oral exposure to NMs can cause neurotoxicity and aggravate the pathogenesis of AD. It was demonstrated that neither synthetic amorphous SiO2 nor CeO2 increases amyloid-β plaque formation, neuroinflammation and oxidative stress in 5xFAD Alzheimer model mice in a subchronic dietary exposure design. Behavioural analyses also revealed an absence of spatial working memory deficits and motor coordination impairments. Surprisingly, the subchronic exposure to 1% CeO2 containing feed pellets resulted in a marked inhibition of plaque burden in the 5xFAD mice and increased locomotor activity. Summarizing the results, the findings from the present study suggest that long-term oral exposure to synthetic amorphous silica NMs, which find wide applications in the food sector, has no major adverse health impact on the central nervous system, specifically regarding the development or progression of the neurodegenerative Alzheimer’s disease. The observations with CeO2 warrant further investigations to explore if long-term dietary administration of this redox-active NM could have beneficial effects in AD.

Methods
Nanomaterials
The CeO2 JRC reference nanomaterial NM-212 was purchased from the Fraunhofer Institute for Molecular Biology and Applied Ecology (IME, Schmallenberg, Germany). Detailed characteristics of the CeO2 NM-212 are provided in the JCR nanomaterial characterisation report [82]. The amorphous fumed SiO2 NM sample was obtained from Sigma-Aldrich, Munich, Germany (#S5130). This sample has been previously characterised in detail [83]. To check the particle size for the material batches applied in this study, the primary particle size distributions of the pristine NMs were determined using scanning electron microscopy (SEM) by measuring 425 primary particles (CeO2) and 500 particles (SiO2), respectively. The CeO2 NMs had a mean size of 35.4 nm ± 17 nm following a log-normal size distribution with a mode diameter of 28.7 nm and sigma = 1.38, obtained by a mathematical fit of the size distribution. The particles displayed a nearly spherical particle morphology. The analysis of the SiO revealed a spherical morphology with a mean size of 12.9 ± 4.9 nm again following a log-normal size distribution with a mode diameter of 11.0 nm and sigma = 1.32. Both materials were present in form of bigger agglomerates consisting usually of several ten to hundreds primary particles.

Study design
The oral exposure studies were performed in heterozygous 5xFAD mice and their nontransgenic C57BL/6J littersmates. The 5xFAD mouse model (Jackson Laboratories) carries five familial AD mutations and is characterized by an early onset of AD-related pathology: the double Swedish mutation (K670N/M671L), which is responsible for the enhanced amyloid production, and mutations which are responsible for altered amyloid precursor protein processing leading to a higher ratio of the more amyloidogenic Aβ production such as the Florida (I716V) and London (V717I) mutations in APP and the mutant presenilin 1 (M146L+I286V) with neuronal expression driven by the neuron-specific mouse Thy-1 promoter [32]. Amyloid deposition starts in the deep layers of the cortex and subiculum at 2 months of age, while memory and motor deficits become detectable from 4 to 6 months of age [32, 33]. The mice were handled according to guidelines of the Society for Laboratory Animals Science (GV-SOLAS) and were housed under standard conditions with access to food and water ad libitum. Lighting was artificial with a sequence of 12 h light and 12 h dark. The study was approved by the Landesamt für Natur, Umwelt und Verbraucherschutz (LANUV, NRW, Germany; Ref. no. 84-02.04.2013.A443).

Nine weeks old female 5xFAD and female C57BL/6J littersmates were exposed ad libitum for 3 or 14 weeks to feed pellets that were loaded with 0.1% or 1% (w/w) SiO2 NM or CeO2, or to control feed pellets (Fig. 7). The study was designed with n = 160 mice, i.e. for the respective sub-studies n = 50 5xFAD mice (n = 10 per treatment group) and n = 30 WT mice (n = 6 per treatment group). One 5xFAD mouse (0.1% SiO2 exposed) died in the first week of exposure and thus were excluded from all analyses. Moreover, in the 3-week sub-study one WT mouse exposed to 1% SiO2 was inappropriately labelled as 5xFAD mouse, whereas in the 14-week study one control 5xFAD mouse was inappropriately classified as WT animal. The study design is shown in Fig. 7. The feed pellets were prepared and provided by ssniff GmbH, Soest, Germany. Additional file 1: Figure S1 shows representative SEM images of the SiO2 and CeO2 NMs (Fig. S1A,B) within prepared feed pellets and by comparison of the pristine NMs (see also 5.1). Energy dispersive x-ray analysis was used to verify the presence of cerium (Additional file 1: Figure S2) and silicon (Additional file 1: Figure S3). One week prior to study start mice were randomized according to age and body weight. During the weeks before dissection, behavioural studies were performed to assess for effects on anxiety, motor performance and spatial working memory. Following sacrifice, brain tissues...
were collected as well as further organs for analyses as described below.

**Behavioural tests**

In alignment with animal ethics, requirements and routine of our animal facility and previous studies [9, 12, 38] all behaviour tasks were performed during daytime, i.e. during the resting phase of the animals. Motor function and grip strength were tested using the string suspension task, where mice are permitted to grab a string that is suspended between two platforms with their forepaws and subsequently allowed to move to one of these platforms [33, 34]. To rate motor performance during 60 s trials, a scoring system from 0 to 7 was used which was adapted from [84] and described in detail in our previous work [12, 38]. Spatial working memory by spontaneous alternation behaviour was assessed using an open arm cross (X)-maze task as described in Jawhar and colleagues [33] and recent work of our studies [9, 12, 38]. During 5 min test sessions, spontaneous alternation was measured and defined as successful if a mouse visited all of the 4 arms alternately. An impairment in spatial working memory is defined by decrease in spontaneous alternation [85]. Anxiety and exploratory activity was measured using the open field test (Noldus, the Netherlands) [35] as previously described [12]. Increased anxiety was defined by spending less time in the open central area compared to the more hidden border during 5 min test sessions. The balance beam walking assay is used to test motor coordination and balance in rodents as previously described [33, 36]. Therefore, a 50 cm long wooden beam was suspended between two plastic platforms (9 cm × 15 cm) placed above two vertical poles at a height of 40 cm. The mice were released in the middle of the beam and released thereafter allowing the mice to traverse the beam. Performance on the walking assay is quantified by measuring the time it takes for the mouse to escape to one of the platforms during a 60 s trial. The trial was repeated three times in 1 day of testing. If an animal remains on the beam for whole 60 s and does not escape to one of the platforms, the maximum time of 60 s is recorded. All three trials are averaged. To avoid odour distraction, all behaviour tasks were cleaned between trials with 70% ethanol. Behaviour tests were recorded with an infrared camera and analysed with associated software (EthoVision XT 11, Noldus).

**Dissection, tissue preparations and histopathology**

The mice were sacrificed by cervical dislocation, followed by decapitation. Right brain hemispheres were stored in 4% paraformaldehyde (PFA) (Merck, St. Louis, Missouri, USA) for immunohistochemistry. Left brain hemispheres were rapidly dissected into cerebellum, midbrain and cortex including hippocampus, snap frozen in liquid nitrogen and then stored at −80 °C until processing for biochemical analyses. Liver, spleen and kidneys were removed and weighed. Small intestines and colons were removed, flushed with saline and opened, subsequently analysed for weight and length and used to prepare Swiss-rolls...
Histology analyses were performed according to routine procedures (fixation in 4% PFA and paraffine embedding). Sections of small and large intestines, liver and spleen were blindly evaluated by an experienced veterinary pathologist using Haematoxylin and Eosin (H&E) stained sections for these organs and additionally Periodic Acid Schiff (PAS) stained sections for small and large intestine. The slides were evaluated semi-quantitatively applying the following grading score: 0 = no findings; 1 = minimal; 2 = slight; 3 = moderate; 4 = severe; 5 = massive.

Immunostaining of paraffin-embedded brain tissue sections
Immunostaining was performed using antibodies for Aβ 42 (clone G2-11, Cat.No. MABN12, Merck Millipore, Darmstadt, Germany), IBA-1 (Cat No. GTX100042, GeneTex, Irvine, California, USA) and GFAP (Cat No. Z0334, Dako Agilent, Santa Clara, USA). After sacrificing the mice and careful dissection of the brains, the right hemisphere was processed, as follows. For fixation, the tissue was immediately immersed in 4% buffered PFA at 4 °C for a minimum of 24 h and 3 μm paraffin-embedded sections were subsequently cut on glass. The sections were deparaffinized in xylene (Roth, Karlsruhe, Germany) and rehydrated in a series of ethanol (Roth) baths. To block endogenous peroxidases, sections were pre-treated with 0.3% H₂O₂ (Merck, St. Louis, Missouri, USA) in 0.01 M phosphate buffered saline (PBS). Antigen retrieval was generated by boiling slices in 10 mM citrate buffer followed by a 3 min incubation in 0.01 M phosphate buffered saline (PBS). After centrifugation (10,000 g, 15 min, 4 °C) the supernatants were deproteinized with an equal volume of 10% metaphosphoric acid (Merck). The concentration of total glutathione was expressed as (GSH/GSSG). The amount of total and oxidized glutathione was evaluated in cerebellum after homogenization in cold 100 mM phosphate buffer (pH 6.8), containing 0.1 mM EDTA (Merck). After centrifugation (10,000g, 15 min, 4 °C) the supernatants were deproteinized with an equal volume of 10% metaphosphoric acid (Merck) and thereafter with a solution of 4 M triethanolamine (Merck) as described by the manufacturer.

Western blot analysis of IBA-1 and GFAP
For the analysis of protein levels, cortex tissues were homogenized in ~8 volumes of ice-cold 0.01 M PBS in a homogenizing veterinary pathologist using Haematoxylin and Eosin (H&E) stained sections for these organs and additionally Periodic Acid Schiff (PAS) stained sections for small and large intestine. The slides were evaluated semi-quantitatively applying the following grading score: 0 = no findings; 1 = minimal; 2 = slight; 3 = moderate; 4 = severe; 5 = massive.

Immunostaining of paraffin-embedded brain tissue sections
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Aβ extraction from brain samples and ELISA
Water-soluble Aβ levels were analysed by Enzyme Linked Immunosorbent Assay (ELISA) in cortical cytosolic fractions [87]. To evaluate soluble proteins, brain tissues were homogenized in ~8 volumes of ice-cold PBS and supernatants were subsequently frozen at −80 °C until further analysis. The amount of Aβ 40 and Aβ 42 was determined using an ELISA kit (FUJIFILM Wako Chemicals Europe GmbH, Neuss, Germany) according to the manufactures protocol and normalized to the total protein content in the respective sample [pmol g⁻¹ tissue]. Total protein content was determined by Pierce™ BCA Protein Assay Kit (Thermo Scientific, Waltham, Massachusetts, USA) as described by the manufacturer.

Oxidative stress markers
Lipid peroxidation was determined in midbrain tissues by the reaction of MDA with thiobarbituric acid (TBA) to form a colorimetric (532 nm)/fluorometric (λₘₐₓ = 532/λₑₐₘ = 553 nm) product, proportional to the MDA present. The amount of MDA was evaluated with the MDA kit (Merck) according to the manufactures protocol. The amount of total and oxidized glutathione was evaluated in cerebellum after homogenization in cold 100 mM phosphate buffer (pH 6.8), containing 0.1 mM EDTA (Merck). After centrifugation (10,000g, 15 min, 4 °C) the supernatants were deproteinized with an equal volume of 10% metaphosphoric acid (Merck) and thereafter with a solution of 4 M triethanolamine (Merck) to increase the pH of the sample. This assay is based on the catalytic reaction of GSH with 5,5′-dithio-bis (2-nitrobenzoic acid) (DTNB, also named as Ellman’s reagent) that forms the yellow derivate 5-thionitrobenzoic acid (TNB). The concentration of GSH in a sample is proportional to the rate of formation of TNB, measured at 412 nm. The concentration of total glutathione was expressed as nmol tGSH per mg of protein. In addition, oxidized GSH (GSSG) was measured using 2-vinylpyridine for masking GSH which is rapidly reduced in two GSH by glutathione reductase and NADPH. The ratio of reduced glutathione to oxidized glutathione was expressed as (GSH/GSSG). Total protein content was determined by Pierce™ BCA Protein Assay Kit (Thermo Scientific, Waltham, Massachusetts, USA) as described by the manufacturer.
potter tissue grinder. The homogenate was centrifuged in a microcentrifuge for 45 min at 12,500 rpm and 4 °C. The amount of protein in the supernatant was evaluated with the BCA kit (Thermo Scientific) according to the manufactures protocol. The samples were prepared with equal amounts of protein (40 µg) and loaded on a 4–12% precast NUPAGE gel (Invitrogen Thermo Scientific) and were separated at 180 V in a Mini-PROTEAN II tank (BIO-RAD, Hercules, California, USA). After electrophoresis the proteins were blotted on a 0.45 µm pore diameter nitrocellulose transfer membrane (Whatman, Schleicher & Schuell) at 250 mA for 45 min in a Mini Trans-Blot tank (BIO-RAD). The membrane was blocked with 5% milk in PBS-T (0.01 M PBS and 0.05% Tween-20) for 30 min. After the blocking, the membrane was incubated overnight at 4 °C with the primary antibody, i.e. (GFAP (Cat No. ab7260, Abcam, 1:1000) or IBA-1 (Cat No. GTX100042, Gentex, 1:1000). Next day the membrane was washed with PBS-T and was incubated 1 h at room temperature with the horseradish peroxidase-conjugated secondary antibody and washed again 5 times with PBS-T. For the detection of the proteins the electrochemiluminescence (ECL) solutions were applied (GE Healthcare Amersham, Fisher Scientific, Schwerte, Germany), and the visualization was performed with the FluorChem 8900 (Biozym, Hessisch Oldendorf, Germany). Quantification of protein expression was done using the ImageJ software (National Institutes of Health, Bethesda, USA).

Statistical analyses
All data are shown as mean and standard error of mean (SEM) unless specified otherwise, with the number of animals indicated in the figure legends for each endpoint. Treatment related effects were analysed using one-way analysis of variance (ANOVA) followed by Dunnett’s post hoc evaluation of control groups versus NMs exposed groups. For the evaluation of ordinal data, indicated as scatterplots with median values, the Kruskal–Wallis test with Dunn- Bonferroni post hoc analysis was used. Analysis were performed using SPSS statistics (V25 IBM Corporation, USA).

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s12989-022-00461-2.

Additional file 1. Figure S1 shows representative SEM images of the SiO2 and CeO2 NMs (Fig. S1A,B) within prepared feed pellets and by comparison of the pristine NMs (see also S1). Figure S2, Figure S3.

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Authors’ contributions
AS participated in the coordination of the study, the interpretation of results, carried out the behaviour studies, the brain tissue sectioning, plaque load quantification and amyloid beta ELISAs, provided advice regarding GSH analyses, performed MDA, GRP and IBA-1 analyses, and drafted the manuscript. TW participated in the planning and coordination of the study, carried out the behaviour studies, supervised brain tissue sectioning and processing, provided advice regarding brain tissue analyses for plaque load quantification, amyloid beta ELISA, participated in the interpretation of the results and has been involved in critically revising the manuscript. JK participated in conceiving the study and participated in the tissue dissection and body and organ weight analyses. HJH participated in the coordination of the study and brain tissue sectioning. BS carried out scanning electron microscope (SEM) analyses and the interpretation of the results. MR carried out the histopathology analysis and the interpretation of the results. CA participated in the design, planning and coordination of the study, supervised tissue sectioning and processing. PFRS devised and coordinated the project, participated in the design and planning of the study, organ weight analyses, interpretation and the statistical analyses of the results, is co-writer and corresponding author of the manuscript. All authors have read, reviewed, commented and approved the final version of the manuscript.

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Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations
Ethics approval and consent to participate
The mice were handled according to guidelines of the Society for Laboratory Animals Science (GV-SOLAS). The study was approved by the Landesamt für Natur, Umwelt und Verbraucherschutz (LANUV, NRW, Germany; Ref. no. 84-02.04.2013.A443).

Consent for publication
Not applicable (no human data presented).

Competing interests
The authors declare that they have no competing interests.

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