Scandinavian Journal of Laboratory Animal Science

2026, Volume 52, Number 3
ISSN 2002-0112

Original article

When middle-aged is not old enough: the challenges of using middle-aged pigs in preclinical neuro studies

By: Aage Kristian Olsen Alstrup[1,], Christian Stald Skoven[2], Andreas Nørgaard Glud[3,4], Brian Hansen[2], Peter M. H. Heegaard[5], Dariusz Orlowski[4]*

[1]Department of Nuclear Medicine, Department of Clinical Medicine, Aarhus University, Palle Juul-Jensens Boulevard 165, DK-8200 Aarhus N
[2]CFIN, Aarhus University, Department of Clinical Medicine, Aarhus University, Palle Juul-Jensens Boulevard 99, DK-8200 Aarhus N
[3]Department of Clinical Medicine, Aarhus University, Palle Juul-Jensens Boulevard 99, DK-8200 Aarhus N
[4]Department of Neurosurgery, Research group CENSE, Aarhus University Hospital, Palle Juul-Jensens Boulevard 165, DK-8200 Aarhus N
[5]DTU Ossabaw Facility, Department of Health Technology, Technical University of Denmark, Henrik Dams Alle 202, DK-2800 Kgs. Lyngby

Correspondence:
Aage Kristian Olsen Alstrup
aagealst@rm.dk

Summary

Many brain disorders emerge in old age, highlighting the importance of using aged laboratory animals to obtain reliable and translatable research findings. While this approach is feasible in rodent models, such as mice and rats, due to their short lifespan, accessing aged animals poses a significant challenge in larger experimental animal models such as pigs. In this study, we compared volumes of whole brains and selected brain structures from young adult Göttingen minipigs (7 months-1 year; N=4) with brains from middle-aged minipigs (4-11 years; N=4), in addition to a single Ossabaw pig (8 years; N=1). All nine pigs were females, and the middle-aged pigs were the oldest ones to which we had access. The brains were removed and stored in formalin until they were structurally scanned in a preclinical magnetic resonance imaging (MRI) scanner. The total volumes of each brain were determined, in addition to the volumes of three structures, namely the hippocampus, striatum, and amygdala. The mean volume of the middle-aged Göttingen minipig brains (78,770 mm3 +/- 3,331 mm3) were 19% larger than for the young minipig brains (65,990 mm3 +/- 1,346 mm3) (p=0.002). In contrast, the Ossabaw pig's brain was significantly larger (121,767 mm3) than both the young (85%) and aging Göttingen minipig brains (55%), reflecting the bigger body size of the Ossabaw pig. The average volume fractions of the structures analyzed were slightly lower for the middle-aged minipigs compared to the young, although this difference was not significant for either the hippocampus (p = 0.299), striatum (p = 0.149), or amygdala (p = 0.406). Qualitative MRI assessment did not reveal brain atrophy or ventricle enlargement in the middle-aged pig brains. The average volume fractions in the Ossabaw pig were comparable to those observed in the Göttingen minipigs. Even the oldest pig brains in this study showed no clear MRI-based indicators of aging-related neurodegeneration. These findings, therefore, highlight the challenges of obtaining aged pig brains and address a real, practical challenge for future aging research using large animal models.

Introduction

The risk of degenerative brain diseases increases with age. These disorders rarely appear before the age of 50 years, after which the incidence increases (Ascherio and Schwarzshild 2016; Boehme et al. 2017). Brain aging is associated with structural, biochemical, metabolic, and electrophysiological changes (Lee and Kim 2022); moreover, those processes do not follow a linear trajectory and their dynamics vary across the lifespan (Dohm-Hansen et al. 2024). Alzheimer's disease, Parkinson's disease, and stroke are degenerative brain disorders that occur almost exclusively in older individuals (Ascherio and Schwarzshild 2016; Boehme et al. 2017; Masters et al. 2015). Consequently, there is growing acknowledgement that laboratory animals used to study age-related neurodegenerative disorders should themselves be aged (Cohen 2018; Holtze et al. 2021; Padmanabhan and Gotz 2023; Bartolomucci et al. 2024). For example, the gut-first hypothesis of Parkinson's disease, which claims that the disease can originate in the intestines, could not be meaningfully evaluated until aging rodents were used (Kim et al. 2019; van den Berge et al. 2019; Wood 2019). Subsequently, a rat study demonstrated that gut-first does not occur in younger laboratory animals, but emerges in older ones (van den Berge et al., 2021). A systematic review further concluded that Parkinson's models using aged laboratory animals produce more valid and translationally relevant results (Klæstrup et al. 2022). Despite this evidence, young laboratory animals continue to be used widely for research on age-related disorders.

While aged mice and rats are relatively accessible due to their short lifespan of approximately 1.5–2 years (Suter et al. 1979), maintaining colonies to old age remains costly. Obtaining aged large animal models is far more problematic, as species such as pigs, sheep, and goats have life expectancies of 15–27 years (Klein 2019), and the associated long-term housing costs are substantial, resulting in significant challenges when attempting to obtain such animals for research. Consequently, most large animal studies employ juvenile or young adult subjects. Furthermore, for some animals, like the Landrace pigs, the weight and size of the older animals may also be a problem in research, as they will no longer fit into the scanners, which limits their use to only younger ones. This raises a critical question: Are these younger animals physiologically and neuroanatomically old enough to model late-onset neurodegeneration? Specifically, do they exhibit signs of neuroanatomical aging, such as cortical or subcortical brain atrophy, ventricular enlargement, or region-specific shrinkage?

To address this question, we examined the brains of minipigs representing the oldest individuals currently accessible for research in Denmark. Notably, we obtained middle-aged pigs (8 and 11 years old), and younger adult minipigs. We were not able to find pigs older than these, and if such were to be found, they would be extremely rare in the Danish research sphere. This allowed us to assess whether aging-related neuroanatomical changes are present in naturally aged minipigs of these ages.

Materials and Methods

Animals

This study did not require any laboratory animal permit, as experiments were only conducted on brains extracted from already euthanized pigs. The young adult Göttingen minipig scans were performed on brains stored in formalin in our laboratory. These brains came from the pigs that were used in various experiments as control animals and later euthanized, and their brains were explanted to be used as a testing/teaching tissue. These four young adult Göttingen minipigs came from the large animal barns at Aarhus University (Påskehøjgaard, Aarhus, Denmark). The four middle-aged older Göttingen, and the single middle-aged Ossabaw pig had never been used for experiments, and they came from Aarhus University (Foulum, Viborg, Denmark) and Technical University of Denmark (DTU Ossabaw Facility, Lyngby, Denmark), respectively. The housing conditions varied between the three barns, but for all three places the legal requirements were met: e.g., room temperature of 21-24 OC, 45-65% humidity, and a minimum of 8 air changes per hour. The minipigs were fed restrictively with minipig food (unknown brand). They were all acclimatized before euthanasia. Their health status was Specific Pathogen Free.

Brain sampling

Brains were sampled from four young adult female Göttingen minipigs (7 months - 1 year; 16-19 kg), four middle-aged female Göttingen minipigs (4-11 years; 41-47 kg) and one female Ossabaw minipig (8 years; 115 kg). The pigs were sedated (pig Zoletil mixture, IM) and subsequently euthanized with an overdose of pentobarbital (100 mg/kg), intravenously. The brains were immediately removed by sawing through and breaking the skull apart and stored refrigerated (5°) in a 10% buffered formalin solution (VWR, Denmark) until the MRI scan. The oldest (11-year-old Göttingen minipig) was perfusion-fixed using 5L of 10% buffered formalin (VWR, Denmark). This procedure could not be practically performed on the other pigs. Instead, the brains were immersion fixed in 10% buffered formalin after removal. The brains were collected over three years but scanned sequentially within one week.

High field magnetic resonance imaging

Before imaging, the meninges were removed with scissors and forceps, the samples were briefly rinsed in phosphate-buffered saline (PBS) before being immersed in fresh PBS for at least 1 h, to increase signal

by removal of excess fixative (Shepherd et al. 2009). Due to the short scan duration (~2 hours), it was sufficient to place the sample in a nitrile glove, maintaining tissue moisture for the duration of the scan. An MRI scan was performed using a 9.4 T preclinical system (BioSpec 94/20, Bruker Biospin, Ettlingen, Germany) equipped with a bore-mounted 86 mm quadrature transmit-receive coil, similar to a previous study (Kuang et al. 2025). To increase consistent positioning, the samples were placed on an in-house 3D-printed (Prusa Mini+; Prusa Research, Prague, Czech Republic), base plate for pig brains (Thermoplastic polyurethane; 85A SoftFlex, KungFuFlex), inside an in-house 3D-printed pig brain container (PolyLactic Acid; 3DE Max, 3D-eksperten, Nørresundby, Denmark). A fish oil capsule fiducial marker was used to ensure correct left-right identification in the images afterwards. To reduce sample vibrations, the prepared sample was fitted into the coil using a custom polyethylene foam cylinder. A high-resolution B0 map was obtained (matrix: 180 x 180 x 180, Field of View (FOV): 90mm x 90mm x 90mm; 10min 48s) for shimming using Bruker's MAPSHIM. One structural dataset was acquired per sample: A 3D rapid acquisition with relaxation enhancement (RARE) sequence with a 300 × 300 µm in-plane resolution, 600 µm slice thickness. The scan parameters used were effective echo time = 23 ms, repetition time = 1200 ms, 3 averages, and a RARE factor = 12. For the minipigs: 128 slices were sufficient to image the whole brain, resulting in an FOV of 60 mm x 54 mm x 76.8 mm (matrix: 200x180x128) and a scan time of 1h 55min 12s. For the Ossabaw pig: 144 slices were obtained (due to the larger size), resulting in an FOV of 60 mm x 54 mm x 86.4 mm (matrix: 200x180x144) and a scan time of 2h 9min 36s.

Data processing and analysis

The datasets were denoised (Veraart et al. 2016) and N4 bias field corrected (Tustison et al. 2010) as per the previous procedure (Knopper et al. 2024). Imaging data were analyzed to determine the total brain volumes (mm^3), as well as the left and right hippocampus, striatum, and amygdala (mm^3), and their average volume fractions (%). The volumes were estimated using ITK-SNAP software (Yushkevich et al. 2006) (v. 4.0.1, March 20, 2023), with semi-automated segmentation (active contour segmentation mode) for the whole brain volumes and manual segmentations of the hippocampus, striatum, and amygdala. The same researcher performed all segmentations without prior knowledge of the animal's age. The images were segmented using an orthogonal viewer (Initial manual segmentation in the coronal plane, followed by corrections, when necessary, in the sagittal and horizontal planes). The segmentations were guided by available pig brain atlases (Felix et al. 1999; Orlowski et al. 2019; Saikali et al. 2010) and the online minipig brain atlas (available at cense.au.dk). The volume data were obtained from the volumes and statistics module in the ITK-SNAP software. The volumes were estimated for long-fixated brains, and therefore, the volumes did not fully represent the in vivo volumes due to shrinkage caused by fixation (de Guzman et al. 2016).

Statistical analysis of data

The total brain volume was compared between the young and the middle-aged Göttingen minipigs by use of the Student t-test in Microsoft Excel. In addition, the relative brain volume (average volume fractions) of the three brain structures, hippocampus, striatum, and amygdala (right and left summed), was calculated and compared across groups with the same Student t-test. A p-value of 0.05 was considered significant. The results from the Göttingen minipigs were also compared with the same variables from the single Ossabaw pig brain.

Results

Qualitative assessment of the obtained MRI scans did not reveal any obvious signs of brain aging, such as atrophy, white matter changes, or enlargement of sulci and ventricles. As shown in Table 1, the volumes of the middle-aged Göttingen minipig brains (78,770 mm^3 ± 3,331 mm^3; mean ± S.D.) were 19 percent larger than for the young minipig brains (65,990 mm^3 ± 1,346 mm^3; mean ± S.D.) (p=0.002). In comparison, the Ossabaw pig's brain volume (121,767 mm^3) was 85% and 55% larger than the young adult and the middle-aged Göttingen minipig brains, respectively. Table 1 shows volumes of the left and right hippocampus, striatum, and amygdala. Statistically significant differences were not found between the right and left hippocampi (p=0.83), striata (p=0.94), nor amygdalae (p=0.74), and therefore left-right-data was pooled in the following. The hippocampus averaged 647 mm^3± 25 mm^3 in the young adult minipigs, while it was 13 % larger in the older minipigs, which averaged 730 mm^3 ± 36 mm^3 (p=0.0095). No statistically significant differences were observed in striatum volume between the young adult (1080 mm^3 ± 25 mm^3) and the middle-aged minipigs (1175 mm^3 ± 92 mm^3; p=0.10), while the amygdala was only borderline smaller in young adults (270 mm^3 ± 14 mm^3) than in middle-aged minipigs (312 mm^3 ± 31 mm^3; p=0.05). In comparison, the three brain structures were equivalently larger in the Ossabaw miniature pig..

The relative volumes of hippocampus, striatum, and amygdala (left and right sides summed) in relation to the total brain volume are shown in Figure 1. The average volume fraction was slightly lower for the middle-aged minipigs compared to the young, but the differences were not significant for either the hippocampus (p=0.30), striatum (p=0.15), or amygdala (p=0.41). The average volume fractions in the Ossabaw pig were roughly on par with what was found in the Göttingen minipigs (Figure 1). MR images from the oldest (11 years) Göttingen minipig are shown in Figure 2.

Table 1. Total brain volume, as well as hippocampus, striatum, and amygdala volumes in the nine pigs.

		Volumes [mm^3]			
Pig bred	**Age, weight and wet brain weight**	**Brain, total**	**Hippocampus**	**Striatum**	**Amygdala**
Göttingen minipigs, middle-aged	4 years; 41.2 kg; 78 grams	80,580	L: 676; R: 689; mean: 683	L: 1,113; R: 1123; mean: 1118	L: 282; R: 281; mean: 282
	6 years; 47.0 kg; 78 grams	81,310	L: 711; R: 727; mean: 719	L: 1,250; R: 1288; mean: 1269	L: 341; R: 342; mean: 342
	4 years; 41.2 kg; 76.5 grams	79,250	L: 726; R: 787; mean: 757	L: 1,086; R: 1066; mean: 1,076	L: 286; R: 291; mean: 289
	11 years; 41.9 kg; 76 grams	73,940	L: 743; R: 776; mean: 760	L: 1,230; R: 1,240; mean: 1,235	L: 341; R: 327 mean: 334
Göttingen minipigs, young adults	7 months; 19 kg; unknown	66,460	L: 635; R: 679; mean: 657	L: 993; R: 1099; mean: 1046	L: 287; R: 272; mean: 280
	7 months; 17 kg; unknown	66,695	L: 615; R: 611; mean: 613	L: 1,076; R: 1,098; mean: 1,087	L: 286; R: 283; mean: 285
	7 months; 16 kg; unknown	63,983	L: 647; R: 643; mean: 646	L: 1,099; R: 1,111; mean: 1,105	L: 295; R: 229 mean: 262
	1 year; 18 kg; unknown	66,820	L: 660; R: 684; mean: 672	L: 1,133; R: 1,032; mean: 1,083	L: 243; R: 267; mean: 255
Ossabaw, middle-aged	8 years; 115 kg; 124 grams	121,767	L: 1,256; R: 1,254; mean: 1,255	L: 1,813; R: 1,821; mean: 1,817	L: 381; R: 387; mean: 384

L: left. R: Right.

Discussion

The human brain starts to show signs of aging (white matter lesions and atrophy) between the ages of 40 and 50. Aging signs accelerate with age, high blood pressure and unhealthy lifestyle factors (Yang et al. 2024). In this study, we compared total brain volumes and selected substructures in young adult and middle-aged Göttingen minipigs, with the additional inclusion of a single Ossabaw pig. If it were possible to make a direct comparison, the age of the examined pig brains would correspond in human years to 40-50 years or younger, but it is unknown how the pig brain deteriorates over time, and this adds to the uncertainty in using the pig brain, when an aged brain is required for scientific research. Contrary to expectations, even the oldest minipigs examined (up to eleven years of age) showed no clear neuroanatomical signs of aging, such as cortical or subcortical brain atrophy, ventricular enlargement, or region-specific shrinkage, features that are well documented in aging humans and observed in other species with shorter lifespans (Blinkouskaya et al. 2021). Instead, total brain volumes were larger in middle-aged Göttingen minipigs compared with young adults, and subcortical structures such as the hippocampus, striatum, and amygdala were either stable or showed pro-

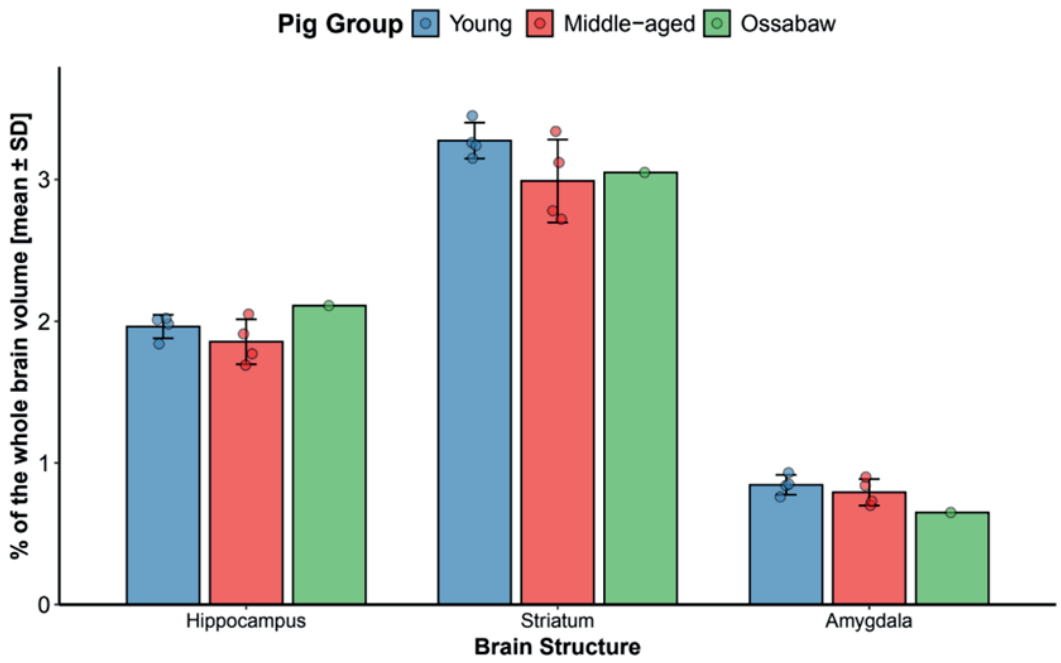

Figure 1. Segmentation in percent of whole brain volumes of three brain regions (hippocampus, striatum, and amygdala) in the middle-aged Göttingen minipigs (N=4), young adult Göttingen minipigs (N=4), and Ossabaw pig (N=1). Data means + S.D.

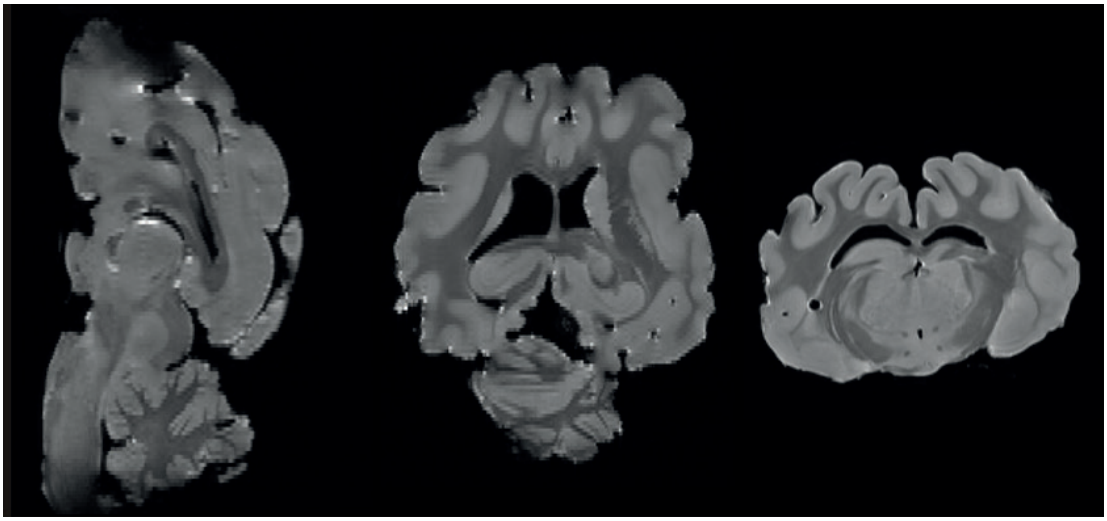

Figure 2. A structural MRI dataset (T2 TurboRARE) for the 11-year-old Göttingen minipig. The center slices are shown for the sagittal (left), axial (center) and coronal (right) views.

portional increases. The Ossabaw pig, despite its larger body and brain weight, displayed a similar volumetric pattern. While total brain volume was lower in the 11-year-old than in the 4-year-old Göttingen minipigs; no volume loss was observed in the examined subcortical structures.

These findings highlight a fundamental limitation in using pigs as translational models for aging research. Even the "oldest" laboratory pigs accessible are not physiologically aged in a neurobiological sense. Pigs have a lifespan of 15–20 years (Klein 2021), and our middle-aged Göttingen minipigs, aged 4–11 years, may represent middle age rather than true senescence. This creates a translational gap, as many neurodegenerative disorders in humans manifest only after midlife (Masters et al. 2015; Boehme et al. 2017). Thus, conventional laboratory pigs may not yet exhibit the neurobiological hallmarks relevant to late-life neurodegeneration.

Our observations are consistent with reports noting that obtaining aged large animals, including pigs, sheep, and goats, is difficult (Holtze et al. 2021). This challenge is partly due to the high costs associated with long-term housing, husbandry demands, as well as the limited duration of research projects and grants, which

typically do not extend over the decades required for animals to reach their natural old age. Consequently, most large animal studies rely on juvenile or young adult subjects, which may limit translational validity and potentially introduce bias findings when studying age-related neurological disorders.

From a translational perspective, pigs/minipigs remain highly valuable: their gyrencephalic brains, human-like gray/white matter ratio, and relatively large brain size (contrary to small, lissencephalic rodent brains) makes them particularly suitable to be used for neurosurgical and neuromodulation studies. Likewise, the size of the minipig body allows the use of standard clinical imaging modalities like CT or MRI (Tohyama and Kobayashi 2018; Lunney et al. 2021; Sørensen et al. 2011). The size of the pig brain makes it useful, for instance, for stroke research (Kuang et al. 2025), deep brain stimulation studies using human-intended electrodes (Orlowski et al. 2017), or PET studies in e.g., Parkinson's disease models (Lillethorup et al. 2018). For aging studies, the pig may also be useful. Age is a leading predictor of disease (An et al. 2022), consequently, understanding which biological parameters change with age and how these factors evolve is crucial. Animal model validity is, therefore, a main concern in the study of the aging brain. The human brain is estimated to lose roughly 5% of its volume per decade after the age of 40 (Markov et al. 2022). Based on observations, veterinarians have established correlation tables to convert pig age to human age equivalents by multiplying the pig's age in years by five to estimate its human age equivalent (https://www.minipiginfo.com/estimating-the-age-of-a-mini-pig.html). Based on this, the oldest pig included in this study (11 years of age) would correspond approximately to a human aged 50-60 years. This is, of course, a rough estimate as pig age equivalence may be more reliable for young pigs, as pigs grow rapidly and mature early (Tohyama and Kobayashi 2018). If, however, one assumes similar aging patterns in the human and pig brain it is therefore surprising that more atrophy is not seen in the rather middle-aged pigs investigated here. Recent studies indicate that the aging process in the brain is accelerated by chronic, high levels of proinflammatory immune factors (Markov et al. 2022; Tamatta et al. 2025). It may therefore be that the controlled environments that laboratory pigs live in shield them from some of the factors that drive aging-related atrophy in the human brain. Usually, the well-controlled conditions of animal studies are considered an advantage in the effort to isolate disease effects, but here it would seem that the lack of environmental factors should be considered in animal-based studies aiming to mimic normal human brain aging. If so, this is likely the case for most laboratory animal species, underscoring the need to study brain aging in humans alongside studies in animal models.

From a methodological perspective, the design of our study was suboptimal and demonstrated additional complications. Brains were obtained post-mortem from different sources, with variable fixation methods, and scanned after prolonged immersion fixation. Such inconsistencies may contribute to volume distortions, as formalin fixation is known to cause tissue shrinkage (de Guzman et al. 2016). Furthermore, our small sample size, particularly the single Ossabaw pig, limits its statistical generalization. However, we conducted the study based on a 3R approach to reduce the use of animals, as no animals were euthanized specifically for this study. Even though we collected the oldest research pig brains available, they still showed no signs of aging. Nevertheless, the consistency of the findings across Göttingen minipigs suggests that the lack of atrophy is not an artifact caused by these limitations but rather reflects the biology of pigs at the ages we were able to obtain

Another critical issue is the species-specific trajectory of aging. Aging is a complex, multifactorial process (Cohen 2018). Rodents show pronounced and relatively early onset of brain aging (Radulescu et al. 2021), allowing convenient modeling of human neurodegeneration, even if their brain is non-gyrated.
Gyrated brains from larger mammals such as pigs may age more slowly, with structural and metabolic changes manifesting later in life (Cohen 2018). Thus, pigs may still serve as valuable models of other aspects of human neurobiology, such as the study of brain structure, connectivity, physiology, or response to various factors, as well as symptomatic modeling of the various diseases (Bjarkam et al. 2017; Orlowski et al. 2017; Bech et al. 2018; Lillethorup et al. 2018; Orstrup et al. 2019; Winterdahl et al. 2019; Bech et al. 2020; Zaer et al. 2020; Zaer et al. 2022; Kuang et al. 2025), but their utility in aging research is limited unless access to old animals becomes easier. Moreover, studies of aging should be carefully planned and adjusted for every research question, including the choice of experimental animal models.

The future solution of the problems associated with studying aging using large animal models may include developing collaborative repositories of aged large animal tissues, integrating naturally aged farm or companion animals into research, or applying accelerated-aging interventions (e.g., genetic or metabolic manipulations) (Azman and Zakaria 2019; Liu et al. 2020; Cai et al. 2022) in pigs to approximate human late-life neurobiology. Alternatively, studies may need to rely on cross-species comparative approaches, integrating rodent models for aging with pig or non-human primate models for anatomy and network-level analyses. Another approach could involve large-scale epidemiological studies in human populations, such as NHANES (Crimmins et al. 2008), and well-established longitudinal cohorts like ELSA (The English Longitudinal Study of Ageing), DanACo (The Danish Aging and Cognition) and SHARE (The Survey of Health, Ageing and Retirement in Europe) (Wigmore et al. 2017; Ruiz-Adame et al. 2023; Gronkjaer et al. 2024; Rosenau

et al. 2024), combining biomarker identification (Moqri et al. 2024) across multiple physiological domains with population-level and correlational studies of age-related diseases.

To conclude, although pigs hold promise as translational models for neuroscience, our findings indicate that currently accessible middle-aged animals do not exhibit the anatomical hallmarks of brain aging. Even at 11 years of age, Göttingen minipigs lack the structural signatures of neurodegeneration. Thus, critical aspects of late-life neurodegeneration cannot be captured in available pig models unless studies can access substantially older animals or novel aging-acceleration approaches are adopted.

Acknowledgements

No financial funding was obtained for this study. The authors would like to thank the animal facility at the Foulum Research Center, Aarhus University, as well as the DTU European Ossabaw facility for donating the brains for this study. Thanks to Michele Gammeltoft for linguistic proofreading.

Conflict of interest

The authors declare that they have no conflicts of interest.

References

An, S., Ahn, C., Moon, S., Sim, E.J., Park, S.-K., (2022). Individualized biological age as a predictor of disease: Korean genome and epidemiology study (KoGES) cohort. *Journal of Personalized Medicine*. **12**(3), 555. doi: 10.3390/jpm12030505

Ascherio, A., Schwarzshild, M.A., (2016). The epidemiology of Parkinson´s disease: risk factors and prevention. *The Lancet Neurology*. **15**(12), 1257-1272. doi: 10.1016/S1474-4422(16)30230-7.

Azman, K.F., Zakaria, R., (2019). D-Galactose-induced accelerated aging model: an overview. *Biogerontology*. **20**(6), 763-782. doi: 10.1007/s10522-019-09837-y.

Bartolomucci, A., Kane, A.E., Gaydosh, L., Razzoli, M., McCoy, B.M., Ehninger, D., Chen, B.H., Howlett, S.E., Snyder-Mackler, N., (2024). Animal models relevant for geroscience: Current trends and future perspectives in biomarkers, and measures of biological aging. *Journal of Gerontology A - Biological Sciences and Medical Sciences*. **79**(9), 135. doi: 10.1093/gerona/glae135.

Bech, J., Glud, A.N., Sangill, R., Petersen, M., Frandsen, J., Orlowski, D., West, M.J., Pedersen, M., Sorensen, J.C.H., Dyrby, T.B., Bjarkam, C.R., (2018). The porcine corticospinal decussation: A combined neuronal tracing and tractography study. *Brain Research Bulleting*. **142**, 253-262. doi: 10.1016/j.brainresbull.2018.08.004.

Bech, J., Orlowski, D., Glud, A.N., Dyrby, D.B., Sorensen, J.C.H., Bjarkam, C.R., (2020). Ex vivo diffusion-weighted MRI tractography of the Gottingen minipig limbic system. *Brain Structure and Functions*. **225**(3), 1055-1071. doi: 10.1007/s00429-020-02058-x.

Bjarkam, C.R., Glud, A.N, Orlowski, D., Sorensen, J.C.H., Palomero-Gallagher, N., (2017). The telencephalon of the Gottingen minipig, cytoarchitecture and cortical surface anatomy. *Brain Structure and Functions*. **222**(5), 2093-2114. doi: 10.1007/s00429-016-1327-5.

Blinkouskaya, Y., Cacoilo, A., Gollamudi, T., Jalalian, S., Weickenmeier, J., (2021). Brain aging mechanisms with mechanical manifestations. *Mechanisms of Ageing and Development*. **200**, 111575. doi: 10.1016/j.mad.2021.111575.

Boehme, A.K., Esenwa, C., Elkind, M.S.V., (2017). Stroke risk factors, genetics, and prevention. *Circulation Research*. **120**(3), 472-495. doi: 10.1161/CIRCRESAHA.116.308398.

Cai, N., Wu, Y., Huang, Y., (2022). *Induction of accelerated aging in a mouse model. Cells*. **11**(9),1418. doi: 10.3390/cells11091418.

Cohen, A.A., (2018). Aging across the tree of life: The importance of a comparative perspective for the use of animal models in aging. *Biochimica et Biophysica Acta Molecular Basis of Diseases*. **1864**(9 Pt A), 2680-2689. doi: 10.1016/j.bbadis.2017.05.028.

Crimmins, E., Vasunilashorn, S., Kim, J.K., Alley, D., (2008). Biomarkers related to aging in human populations. *Advances in Clinical Chemistry*. **46**, 161-216. doi: 10.1016/s0065-2423(08)00405-8.

de Guzman, A.E., Wong, M.D., Gleave, J.A., Nieman, B.J., (2016). Variations in post-perfusion immersion fixation and storage alter MRI measurements of mouse brain morphometry. *Neuroimage*. **142**, 687-695. doi: 10.1016/j.neuroimage.2016.06.028.

Dohm-Hansen, S., English, J.A., Lavelle, A., Fitzsimons, C.P., Lucassen, P.J., Nolan, Y.M., (2024). The 'middle-aging' brain. *Trends in Neuroscience*. **47**(4):259-272. doi:10.1016/j.tins.2024.02.001.

Félix, B., Léger, M.E., Albe-Fessard, D., Marcilloux, J.C., Rampin, O., Laplace, J.P., (1999). Stereotaxic atlas of the pig brain. *Brain Research Bulletin*. **49**(1-2), 1-137. doi: 10.1016/s0361-9230(99)00012-x. PMID: 10466025.

Gronkjaer, M., Mortensen, E.L., Wimmelmann, C.L., Flensborg-Madsen, T., Osler, M., Okholm, G.T., (2024). The Danish aging and cognition (DanACo) cohort. *BMC Geriatrics*. **24**(1), 238. doi: 10.1186/s12877-024-04841-5.

Holtze, S., Gorshkova, E., Braude, S., Cellerino, A., Dammann, P., Hildebrandt, T.B., Hoeflich, A., Hoffmann, S., Koch, P., Terzibasi Tozzini, E., Skulachev, M., Skulachev, V.P., Sahm, A., (2021). Alternative animal models of aging research. F*rontiers in Molecular Bioscience*. **8**, 660959. doi: 10.3389/fmolb.2021.660959.

Kim, S., Kwon, S.-H., Kam, T.-I., Panicker, N., Karuppagounder, S.S., Lee, S., Lee, J.H., Kim, W.R., Kook, M., Foss, C.A., Shen, C., Lee, H., Kulkarni, S., Pasricha, P.J., Lee, G., Pomper, M.G., Dawson, V.L., Dawson, T.M., Ko, H.S., (2019). Transneuronal propagation of pathologic alpha-synuclein from the gut to the brain models Parkinson´s disease. *Neuron*. **103**(4), 627-641. doi: 10.1016/j.neuron.2019.05.035.

Klein, V., (eds.) (2019). Cummingham´s textbook of veterinary physiology. 6th. Ed.: Saunders. DOI: 10.1016/C2015-0-06149-4.

Klæstrup, I.H., Just, M.K., Holm, K.L., Alstrup, A.K.O., Romero-Ramos, M., Borghammer, P., Van Den Berge, N., (2022). Impact of aging on animal models of Parkinson´s disease. *Frontiers in Aging Neuroscience*. **14**, 909273. doi: 10.3389/fnagi.2022.909273.

Knopper, R.W., Skoven, C.S., Eskildsen, S.F., Østergaard, L., Hansen, B., (2024). The effects of locus coeruleus ablation on mouse brain volume and microstructure evaluated by high-field MRI. *Frontiers in Cellular Neuroscience*. **18**, 1498133. doi: 10.3389/fncel.2024.1498133.

Kuang, V.H., Skoven, C.S., Arvin, S., Fitting, L.M., Drasbek, K.R., Hansen, B., Orlowski, D., Sorensen, J.C.H., (2025). A large animal model for focal stroke: Photothrombotic lesion in the cortex of Danish Landrace pigs. *Journal of Neuroscience Methods*. **418**, 110408. doi: 10.1016/j.jneumeth.2025.110408.

Lee, J., Kim, H.J., (2022). Normal aging induces changes in the brain and neurodegeneration progress: Review of the structural, biochemical, metabolic, cellular, and molecular changes. *Frontiers in Aging Neuroscience*. **14**, 931536. doi:10.3389/fnagi.2022.931536.

Lillethorup, T.P., Glud, A.G., Alstrup, A.K.O., Noer, O., Nielsen, E.H.T., Schacht, A.C., Landeck, N., Kirik, D., Orlowski, D., Sorensen, J.C.H., Doudet, D.J., Landau, A.M., (2018). Longitudinal monoaminergic PET imaging of chronic proteasome inhibition in minipigs. *Scientific Reports*. **8**(1), 15715.

Liu, B., Liu, J., Shi, J.S., (2020). SAMP8 mice as a model of age-related cognition decline with underlying mechanisms in Alzheimer's disease. *Journal of Alzheimers Disease*. **75**(2), 385-395. doi: 10.3233/JAD-200063.

Lunney, J.K., Goor, A., Walker, K.E., Hailstock, T., Franklin, J., Dai, C., (2021). Importance of the pig as a human biomedical model. *Science Translational Medicine*. **13**, 621. doi: 10.1126/scitranslmed.abd5758.

Markov, N.T., Lindbergh, C.A., Staffaroni, A.M., Furman, D., (2022). Age-related brain atrophy is not a homogenous process: Different functional brain networks associate differentially with aging and blood factors. *The Proceedings of the National Academy of Sciences*. **119**(49): e2207181119. doi: 10.1073/pnas.2207181119.

Masters, C.L., Bateman, R., Blennow, K., Rowe, C.C., Sperling, R.A., Cummings, J.L., (2015). Alzheimer´s disease. *Nature Reviews Disease Primers*. **1**, 15056. doi: 10.1038/nrdp.2015.56.

Moqri, M., Herzog, C., Poganik, J.R., Ying, K., Justice, J.N., Belsky, D.W., Higgins-Chen, A.T., Chen, B.H., Cohen, A.A., Fuellen, G., Hagg, S., Marioni, R.E., Widschwendter, M., Fortney, K., Fedichev, P.O., Zhavoronkov, A., Barzilai, N., Lasky-Su, J., Kiel, D.P., Kennedy, B.K., Cummings, S., Slagboom, P.E., Verdin, E., Maier, A.B., Sebastiano, V., Snyder, M.P., Gladyshev, V.N., Horvath, S. Ferrucci, L., (2024). Validation of biomarkers of aging. *Nature Medicine*. **30**(2), 360-372. doi: 10.1038/s41591-023-02784-9.

Orlowski, D., Michalis, A., Glud, A.N., Korshoj, A.R., Fitting, L.M., Mikkelsen, T.W., Mercanzini, A., Jordan, A., Dransart A., Sorensen, J.C.H., (2017). Brain tissue reaction to deep brain stimulation - A longitudinal study of DBS in the Goettingen minipig. *Neuromodulation*. **20**(5), 417-423. doi: 10.1111/ner.12576.

Orlowski, D., Glud, A.N., Palomero-Gallagher, N., Sørensen, J.C.H., Bjarkam, C.R., (2019). Online histological atlas of the Göttingen minipig brain. *Heliyon*. **5**(3), e01363. doi: 10.1016/j.heliyon.2019.e01363.

Orstrup, L., Tvilling, H.L., Orlowski, D., Zaer, H., Bjarkam, J.R., von Voss, P., Andersen, P.S., Christoffersen, B.O., Hedemann Sorensen, J.C, Laursen, T., Thygesen, P., Lykkesfeldt J., Glud, A.N., (2019). Towards a Gottingen minipig model of adult onset growth hormone deficiency: evaluation of stereotactic electrocoagulation method. *Heliyon*. **5**(11), e02892. doi: 10.1016/j.heliyon.2019.e02892.

Padmanabhan, P., Gotz, J., (2023). Clinical relevance of animal models in aging-related dementia research. *Nature Aging*. **3**(5), 481-493. doi: 10.1038/s43587-023-00402-4.

Radulescu, C.I., Cerar, V., Haslehurst, P., Kopanitsa, M., Barnes, S.J., (2021). The aging mouse brain: cognition, connectivity and calcium. *Cell Calcium*. **94**: 102358. doi: 10.1016/j.ceca.2021.102358.

Rosenau, C., Kohler, S., van Boxtel, M., Tange, H., Deckers, K., (2024). Validation of the updated "LIfestyle for BRAin health" (LIBRA) index in the English longitudinal study of ageing and Maastricht aging study. *Journal of Alzheimers Disease*. **101**(4), 1237-1248. doi: 10.3233/JAD-240666.

Ruiz-Adame, M., Ibanez, A., Mollayeva T., Trepel D., (2023). Association between neuroticism and dementia on healthcare use: A multi-level analysis across 27 countries from the survey of health, ageing and retirement in Europe (SHARE). *Journal of Alzheimers Disease*. **95**(1), 181-193. doi: 10.3233/JAD-230265.

Saikali, S., Meurice, P., Sauleau, P., Eliat, P.A., Bellaud, P., Randuineau, G., Vérin, M., Malbert, C.H., (2010). A three-dimensional digital segmented and deformable brain atlas of the domestic pig. *Journal of Neuroscience Methods*. **192**(1), 102-9. doi: 10.1016/j.jneumeth.2010.07.041.

Shepherd, T.M., Thelwall, P.E., Stanisz, G.J., Blackband, S.J., (2009). Aldehyde fixative solutions alter water

relaxation and diffusion properties of nervous tissue. *Magnetic Resonance in Medicine*. **62**(1), 26-34. doi: 10.1002/mrm.21977.

Sørensen, J.C., Nielsen, M.S., Rosendal, F., Deding, D., Ettrup, K.S., Jensen, K.N., Jørgensen, R.L., Glud, A.N., Meier, K., Fitting, L.M., Møller, A., Alstrup, A.K.O., Ostergaard, L., Bjarkam, C.R., (2011). Development of neuromodulation treatments in a large animal model--do neurosurgeons dream of electric pigs? *Progress in Brain Research*. **194**, 97-103. doi: 10.1016/B978-0-444-53815-4.00014-5.

Suter, P., Luetkemeier, H., Zakova, N., Christen, P., Sachsse K., Hess, R., (1979). Lifespan studies on male and female mice and rats under SPF-laboratory conditions. *Archives of Toxicology*. **2**, 403-407.

Tamatta, R., Pai, V., Jaiswal, C., Singh, I., Singh, A.K., (2025). Neuroinflammaging and the immune landscape: The role of autophagy and senescence in aging brain. *Biogerontology*. **26**(2), 52. doi: 10.1007/s10522-025-10199-x.

Tohyama, S., Kobayashi, E., (2018). Age-appropriateness of porcine models used for cell transplantation. Cell *Transplantation*. **28**(2), 2024-228. doi: 10.1177/0963689718817477.

Tustison, N.J., Avants, B.B., Cook, P.A., Zheng, Y., Egan, A., Yushvich, P.A., Gee, J.C., (2010). N4ITK: improved N3 bias correction. *IEEE Transaction on Medical Imaging*. **29**(6): 1310-1320. doi: 10.1109/TMI.2010.2046908.

Van Den Berge, N., Ferreira, N., Gram, H., Mikkelsen, T.W., Alstrup, A.K.O., Casadei, N., Tsung-Pin, P., Riess, O., Nyengaard, J.R., Tamgüney, G., Jensen, P.H., Borghammer, P., (2019). Evidence for bidirectional and trans-synaptic parasympathetic and sympathetic propagation of alpha-synuclein in rats. *Acta Neuropatology*. **138**(4), 535-550. doi: 10.1007/s00401-019-02040-w.

Van Den Berge, N., Ferreira, N., Mikkelsen, T.W., Alstrup, A.K.O., Tamgüney, G., Karlsson, P., Terkelsen, A.J., Nyengaard, J.R., Jensen P.H., Borghammer, P., (2021). Ageing promotes pathological alpha-synucleinpropagation and autonomic dysfunction in wild-type rats. *Brain*. **133**(6), 1853-1868. doi: 10.1093/brain/awab061.

Veraart, J., Novikov, D.S., Christians, D., Ades-Aron, B., Sijbes, J., Fieremans, E., (2016). Denoising of diffusion MRI using random matrix theory. *Neuroimage*. **142**, 394-406. doi: 10.1016/j.neuroimage.2016.08.016.

Wigmore, E.M., Clarke, T.K., Howard, D.M., Adams, M.J., Hall, L.S., Zeng, Y., Gibson, J., Davies, G., Fernandez-Pujals, A.M., Thomson, P.A., Hayward, C., Smith, B.H., Hocking, L.J., Padmanabhan, S., Deary, I.J., Porteous, D.J., Nicodemus, K.K., McIntosh, A.M., (2017). Do regional brain volumes and major depressive disorder share genetic architecture? A study of generation Scotland (n=19 762), UK Biobank (n=24 048) and the English longitudinal study of ageing (n=5766). *Translational Psychiatry* 7(8), e1205. doi: 10.1038/tp.2017.148.

Winterdahl, M., Noer, O., Orlowski, D., Schacht, A.C., Jakobsen, S., Alstrup, A.K.O., Gjedde, A., Landau, A.M., (2019). Sucrose intake lowers mu-opioid and dopamine D2/3 receptor availability in porcine brain. *Scientific Reports*. **9**(1), 16918. doi: 10.1038/s41598-019-53430-9.

Wood, H., (2019). New models show gut-brain transmission of Parkinson disease pathology. *Nature Reviews Neurology*. **15**: 491. doi: 10.1038/s41582-019-0241-x.

Yushkevich, P.A., Piven, J., Hazlett, H.C., Smith, R.G., Ho, S., Gee, J.C., Gerig, G., (2006). User-guided 3D active contour segmentation of anatomical structures: significantly improved efficiency and reliability. *Neuroimage*. **31**(3), 1116-1128. doi: 10.1016/j.neuroimage.2006.01.015.

Yang, Z., Wen, J., Erus, G., Govindarajan, S.T., Melhem, R., Mamourian, E., Cui, Y., Srinivasan, D., Abdulkadir, A., Parmpi , P., Wittfeld, K., Grabe, H.J., Bülow, R., Frenzel, S., Tosun, D., Bilgel, M., An, Y., Yi, D., Marcus, D., LaMontagne, P., Benzinger, T.L.S., Heckbert, S.R., Austin, T.R., Waldstein, S.R., Evans, M.K., Zonderman, A.B., Launer, L.J., Sotiras, A., Espeland, M.A., Masters, C.L., Maruff, P., Fripp, J., Toga, A.W., O'Bryant, S., Chakravarty, M.M., Villeneuve, S., Johnson, S.C., Morris, J.C., Albert, M.S., Yaffe, K., Völzke, H., Ferrucci, L., Bryan, R.N., Shinohara, R.T., Fan, Y., Habes, M., Lalousis, P.A., Koutsouleris, N., Wolk D.A , Resnick, S.M., Shou, H., Nasrallah, I., Davatzikos, C., (2024). Brain aging patterns in a large and diverse cohort of 49,482 individuals. *Nature Medicine*. **30**(10), 3015-3026. doi: 10.1038/s41591-024-03144-x.

Zaer, H., Fan, W., Orlowski, D., Glud, A.N., Jensen, M.B., Worm, E.S., Lukacova, S., Mikkelsen, T.W., Fitting, L.M., Jacobsen, L.M., Portmann, T., Hsieh, J.Y., Noel, C., Weidlich, G., Chung, W., Riley, P., Jenkins, C., Adler Jr, J.R, Schneider, M.B., Sorensen J.C.H., Stroh, A., (2022). Non-ablative doses of focal ionizing radiation alters function of central neural circuits. *Brain Stimulation*. **15**(3), 586-597. doi: 10.1016/j.brs.2022.04.001. doi: 10.1016/j.brs.2022.04.001.

Zaer, H., Glud, A.N., Schneider, B.M., Lukacova, S., Hansen, K.V., Adler, J.R., Hoyer, M., Jensen, M.B., Hansen, R., Hoffmann, L., Worm, E.S., Sorensen, J.C.H., Orlowski, D., (2020). Radionecrosis and cellular changes in small volume stereotactic brain radiosurgery in a porcine model. *Scientific Reports*. **10**(1), 16223. doi: 10.1038/s41598-020-72876-w.