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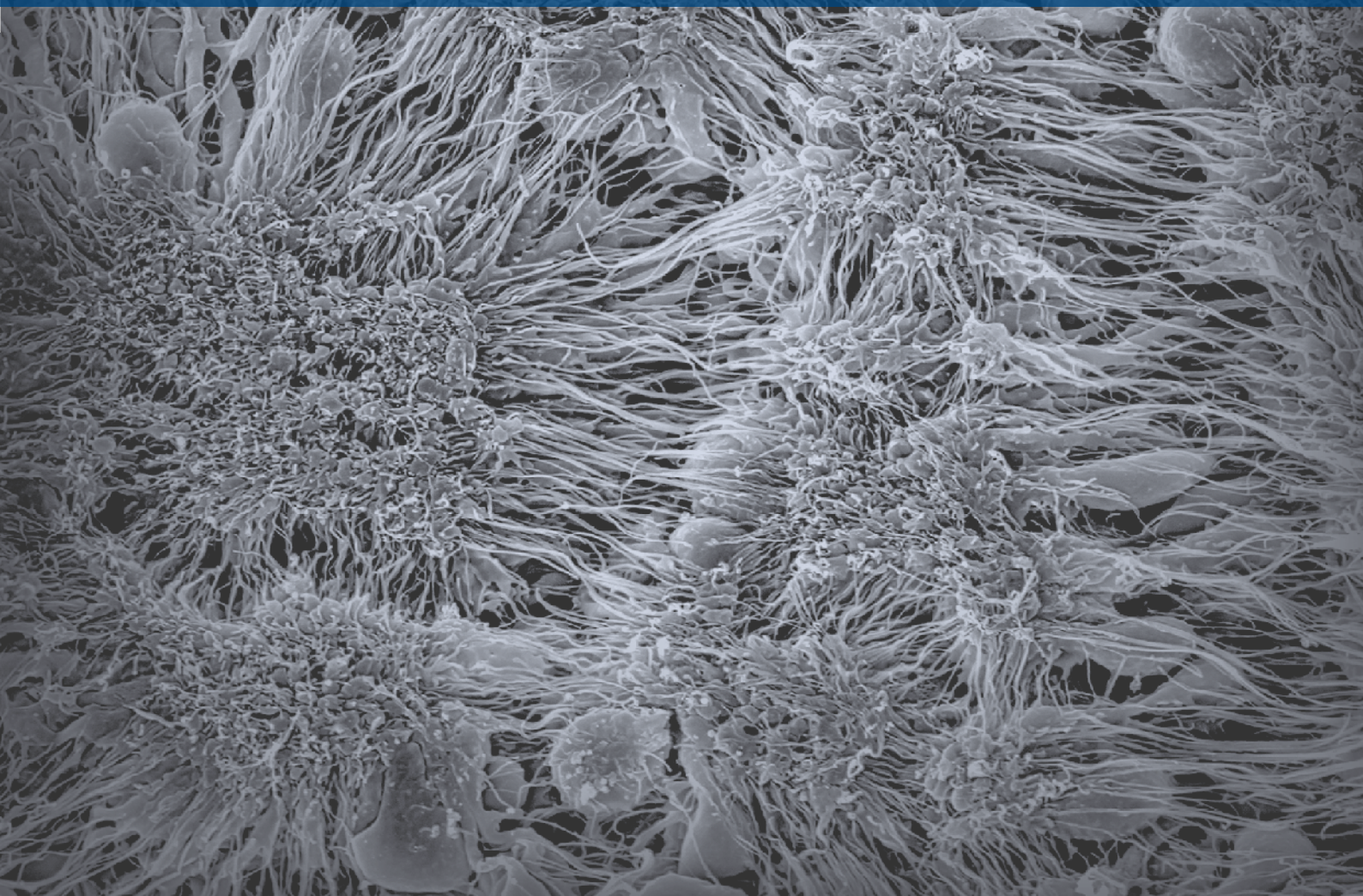
Brain organoids—model systems of the human brain



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The image on the cover shows the surface of a brain organoid under the scanning electron microscope. Credits: Katherina Psathaki & Thomas Rauen

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Brain organoids—model systems of the human brain

Contents

Summary and conclusions

The fundamental developmental processes and functional mechanisms of the human brain remain poorly understood. Important prerequisites still need to be met to enable the successful and precise treatment of many neurological and psychiatric diseases with minimal side effects. However, attaining such prerequisites calls for considerations that are neither practical nor ethical for conducting research on the living human brain. As the human brain contains biologically unique structures, many research questions can be answered only to a limited extent using animal models. In light of these limitations, the field of brain organoids[1] presents a promising alternative for research.

Organoids are derived from stem cells and grow as three-dimensional tissue structures *in vitro*, i.e., outside of the human body and imitate the cellular architecture and specific functional aspects of an organ. Organoids exist for different human organs, such as brain organoids for the human brain, consisting of nerve and glial cells. However, a brain organoid does not represent the whole human brain, but only specific regions of the brain. Currently, brain organoids have the potential to reach the size of a pea at most.

Brain organoids provide new insights into early brain development and the development of neurological and psychiatric diseases. They also enable the study of the effects of drugs, toxins, germs, or viruses on human brain cells and on the brain. Their applicability has been demonstrated by the use of brain organoids to provide evidence for a causal link between a Zika virus infection and the development of microcephaly. As a brain organoid contains the genetic information of the individual from whom the tissue cells were derived, research with brain organoids has the potential for discoveries specific to individual patients, such as the action of a certain drug.

Brain organoids can offer a valuable modelling system for studying part of the human brain, but they have inherent limitations. For one, the neural tissue structure of brain organoids does not exhibit the density and complexity of the human brain. In addition, although different brain region-specific organoids can be combined into “assembloids”, it is questionable whether these organoid assemblies can model the highly complex interaction of different regions of the human brain. Also, although brain organoids can model to some extent the early stages of brain development, they cannot model the later stages—as well as numerous neurodegenerative processes that naturally occur later in life.

1 Unless stated otherwise, reference is always to *human brain* organoids in the following.

One of the reasons for the limitations of brain organoids is their insufficient supply of nutrients. Unlike the human brain, brain organoids are not connected to a circulatory system, per se, and the cells are not fully supplied with nutrients, so they may die after a few months of growth. To provide a sufficient supply of nutrients, a human brain organoid can be transplanted into the brain of an animal, usually a mouse.

However, there are natural limitations to such experiments: on the one hand, the expansion of human brain organoids in a rodent is limited by the available anatomical space and the animal's comparatively short lifespan. To enable a longer maturation period, brain organoids could theoretically be transplanted into the brain of a large, long-living organism, for example a domestic pig or a primate. In addition, it is conceivable to transplant a brain organoid into the recipient animal at a very early stage of development, which would allow for much greater integration of the human cells into the functional circuits of the animal brain. However, it is currently unknown whether such experiments would indeed enable better formation of structural and functional units and what degree of complexity and maturity of cells and circuits could be achieved.

Brain organoids develop in culture media, with factors and molecules that are subject to free diffusion, i.e., non-controllable mixing. Although the nerve cells in the brain organoid arrange themselves roughly in the layered architecture typical for the brain, specific messengers that would lead to regional specification are absent. In addition, this early architecture is lost *in vitro* over time. Although individual cells continue to develop as in the foetal brain, the brain organoid does not even come close to achieving the ordered structure of the human brain—and this is likely to persist even if future research could produce a more ordered emergence of regions of the brain.

Furthermore, specific sensory stimuli need to be present within a certain time window in order to elicit the functional microarchitecture of the corresponding brain regions. So far, brain organoids lack such sensory impressions. Brain organoids could be provided with multiple sensors in the future, for example, the visual or pain-sensitive sensors and motor effectors. However, which stimuli brain organoids could be given and how these stimuli will be processed are separate questions that are not easy to answer. The sensation of pain is a complex process that involves numerous different brain areas *in vivo*, and even if corresponding brain organoid receptors could be stimulated, the assessment of the sensation of pain in such organoids is not currently possible.

Ethical considerations

The production of—and research into—these novel entities can lead to unease and concerns regarding the transgression of ethically formulated limits of action, as these entities involve cell groups that form the biological substrate of the human brain, which is being exploited in an extremely artificial manner. Although there is an immense categorical distance between a tiny brain organoid that exists in isolation and a human brain that is integrated into a body, there is nonetheless great potential for using these new research tools toward improving our understanding and treatment of brain functions or disorders.

The central issue of international bioethical debate is whether, and to what extent, human brain organoids are or could be subject to the moral obligation to protect them. That is, do we have to treat them in any particular way *for their own sake* (keyword: moral status)? The prevailing opinion is that such claims for protection are generally applicable only when an organoid has consciousness or *sentience*, at least in a minimal way—a condition that is currently *not clearly* met according to general opinion. For the development of functions that can be given the attribute "conscious", the biological structures involved need to have a sufficient size, complexity, and differentiation, which is currently not the case with brain organoid structures.

While the question of whether we have the moral obligation to protect brain organoids cannot be answered conclusively at the present time, a sceptical prognosis can be made with respect to future brain organoids of manifold and increasing complexity. For this purpose, it is even more important to first look for ways to identify the threshold of consciousness and sentience, which is proving to be difficult. Despite much progress in clarifying the necessary neurobiological conditions, there are still deep gaps in our knowledge of as well as different opinions about variants, the extent, and the biological realisation of consciousness.

Closely linked to the issue of possible consciousness or sentience of future brain organoids and the associated duty of protection, is the specific question of whether such brain organoids, which would potentially be sensitive to pain, should be used at all or whether they should be used for important research projects under strict conditions (similar to animal protection guidelines). Further debates and considerations may be required here.

Legal classification

It is occasionally argued that at least highly developed brain organoids should be granted similar protection to human embryos. If the incipient development of the nervous system is considered to be decisive for the embryo's right to protection, then highly developed brain organoids are equated to embryos. However, as brain organoids, unlike embryos, cannot develop into an organism, i.e., a holistic organic functional system or even a human being, similar protection, as provided for embryos in the German Embryo Protection Act (ESchG), cannot be derived from the applicable law or the constitution. Brain organoids *in vitro* can presumably, at best, reproduce the functions of individual brain regions. Even if this were to change in the future, being human is not limited to the possession of individual characteristics such as pain perception or consciousness and a constitutional request for protection cannot therefore be linked to individual characteristics. Overall, even highly developed brain organoids cannot be attributed a comparable status to embryos *in vitro* from a constitutional perspective. They are not legal subjects, but legal objects.

However, living humans are legal subjects: their bodies are not legal objects, in particular they are not something that can be owned. On separating an organic structure from the body, the now independent, not totipotent body substance—i.e., not capable of forming an autonomous organism—falls under the property law of the German Civil Code (BGB). It may be a case of ownership. If "a new movable object has been produced

by processing or transforming one or more substances [the original cells]" (Section 950, BGB), the researcher producing them acquires original ownership. This also applies if the new object is an organoid.

Irrespective of the question of ownership, people whose body cells are to be used for producing a new entity have, to some degree, the right to determine, or at least jointly determine, the use of the said cells. If for example the donor can be identified and it is possible to determine information about the donor from the cells, then the right of the donor to self-determination is affected. In principle, the donor then needs to be informed in an appropriate manner about the use of their cells and give their consent. In addition, data protection measures are required and if possible anonymisation, or at least pseudonymisation, and the secure safekeeping of the corresponding key.

Beyond the regulations of the German Animal Protection Act, there are currently no special regulations for transplanting human brain organoids into living animals. However, for the production and use of human-animal chimeras, various amendments to the current law are recommended, particularly with respect to the interdisciplinary and professional evaluation of corresponding research projects by competent ethics committees.

Conclusions

In consideration of the above, the authors of this work have arrived to the following conclusions:

1. Research on and with brain organoids *in vitro* does not as such raise any ethical and legal question that would require regulation in the foreseeable future. The conditions in which human cells can be used to produce brain organoids are also sufficiently regulated.

2. Research *in vivo* in which brain organoids from human neuronal cells are transplanted into animals is regulated by the German Animal Protection Act (TierSchG). The Ethics Committees stipulated by the Act should have expertise in the field of brain research for evaluating the research discussed here.

3. As this is a dynamic area of research, it is possible that the current limits of the functional potential of brain organoids could shift in the future. Such developmental possibilities and their ethical, legal, and social relevance must be (i) continuously and realistically assessed and (ii) regulated at an early stage if necessary. Regarding the first aspect, only the procedures of science-internal and science-public discussion can be held responsible. Regarding the second aspect, regulatory and supervisory activities may become necessary one day, and need to be assigned to corresponding bodies such as the Central Ethics Committee for Stem Cell Research (ZES).

1 Introduction

Growing cells *in vitro*, i.e., in a Petri dish, is a widely used technique in biology. Stem cell-derived three-dimensional tissue structures that mimic the cellular architecture and certain functional aspects of an organ can be generated in a suitable tissue culture environment. These structures, called organoids, resemble an organ in some ways. Three characteristics are essential for the definition of an organoid[2]: (i) emergence through self-organisation in a culture medium; (ii) passage through certain developmental stages of the modelled organ; and (iii) the development of essential organ-specific functions.

Such organoids exist for various human organs, including the human brain.

Box 1: Development of organoids from stem cells[3]

Organoids are generated from stem cells, either adult stem cells—i.e., stem cells present in almost all organs—or embryonic stem cells (ES cells) or induced pluripotent stem cells (iPS cells). Adult stem cells can be induced to undergo self-organisation processes by various proteins, known as "growth factors". These processes are like the ones underlying the natural renewal of an organ. ES or iPS cells, on the other hand, are induced to mimic processes of early organ development. In both cases, tissue cultures are generated that contain the cells essentially found in an organ in a near-natural three-dimensional arrangement. These so-called organoids can perform some functions of the corresponding replicated organ.

Brain organoids consist of nerve and glial cells derived from ES and iPS cells, respectively; adult stem cells, on the other hand, are not suitable for the replication of brain tissue. The omission or addition of certain factors leads to the formation of either solely brain region-specific organoids (for example, retinal, midbrain, or forebrain organoids) or organoids consisting of different brain region-specific structures. The development of a human brain organoid is very time-consuming: the culture and development processes required correspond to the time scale of human brain development. This means that the cells in organoids divide and specialise over the course of weeks and months, during which they form complex structures.

In this way, the early stages of the development of human brain tissue can be partially replicated while later developmental stages cannot yet be modelled at all.

2 Baertschi et al. (2020), p. 3.

3 For a general overview of organoids, see the Interdisciplinary Working Group on Genetic Engineering Report of BBAW and German Stem Cell Network (GSCN) (2020).

The basic developmental processes and functional mechanisms of the human brain are still not fully understood, and prerequisites for enabling the enhanced understanding of the pathological development of numerous neurological and psychiatric diseases and treatments thereof are lacking. Although many functions of the human brain can be studied using animal models, fundamental properties unique to the functioning of the human brain can be reproduced only in human models, the so-called brain organoids. Brain organoids are in a sense a cell-biological window that may offer a glimpse into the development of the human brain and organ-specific diseases. The effect of drugs, toxins, or pathogens on the development of the brain and the functioning of human brain cells can be studied using brain organoids.[4] For example, brain organoid models have been used to show the causal relationship between Zika virus infection and the development of microcephaly in the human foetus, helping to elucidate the underlying disease mechanisms and to test the efficacy of various drugs.

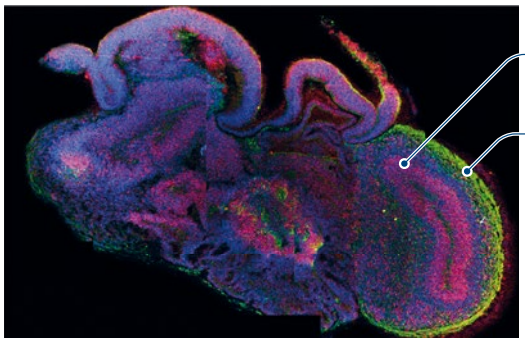
A brain organoid contains the individual genetic information of the human from whom the cells originate. The production of patient-specific brain organoids may also enable precision medicine,[5] allowing for the generation of drug treatment tailored to individual patients. Patient-specific brain organoids can also help us better understand the mechanisms driving the onset and progression of brain brain diseases and disorders.

4 Brain organoids have been used in several studies to better understand the causes of neurological development disorders. This includes modelling lissencephaly, genetic microcephaly, microcephaly following Zika virus infection, macrocephaly, Timothy syndrome (a severe neurodevelopmental disease characterised by an L-type calcium channel mutation), or prenatal drug exposure.

5 *Precision medicine*, also known as *personalised* or *individualised medicine*, considers the characteristics unique to each person and aims to tailor diagnostic and therapeutic procedures to the needs of the individual patient—to increase the efficacy of medical treatments while mitigating or avoiding adverse effects.

Brain organoids

Brain organoids emerge from human embryonic or induced pluripotent stem cells, which are induced to form both neurons and glial cells by pharmacological and electrical stimulation. Glial cells not only form the supporting scaffold for the neurons and electrically insulate them from each other but also contribute to the transmission of signals by releasing neurotransmitters such as glutamate.



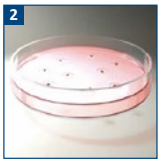
Different cell types (marked in colour) arrange themselves into ordered structures in a brain organoid by means of self-organisation. These structures resemble the layered structure of the human cerebrum.

The development of a brain organoid outlines the most important steps of human brain development. First, cultured stem cells aggregate in a culture vessel and form the three different cell layers, the "cotyledons" of early embryonic development (ectoderm, mesoderm, endoderm). By using special culture media, the stem cells are induced to form only the "neuroectoderm", the region from which the brain develops in embryos.

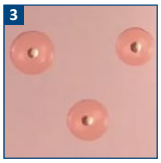
Then, aggregates of these precursor cells are embedded in Matrigel, a gelatinous substance that mimics the embryonic environment. Therein, the precursor cells arrange themselves as in the developing brain, forming neurons and creating three-dimensional structures.

Several thousand stem cells are pipetted into a culture vessel where they sink.

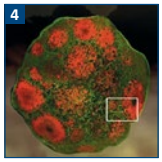
The stem cells aggregate into "embryoid bodies", clumps of cells that undergo the first steps of embryonic development.



Individual "embryoid bodies" are embedded in matrigel.



Organoids embedded in Matrigel grow in a cell culture dish with continuous gentle shaking.



The cells arrange themselves as in the brain and form cavities (ventricles) — a precursor layer (red) and finished nerve cells (green).

Figure 1: Overview of the production and structure of brain organoids.[6]

6 Source of microscopic image in centre: Lancaster et al. (2013).
Source of graphs 1a to 4: Tibor Kulcsar and Jürgen Knoblich, IMBA.

Press reports of the initial phase of research into brain organoids often misleadingly refer to these structures as “mini brains”.[7] This term suggests that a brain organoid is a miniaturised version of a human brain, which is capable of cognitive processes. This is not the case, as described in more detail in Chapter 2. Furthermore, over the course of their development, brain organoids lose the appearance and structure resembling the brain, thus the term “mini brain” leads to misconceptions and false expectations. A more accurate alternative would be to refer to this structure as a “cerebral organoid”. While this term is more accurate, it is less accessible, and thus, could complicate public discussion. Therefore, the term “brain organoid” is the most accurate term and is used in this statement.

The replication of human brain structures for research purposes still raises ethical issues, many of which have already been addressed in other bioethical debates over the past decade. Important ethical questions include: how far should the *in vitro* development of brain tissue go, and are there ethical limits that should not be overstepped? Are brain organoids categorically deserving of protection or do they require protection from a certain stage onward? Is it possible that such cell cultures may develop ethically relevant characteristics of human individuality and thus be deserving of protection from legal and ethical perspectives? Are the research and handling of brain organoids a threat to society? These and other issues will be addressed in Chapter 4.

7 See evidence in Taupitz (2021).

2 Brain organoids—the basics and areas of application

2.1 What can and cannot be modelled in brain organoids?

2.1.1 Neuronal networks

The human brain contains approximately 86 billion neurons[8] organised in extraordinarily complex networks. Such networks are formed by linking thin, highly branched nerve cell processes, known as axons and dendrites (see Figure 2).[9] Each individual nerve cell forms contacts with approximately 1,000 other new cells via its axon. These neuronal contact zones, called synapses, are used to transmit information between the neurons. A synapse can thus be considered as the smallest functional unit of the brain, with the human brain containing up to 100 trillion synapses—about a thousand times more than the number of stars in our galaxy.

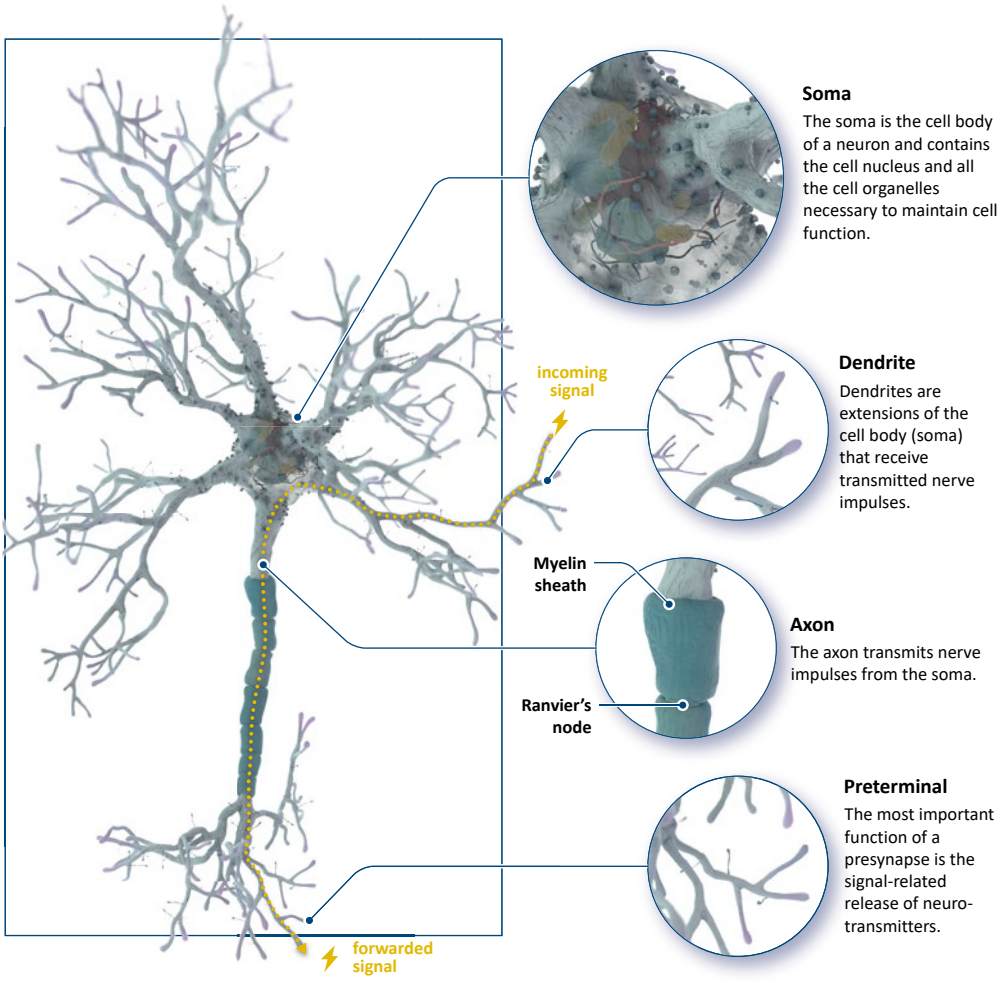


Figure 2: Structure and function of a nerve cell.

8 Herculano-Houzel (2009).

9 Its diameter is around 50 to 300 nanometres. See Braitenberg & Schüz (2014); Helmstaedter (2013); Motta et al. (2019).

Box 2: Excitatory and inhibitory synapses in the cerebral cortex

The human brain can be divided into different regions, including the cerebrum, the diencephalon, the cerebellum, and the brain stem.

In the human cerebral cortex, about 75 to 80 percent of the nerve cells are excitatory and about 20 to 25 percent inhibitory.[10] In mammals, the inhibitory nerve cells (interneurons) form highly specific synaptic contacts that are specialised for certain regions of the linked nerve cells.[11] Inhibitory nerve cells have multiple functions, for example in preventing epileptic brain activity,[12] sensory processing in the visual system[13], and for the precisely timed synchronisation of brain activity.[14] There is also evidence that their function may be crucial for efficient learning,[15] and they play a role of comparable importance in information processing as excitatory neurons. Lastly, a central research hypothesis is that a faulty balance between excitatory and inhibitory synaptic activity could cause the development of neuropsychiatric diseases.[16]

Current knowledge about information processing in neurons comes largely from animal studies. The question of how far these results can be directly transferred to humans is the subject of numerous studies. Although synapses comprise nearly of the same components regardless of their location within the brain, they differ in their molecular composition based on the brain region and species.[17]

Crucial for the brain's processing power are its electrochemical signal conduction properties—i.e., its neuronal activity—the interaction of different cell types and the complexity of cell connections (see Figure 3). Complex neuronal networks are formed at an early stage of human development and are structured via both genetic determinants and sensory signals arising from the environment.[18] A foetus's brain becomes increasingly structured while processing environmental stimuli such as sounds, touch, and positional changes, allowing for the maturation of the interplay between the stimulus and the response. Thus, over the course of human brain development, extensive neuronal networks emerge that store and process information and link it with existing information.

10 Beaulieu et al. (1992); Hornung & Tribolet (1994).

11 Kubota et al. (2016).

12 Farrell et al. (2019).

13 Ferster & Miller (2000).

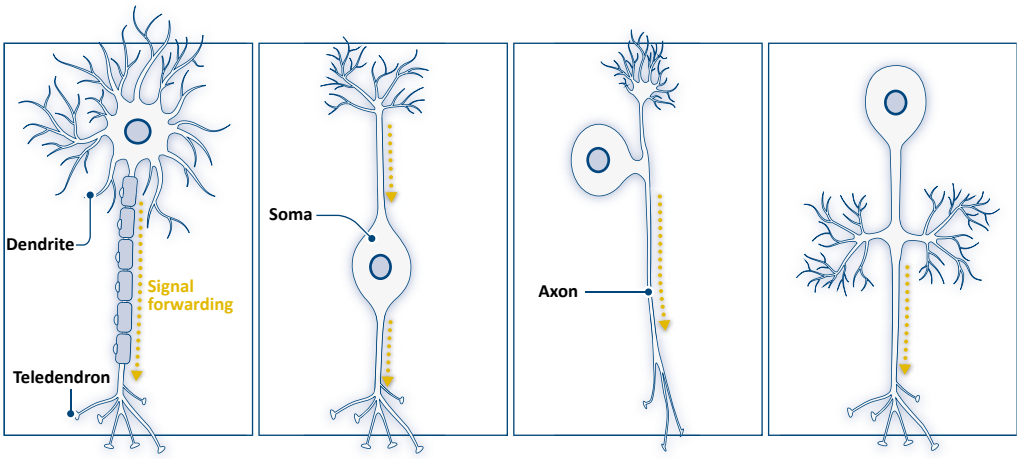
14 Pouille (2001); Wehr & Zador (2003).

15 Letzkus et al. (2011).

16 Cline (2005); Marín (2012); Rubenstein & Merzenich (2003); Selten et al. (2018).

17 Beaulieu-Laroche et al. (2018); Beed et al. (2020); Benavides-Piccione et al. (2019).

18 Power et al. (2010); Vogel et al. (2010).



Multipolar neuron

Multipolar nerve cells consist, for example, of motor neurons, as found in the central nervous system or in the anterior horn of the spinal cord. The surface of the cell body and the dendrites are the receptor zones for these cells. The conducting mechanism consists of the axon, which branches in its end portion into the "tree" (telodendron).

Bipolar neuron

A bipolar nerve cell is a nerve cell with two separate processes, with only one dendrite and one axon. Bipolar nerve cells are specialised neurons for relaying specific senses. They are found mainly in the retina and in the ear (ganglion cells of the inner ear).

Pseudounipolar neuron

A pseudounipolar nerve cell is a nerve cell whose dendrite and axon have merged to form a nerve cell process that divides shortly thereafter. These are sensitive neurons of the peripheral nervous system that are found in the sensitive head ganglia and spinal ganglia.

Unipolar neuron

A unipolar neuron is a nerve cell that has one component, usually the axon, and no dendrites. The best-known example in humans are nerve cells of the first neuron of the retina (rods and cones).

Figure 3: Overview of different nerve cell types in the human body.

To date, we have been able to observe the proliferation, growth, and ordered positioning of nerve cells and the formation of nerve fibres in organoids. Organoid neurons resemble neurons in the human brain in their biochemical, morphological, and physiological properties. However, organoids exhibit increased glycolysis (breakdown of sugar for energy production), stress, and apoptosis (controlled cell death) compared to the brain *in vivo*.[19] This is likely a result of the artificial culture conditions of brain organoids, such as the atmospheric oxygen content, which differ from the natural habitat of the developing human brain. Furthermore, the specificity of certain cellular subtypes, particularly highly specialized types of interneurons (inhibitory neurons), is not as pronounced in brain organoids, impeding the further maturation of these cells. In addition, dysregulation of the maturation programs of progenitor cells is also evident in organoids.[20]

The density and complexity of neuronal processes and synapses in organoids is significantly lower than those of the human brain, and it is unknown whether local circuits in organoids share structural similarities to those of animal and human brains. Although a brain organoid forms more extensive connections with the tissue of the host when transplanted into the brain of an animal or when combined with other organoids *in vitro*, the actual degree of precision of such connections is yet unclear.[21]

Specific interconnections of individual neurons within the neural network are crucial for the establishment of all higher functions of the nervous system in a living organism.

19 Mostajo-Radji et al. (2020).

20 Andrews & Kriegstein (2022).

21 Dong et al. (2020); Marton & Paşca (2020).

However, it is not yet known whether neurons in brain organoids can establish sufficiently complex and plastic connections to create circuits comparable to those found in the human brain, or whether they connect with each other randomly. Currently, as brain organoids also lack sensory input, they do not undergo complex development comparable to that of the foetal brain.

Neurons in two-dimensional cell cultures, generated from human stem cells, show robust synaptic activity in electrophysiological measurements,[22] but they often do not achieve the same electrophysiological complexity as neurons during natural development *in vivo*.

Unlike the human brain, which has a suitable tissue environment (including extracellular matrix, glial cells, etc.) and permits the development of neurons over a long period of time, two-dimensional cell cultures cannot replicate the *in vivo* environmental conditions and developmental periods, providing an explanation for the less pronounced complexity of two-dimensional cell cultures.

Three-dimensional brain organoids, on the other hand, do develop a complex organisation that allows for longer culture duration and hence longer neuronal maturation, with studies detecting electrically active cells that interact in a coordinated manner displaying spontaneous network activity of periodic and regular oscillatory events.[23] However, the degree of complexity of neuronal activity of brain organoids remains unknown. In addition, it is unclear whether the electrophysiological properties of networked neurons in the brain organoid resemble those of the developing human brain. Electrophysiological studies have shown that neuronal networks in brain organoids can develop self-organised activity patterns similar to those of the developing normal brain; moreover, their electrical activity increases and becomes more complex over time.[24] However, it remains to be elucidated whether this maturation of brain organoids reflects normal human brain development.

Even though brain organoids are currently far from being able to adequately replicate the human brain, there are promising observations of cellular processes necessary for learning functions and memory formation.[25] Brain organoids not only offer new insights into early brain development but also may permit research into the pathological mechanisms that underlie learning and memory losses in neurodegenerative diseases.

2.1.2 Glial cells

Glial cells are the only cells of the brain and spinal cord that are not nerve cells—with the exception of endothelial cells forming the blood vessels. They perform numerous functions, such as oligodendrocytes regulating nerve conduction speed by forming fatty insulating layers (myelin) and microglial cells removing protein aggregates or dead cells and performing immune system defence functions in the nerve tissue.

22 Kirwan et al. (2015); Shi et al. (2012).

23 Paşca et al. (2015); Watanabe et al. (2017); Trujillo et al. (2019); Samarasinghe et al. (2021).

24 Mansour et al. (2018); Monzel et al. (2017); Quadrato et al. (2017); Trujillo et al. (2019).

25 Zafeiriou et al. (2020).

Studies have pointed to the great diversity of cell types even within different classes of glial cells, a notion that is postulated as essential for information processing in the brain. Most of the many neuronal contact points in the brain are surrounded by glial cells, which actively coregulate and influence synaptic transfer. The insulation layers of nerve cell processes (axons) by virtue of their thickness and length of their insulation (myelination) also make an essential contribution to the highly specialised processing properties of glial cells in the auditory system, among others.[26]

To date, the formation of some glial cell types (astrocytes, oligodendrocytes)[27] has been confirmed in brain organoids, as has the myelination of nerve cell processes.[28] Due to longer development periods and the differentiated use of signal molecules, it is now possible to reproduce organoids of specific brain regions or neuronal layers of a brain region, which can then form brain region-specific glial cells.[29] Brain organoids have thus been used for investigating the effects of certain drugs on glial cells,[30] the malfunctions of glial cells in models of frontotemporal dementia (Pick's disease),[31] and the causes for neurodevelopmental biological malformations that stem from radial glial cells.[32] As glial cells also play an important role in the blood-brain barrier, organoid models have been developed to study these functions.[33]

It is likely that early developmental stages can be modelled partially; however, later developmental stages, such as from late pregnancy onward, cannot yet be modelled.

2.1.3 Vascularisation

In addition to various glial cell types, brain organoids also lack other non-neuronal cell populations that occur naturally in the brain, such as endothelial cells forming the blood vessels. Without a vascular system and a stable blood and nutrient supply, the size, growth, and developmental capacity of brain organoids are severely limited. Accordingly, as the size of a brain organoid increases, centrally located necrotic, i.e., dying areas, and cellular stress phenomena often develop.[34] However, the limited developmental capacity of such processes severely limits the modelling of later developmental stages in brain organoids, rendering it challenging to conduct research into neurodegenerative diseases occurring later in life.

One approach to enable vascularisation—i.e., blood and nutrient supply of a brain organoid—over the long term, involves the transplantation of human brain organoids into the brain of adult mice.[35] Studies have shown that transplanted brain organoids can be maintained in the mouse brain for more than six months. In this case, the implanted brain organoids are vascularised by vessels of the recipient animal—i.e., cross-linked

26 Ford et al. (2015); Stange-Marten et al. (2017).

27 Dezonne et al. (2017); Marton et al. (2019); Yakoub (2019).

28 James et al. (2021); Shaker et al. (2021).

29 Qian et al. (2020).

30 Dang et al. (2021).

31 Szebényi et al. (2021).

32 Kim et al. (2021).

33 Bergmann et al. (2018); Nzou et al. (2020).

34 Bhaduri et al. (2020); Qian et al. (2019).

35 Daviaud et al. (2018); Mansour et al. (2018).

with blood vessels and subsequently supplied[36] (see also Chapter 2.2.5). Vascularisation occurs spontaneously via the vascular system of the transplant recipient. Alternatively, the implantation of vascular cells into the brain organoid or the induction of vascular cells via corresponding proteins, so called transcription factors, provides an approach to vascularisation *in vitro*. This method aims to reduce potential damage to the nervous tissue due to lack of oxygen, nutrients, and waste removal, thereby improving the development of the organoid.[37]

Box 3: Transplantation of brain organoids and the emergence of chimeras

The transplantation of cells or tissue from one species into an organism of another is a widely used procedure in biological and biomedical research. According to a very broad scientific definition, all results of such experiments—that is, organisms with cells of different species—are called interspecies chimeras ("mixed beings").[38] According to this definition, a human-animal chimera is created by transplanting a human brain organoid into an experimental animal.

According to a more recent, stricter definition, only the transplantation of cells or specific tissue across species into the *embryonic* organism of another species is referred to as chimera production.[39] Such experiments were carried out in the 1920s, when the embryologist Hilde Mangold transplanted embryonic tissue of one newt species into embryos of another newt species. These experiments, which were groundbreaking at the time because they spanned more than one species, made it possible to identify which structures had developed from the donor tissue and which from the host tissue.[40]

Defined either strictly or broadly, the discussion of "chimeras" is problematic in terms of bioethics and public perception, as it touches on the generation of sinister human-animal hybrids that are deeply rooted in cultural history. It is therefore even more important to explain in a transparent, precise, and differentiated manner which recipient animals, developmental stages, functional integration types, and ultimately which phenotypic change potentials are involved in the transplantation of each organoid.[41]

In any case, the transplantation experiments performed for the purpose of brain organoid vascularization are, according to current knowledge, entirely free of humanization potential for the recipient animals.

Despite the paucity of transplantation studies to date, the data available show that vascularisation and thus blood and nutrient supply to transplanted brain organoids is theoretically possible. In addition, vascularisation *in vitro* has been shown to alleviate the supply deficits to brain organoids.[42]

36 Mansour et al. (2018); Shi et al. (2020).

37 Cakir et al. (2019); Shi et al. (2020).

38 See ICSSR (2021), p. 79.

39 National Academies of Sciences, Engineering, and Medicine & Committee on Ethical, Legal, and Regulatory Issues Associated with Neural Chimeras and Organoids (2021), p. 2.

40 Spemann & Mangold (1924).

41 The German Ethics Council (2011) already argued in this direction. For a current and systematic discussion of the normative assessment and regulation of chimera research, see Hyung et al. (2021).

42 Bhaduri et al. (2020).

A functional blood-brain barrier performs an essential role in protecting the brain and the entire central nervous system from exposure to harmful substances circulating in the bloodstream. In brain organoids, features of such a blood-brain barrier can be detected after the induction of endothelial cells *in vitro*. However, it is not yet clear whether human brain organoids that have been pre-vascularised in this way form a functional blood-brain barrier in the rodent brain. Previous studies have shown, however, that the blood-brain barrier can theoretically be restored after the transplantation of neuronal precursor cells from one rodent species into the brain of another individual of the same species.[43]

As an alternative to vascularisation, nutrients could be supplied to brain organoids by means of interconnected organoids of different organ systems (*organ-on-a-chip* technology) and artificial vascular networks that transport fluids.[44]

All of these developments represent important foundations for the maturation and stabilisation of brain organoids over long time periods, providing some of the prerequisite conditions for modelling developmental and disease processes, even beyond the early embryonic stages.

2.1.4 Expansion and folding of the cerebral surface

The brain is divided into different areas that have developed at different time periods over the course of evolution. Language, memory, learning, thinking, imagination, and consciousness are mental functions attributed primarily to the cerebral cortex, the evolutionarily youngest part of the human brain. During evolution, the cerebral cortex has undergone major changes, which in primates, particularly humans, has led to a significant enlargement and increased complexity of the circuitry.[45] The human neocortex, the evolutionarily biologically youngest part of the cerebral cortex, has almost tripled in size compared to that of our closest relative in the animal kingdom, the chimpanzee.[46] One reason for this increase in volume is the presence of more stem cells that undergo more division cycles in the human brain compared with less-complex brains, resulting in the formation of more neurons and glial cells. The result is an exponential and extensive increase in neuronal connections.

As seen in many mammals, large brains are characterised by a folded cerebral cortex. This folding allows for not only an increase in surface area but also a reduction in the length of the axonal connections between the cortical areas. Areas that communicate intensively with one another and need to connect more strongly can be brought closer together with folding. The folding patterns therefore reflect the connectivity architecture of the cerebral cortex and exhibit species-specific characteristics. However, there is no simple relationship between the degree of folding of the cerebral cortex and the differentiation of brain power. Brains without folds (*lissencephalic*) are found not only in rodents but also in small primates such as marmosets (tufted monkeys), which are primarily native to the Amazon basin. On the other hand, the brains of elephants or dolphins have considerably more brain convolutions than the human brain. However, the complexity of the connecting architecture is essential for the brain's performance.

43 Brandner et al. (1998).

44 Browne et al. (2021); Shirure et al. (2021).

45 Namba & Huttner (2017); Rakic (2009).

46 Heide & Huttner (2020).

Developmental disorders that lead to a severe reduction in the volume of the cerebral cortex and the number of axonal connections also result in a lack of folding in the human brain. Such pathologies are summarised by the term lissencephaly and are associated with abnormal wiring patterns and reduced brain performance. They can occur following infections such as with the Zika virus.

Many studies indicate that the folding of the cerebral cortex is based on a complex interplay between the structure's size, thickness, and the number of different areas and their connections. It is assumed that the elastic tensile forces of the axonal connections are one of the causes of the folding.[47]

Unlike the human cortex, which begins to fold in the foetus mid-pregnancy, cortical brain organoids do not typically fold, regardless of how long they are kept in culture. Human brain organoids are *lissencephalic*, like the mouse brain, which has a smooth (*lissencephalic*) cerebral cortex with no convolutions or furrows. It remains to be clarified as to why human cortical brain organoids do not fold, although it has been possible to model some aspects of cerebral folding in human brain organoids.[48] While it has been assumed that the organoid's lack of folding stems from the absence of essential structural properties of the connections between the neurons, it does not preclude other explanations.

2.2 Areas of application

2.2.1 Modelling normal human brain development

Brain organoids can partially mimic the spatio-temporal dynamics of neuron and glial cell formation, the formation of regional neuronal circuits, and the integration of glial cells into a neuronal network.[49] This allows for the study of principles and mechanisms of brain development and the experimental testing of neuroscientific hypotheses. For example, it is assumed that brain morphology and the characteristic interconnection of individual neurons and different regions are mediated by spatio-temporal gradients of signalling substances.[50] The control of neuronal development and connectivity occurs via multiple signalling mechanisms. The detailed investigation and experimental manipulation of the development of brain organoids can provide insights into the individual contributions of these mechanisms.[51] Accordingly, brain organoids are also suitable for investigating the emergence of neuronal developmental disorders.

47 It also provided convincing evidence that certain components of the extracellular matrix as well as hyaluronic acid can cause the folding of the human cerebral cortex (Long et al. 2018).

48 Karzbrun & Reiner (2019); Li et al. (2017).

49 It has also been shown that human brain organoids also undergo the gene expression programmes of the foetal human brain to a large extent (Camp et al. 2015).

50 Beul et al. (2018); Goulas et al. (2019).

51 Suzuki & Vanderhaeghen (2015).

Box 4: Regular human brain development

Normal development of the human brain proceeds at a cellular level in the following, roughly outlined steps, which partly overlap.

Induction: Part of the ectoderm (the outer cell layer of the embryo) is converted into tissue from which neurons can develop, forming the neural plate on the back of the embryo. This induction is controlled by multi-layered interactions between different signalling molecules. With folding, the neural plate forms a tube, known as the neural tube, for the subsequent development of nerve tissue. This means that the polarisation and structuring of the nervous system occur at a very early stage.

Proliferation: This is followed by a phase of cell division, whereby local stem cells multiply by symmetrical division, or in the case of asymmetrical division a daughter cell emerges from the generative process and gives rise to a more specialised precursor cell. This precursor divides further into several nerve cells (neurogenesis).[52]

Migration, aggregation, differentiation, and linkage: Young nerve cells migrate along radial glial cells to respective predetermined target areas where they form aggregated cellular populations. As soon as the neurons reach their target area, they develop their characteristic morphology—forming cellular processes (dendrites and axons), connecting the neurons, and forming local circuits as well as long-range connections.

Further specification of circuits by apoptosis and selection. There is initially an overproduction of neurons, some of which connect relatively non-specifically. Through activity-based mechanisms, some neurons die (programmed cell death, apoptosis) with the degradation of certain synaptic connections and the stabilisation of others. In addition, the cortical folding forms the characteristic shape of the human brain.

The processes outlined here involve a complex interplay of many factors—such as gene expression, signalling cascades, biomechanical self-organisation, interaction with non-neuronal cells, and multi-layered intrinsic and extrinsic factors. While all these developmental conditions are present in the *in vivo* environment of the embryo, they can be imitated artificially but only in an incomplete manner *in vitro*, resulting in manifold limitations of the organoid models.

To date, research with brain organoids has led to a better understanding of brain development at the cellular level. In brain organoids, populations of neuronal precursor cells form and develop into mature subtypes of cortical neurons. In addition, brain organoids show characteristic cytoarchitectural organisational forms with the incipient formation of an early layered cortex structure, consisting of radial glial cells, neuronal stem cells, and early cortical neurons.[53] While brain organoids in their macroscopic shape and organisation primarily represent early prenatal development, single cells contained therein can reach stages that correspond in certain aspects to the postnatal state *in vivo*. Numerous developmental milestones are reached at the molecular and

52 Namba & Huttner (2017); Rakic (2009).

53 Lancaster et al. (2013); Kadoshima et al. (2013).

cellular levels, suggesting that important components of a cellular *in vivo* developmental programme also persist *in vitro*.[54] These observations underline the great potential of neuronal self-organisation.

Brain organoids also allow for the comparison of the temporal sequence of the development and morphogenesis of the brain in humans and animals such as rodents or primates.[55] Thus, comparison of human and chimpanzee brain organoids has shown a specific extension of stages of the cell cycle in the former,[56] leading to the division and proliferation of neuronal stem cells. This is a mechanism that could contribute to the development of a larger human brain. In addition, the migration patterns of neural stem cells in humans differ from those in chimpanzees and bonobos.[57] Subtle differences in the early morphogenetic development of brain organoids, derived from humans and apes, also help explain the evolutionary expansion of the human brain.[58] Brain organoids in this context even allow for observing the influence of genes that differ in modern humans and extinct human species such as Neanderthals and Denisova humans.[59] Such observations help us to better understand the evolution of the human brain by performing a kind of reverse engineering of the underlying molecular mechanisms.[60]

2.2.2 Modelling disorders of human brain development

Brain organoids are an important model for the study of complex neurodevelopmental and psychiatric diseases,[61] particularly neuropediatric developmental disorders that are associated with epilepsy and mental disorders such as autism spectrum disorders. For a subset of patients these disorders can be traced to rare mutations in individual genes.[62] In this context, brain organoids now enable patient-specific investigations into the effects of these mutations on brain development at the cellular, physiological, and molecular levels, and the development of individualized therapies.[63]

However, most brain diseases arise from the complex, multifactorial interplay of environmental influences and genetic risk factors. Only the combination of many frequently occurring gene variants leads to an increased risk for disease development.[64] Genome-wide association studies have identified many of these gene variants and located them to mostly DNA regions that are specific to the human genome, however, their effects on brain development and brain cell function remain largely unknown. Uncovering the impact of these gene variants is critical to better understand the aetiology of brain diseases and to accelerate the development of effective therapies.

54 Gordon et al. (2021).

55 Lancaster & Knoblich (2014); Marshall & Mason (2019).

56 Specifically this applies to prometaphase and metaphase, Mora-Bermúdez et al. (2016).

57 Marchetto et al. (2019).

58 Benito-Kwiecinski et al. (2021).

59 Trujillo et al. (2021). Criticism see Maricic et al. (2021).

60 Mostajo-Radji et al. (2020); Pollen et al. (2019).

61 Lancaster & Knoblich (2014).

62 Brandler & Sebat (2015); Sanders et al. (2019).

63 Klaus et al. (2019); Klingler et al. (2021).

64 Sullivan & Geschwind (2019).

Such complex, human-specific genetic risk factors can be studied in brain organoids that are generated directly from the cells of affected patients, for example those with autism spectrum disorder or schizophrenia. In this context, such studies have so far been used to ascertain how manipulations of certain genes affect brain development.[65] Experimental approaches using induced pluripotent stem cells from affected and unaffected family members or from diseased and non-diseased individuals are currently undertaken mainly in two-dimensional cell culture models.[66]

In complex diseases, in which a large number of different combinations of gene variants increase the risk equally, it would be important to compare organoids from as many patients as possible as well as from non-diseased control subjects, and if necessary to preselect the combinations according to cumulative genetic risk.[67] In the near future, it is deemed feasible to develop methods that allow for comparison of the function of cells from several different individuals in the same experimental setup and possibly even in the same organoid.[68]

For diseases that could be caused by individual mutations, new methods of gene editing such as the "gene scissors" CRISPR-Cas9, facilitate testing for the potential association between the mutation and disease development.[69] The correspondingly identified mutation could then be corrected in patient stem cells or alternatively also introduced into stem cells of non-diseased control subjects. Such a procedure would allow for the scientifically valid investigation as to whether the corresponding mutation is causally related to the specific symptomatology. Until recently, such causal research on complex tissue has been feasible only in animal experiments. The potential of using CRISPR-Cas9 to edit several gene variants simultaneously offers a way for investigating polygenic diseases, i.e., diseases for which an interaction of different genes is assumed to be a risk factor.[70]

Although brain organoids are an important building block in the research of neurodevelopmental and psychiatric diseases, they cannot or cannot sufficiently (at this point) adequately model many disease-relevant aspects, and their research should therefore best be carried out in combination with other methods. This is particularly relevant for psychiatric diseases, wherein behavioural effects play an important role that cannot be modelled in research with brain organoids. However, some approaches to brain organoid research are addressing the problem. A central element of many psychiatric diseases is the disrupted communication between different brain areas.[71] Even though it is currently not possible to reproduce different brain areas within a brain organoid, attempts are being made to study such communication disorders in so-called

65 Cheffer et al. (2020).

66 Khan et al. (2020).

67 For these studies, it is important to control the variability of the individual brain organoids, in particular differences in the cell composition. Here methods that allow single cell level resolution can enable a more precise comparison of the results in different organoids. This allows technical effects to be better distinguished from patient-specific effects. See Brancati et al. (2020); Camp et al. (2018).

68 Cederquist et al. (2020); Cuomo et al. (2020).

69 Bian et al. (2018); Buchsbaum et al. (2020); Fischer et al. (2019); LaMarca et al. (2018).

70 Matos et al. (2020); Schrode et al. (2019).

71 McTeague et al. (2017); McTeague et al. (2020).

assembloids, structures composed of different region-specific brain organoids. This research could be used to model the interaction of different brain regions and may lead to a better understanding of neuropsychiatric diseases.[72]

Brain organoids make it possible to study not only living, developing brain cells of patients in a cell network but also their response to possible environmental risk factors such as prenatal infections, toxin exposure, or the release of stress hormones. In many cases, both the genetic risk factor and the corresponding environmental influence are needed to trigger disease progression; such an interaction could theoretically be studied in brain organoids to gain insight that cannot be obtained in animal models (no human-specific genetic risk) or in post-mortem tissue (no possibility of dynamically measuring the response to environmental influences). Brain organoids thus also enable studying the effect of specific viral and other infectious diseases on the development of the human brain. A breakthrough study using brain organoids has revealed that Zika virus infection during pregnancy can lead to restricted brain development (microcephaly) in the foetus (see Figure 4).[73]

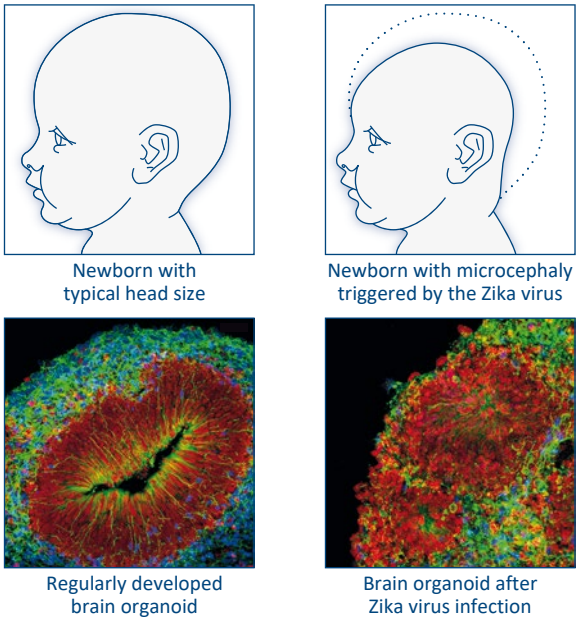


Figure 4: Evidence of an association between Zika virus infection during pregnancy and the development of microcephaly in newborns.[74]

The effect of new human-pathogenic viruses such as SARS-CoV-2 on brain development is also being investigated in brain organoids.[75] Corresponding research could make a crucial contribution to formulating well-founded recommendations for pregnant women during future pandemics much faster than before, even if other important factors such as the human immune system and the placental receptivity of viruses cannot be fully modelled.

72 Bagley et al. (2017); Paşca (2018).

73 Tang et al. (2016).

74 Source of microscopic Figures below: Ming et al. (2017).

75 For example Pellegrini et al. (2020).

2.2.3 Modelling neurodegenerative diseases of the ageing brain

Brain organoids are produced from embryonic stem cells (ES cells) or adult induced pluripotent stem cells (iPS cells). They represent a very early stage of brain development that does not extend beyond the maturation level of a prenatal brain in terms of the observed cell types and tissue architecture. In addition, ES and iPS cells also correspond epigenetically[76] to a very early embryonic stage. This is naturally the case with ES cells originally derived from a blastocyst (embryo in the early developmental stage) while iPS cells lose epigenetic age signatures during the reprogramming of the donor cells.[77] Thus, brain organoids are primarily models for the study of very early developmental stages and prenatal-onset disease processes.

Nevertheless, organoids can also be used for research into diseases of old age, particularly neurodegenerative diseases such as Alzheimer's disease.[78] Many of the neurodegenerative diseases occurring in old age are specific to humans, and as such, human cell culture models are expected to be more appropriate than animal models. Even though brain organoids model only very early developmental stages, they can be used to study mechanistic processes in a three-dimensional, tissue-like structure, for example, the interactions of pathogenetically relevant cells and molecules that are involved in the development of neurodegenerative diseases. Such interactions between nerve cells, glial cells, and the microglia, which function as immune cells of the nervous system, are considered paramount for the pathogenesis of diseases such as Alzheimer's disease. On the other hand, pathological processes in the tissue outside the cells can theoretically be studied in brain organoids. In Alzheimer's disease, for example, there is extracellular accumulation and aggregation of Aß-peptide, the so-called plaque formation in brain tissue, a process that is currently being studied in brain organoids.[79]

2.2.4 Testing substances on brain organoids

The developing foetal brain is particularly sensitive to substances such as toxins or drugs. Brain organoids allow studies into the effects of such substances on brain development *in vitro*. This could also facilitate the search for early therapies or preventative approaches. Research with brain organoids has so far examined the effects of alcohol and specific drugs such as cocaine and methamphetamine.[80] However, brain organoids have already been used to identify new drugs for neuropsychiatric diseases, such as Parkinson's disease, Alzheimer's disease, and autism.[81]

Although research with brain organoids has the potential for studying the effects of drugs or toxins on the complex processes of brain development, certain limitations need to be considered.[82] Often sex differences underlie the effect of substances on brain development, necessitating modelling of the complex interaction between genetic sex

76 The epigenetics of a cell is the activation or deactivation of gene sequences without changing the genetic information of the DNA. Epigenetic characteristics are used to pass on changes in gene function to daughter cells that are not based on mutation or recombination.

77 Lo Sardo et al. (2017).

78 Cenini et al. (2021).

79 Venkataraman et al. (2020).

80 Arzua et al. (2020); Dang et al. (2021); Lee et al. (2017).

81 Struzyna & Watt (2021).

82 For example, certain substances do not reach all areas of the brain organoid equally.

and hormonal processes. Additionally, the generalizability of such brain organoid research would require investigations into brain organoids with different genetic profiles related to genetic sex, disease risks, or ethnic differences.

Brain organoids could also help us understand why certain drugs lead to an improvement in symptoms or cause side effects in some but not all patients.[83] This can be achieved by comparative studies of brain organoids, whose cells originate from therapy-responsive and therapy-resistant patients.[84] Brain organoids can also help us understand the individual causes of rare genetic brain development disorders and help make specific diagnoses that cannot be achieved using other examination methods.[85] In the future, *precision medical therapy* could be achieved in this way by individually testing and optimising medical interventions such as the administration of drugs in patient-specific brain organoids.[86]

2.2.5 Brain organoids as a therapeutic tool?

As human brain organoids that are transplanted into animals become vascularised and can survive for longer time periods (see Chapter 2.1.3), the question of their therapeutic potential is raised. So far transplanted brain organoids have been shown to exhibit only a rudimentary architecture.[87] However, mature neurons within brain organoids show growth of nerve cell processes (axons) into large parts of the animal recipient brain.[88] In addition, the transplanted neurons demonstrate partly synchronous electrical activity.[89] In addition to functional neuronal connections within brain organoids, signals have also been detected in neurons of the recipient brain after electrical stimulation of the transplanted brain organoids, indicating functional connections between the graft and the recipient.[90] Such findings open up possibilities for the development of regenerative therapies, particularly the use of tailor-made brain organoids for modulating endogenous neuronal networks. However, it is unclear whether the partially complex architecture of brain organoids observed *in vitro* can be replicated in the transplant. Considerable biological and technical hurdles need to be overcome before the potential therapeutic application of brain organoids is realised. These include brain organoid standardisation, stereotactic (very precise) transplantation into the recipient brain, vascularisation, and defined interconnection with the neuronal networks of the recipient brain. Potential adverse events include uncontrolled expansion of brain organoids after implantation and functional interference with physiological brain functions, currently precluding the successful therapeutic application of brain organoids.

83 In general, it is important to examine many brain organoids in parallel for the efficient testing of many substances and mapping the outlined variability. The further development of high-throughput methods can make a significant contribution here (Renner et al., 2020).

84 Currently, this type of investigation is mainly carried out with stem cell-based nerve cells in a 2D model (Lago et al., 2021; Pasteuning-Vuhman et al., 2021).

85 Gomes et al. (2020).

86 Lampert et al. (2020).

87 Shi et al. (2020).

88 Mansour et al. (2018).

89 Mansour et al. (2018); Shi et al. (2020).

90 Mansour et al. (2018).

In addition, other methods for the treatment of neurodegenerative diseases are already being clinically tested, including cell replacement strategies such as the transplantation of cell suspensions to replace dopamine-forming neurons in Parkinson's disease.[91] Further procedures currently at the experimental stage even include the direct conversion of non-neuronal cells into nerve cells in the recipient brain.[92] Any therapeutic use of brain organoids would have to be measured against these alternate methods in terms of safety and efficacy.

91 Barker et al. (2018).

92 Rivetti di Val Cervo et al. (2017).

3 Research perspectives

As discussed in Chapter 2, although brain organoid research has already contributed to a better understanding of normal human brain development and various brain diseases, due to their inherent limitations, brain organoids represent a simplified and artificial model of a developing human brain. So far neither the correct topographical arrangement of individual brain regions nor the normal interconnection of these regions can be found *in vitro*. Brain organoids are also much smaller than the human brain and the cellular subtypes are not as numerous. They also lack non-neuronal cells, such as the endothelium of blood vessels and the immunologically important microglia (except for the incipient stages), while the cells present are significantly less mature than their counterparts in the human brain. However, well-founded hope exists that some of these limitations could be overcome in the future using innovative new methods. But what developments are currently possible and where do the natural limits of the brain organoid model lie?

3.1 Differentiation of specific brain areas in brain organoids

Specific regions of the human brain are responsible for specific tasks. The visual cortex is responsible for visual processes, the motor cortex for movement control, and the prefrontal cortex for more complex thought processes.[93] The subdivision and segregation of different brain areas already begins in the neuronal precursor cells.[94] As the neural plate and neural tube start to form, the developing brain of the embryo receives signals from messenger substances secreted by different signalling centres (organisers) and undergoes differentiation into the anterior and posterior areas (anteroposterior specification). After completion of the neural tube, the neuroepithelium undergoes differentiation into the upper and lower areas (dorsoventral specification). During further development, the different brain regions then separate according to a fixed topographical order (see Figure 5). However, in the culture media of brain organoids, factors and molecules diffuse freely and elicit a random (rather than an ordered) arrangement of individual areas.[95]

In the future, novel methods are expected to enable the emergence of ordered brain regions in organoids, for example, via the introduction of artificial signalling centres into the cultures or the build-up of gradients of signalling substances using microfluidic systems. In addition, it is also possible that glial cells will integrate better within organoids and develop further. This may lead to the stabilisation of organoid metabolism and prolongation of organoid development, an important prerequisite for the establishment of more complex neuron interconnections. In this way, brain organoids could more

93 Luo (2016).

94 In the precursor cells, gradients of growth factors establish a positional identity, which is then passed onto the neurons generated by the precursor cells. This principle, known as *protomap hypothesis*, has recently been demonstrated convincingly by gene expression analysis in the foetal brain. Nowakowski et al. (2017).

95 Bhaduri et al. (2020); Renner et al. (2017).

precisely replicate the arrangement of brain regions along the anteroposterior and dorsoventral axes and exhibit structural similarity to the human brain. Initial attempts to form brain axes *in vitro* have already been successfully carried out in two-dimensional cultures[96] as has the dorsoventral differentiation in brain organoids.[97] The use of gradients of individual messenger substances (morphogens) it has been possible to create transitions between cells with different regional identities; however, this has not yet led to the establishment of topographically clearly defined regions—which requires a complex interplay between many messenger substances and further mechanisms, such as cellular sorting phenomena or the establishment of "borders" (the segregation of different cell types and brain regions).

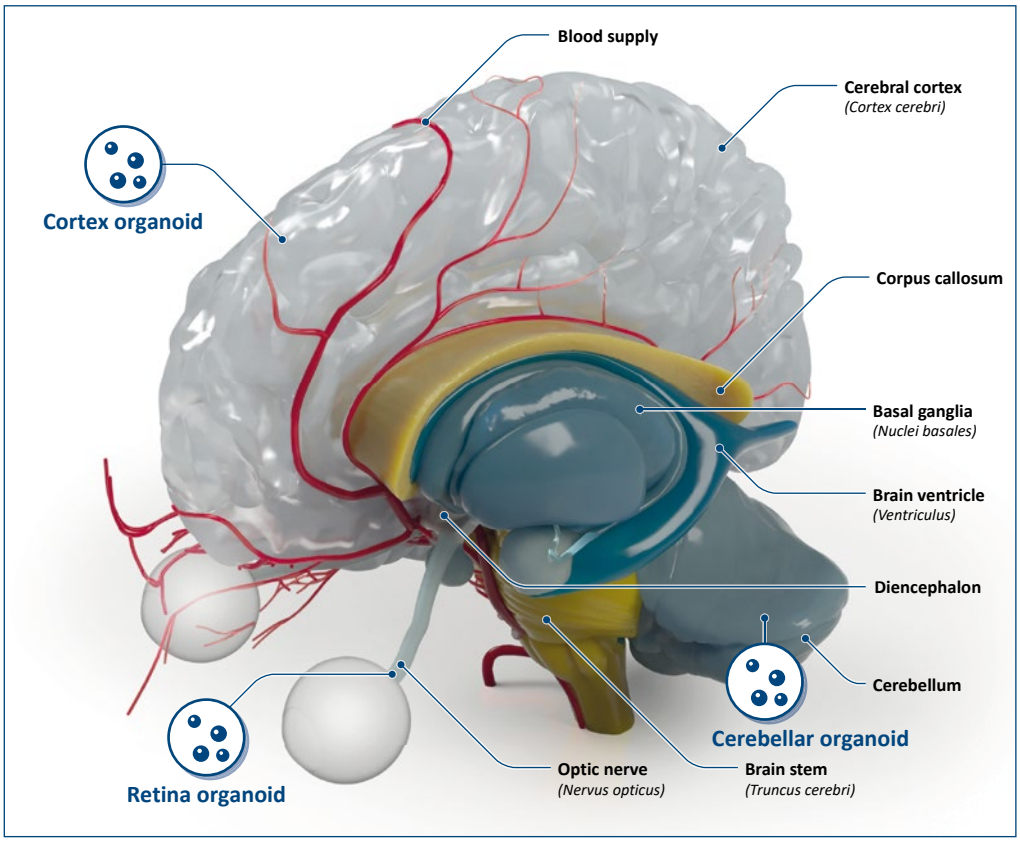


Figure 5: Structure of the human brain and three selected brain organoid types, which represent the corresponding brain regions.

At present, it is not possible to predict whether mapping the ordered architecture of an entire brain in the organoid is within reach. The regionalisation of the brain requires a larger number of different signalling centres, which in turn are arranged in a highly complex, three-dimensional architecture. Animal experiments have shown that specific sensory input is also required and that sensory stimuli must occur within a specific time frame to reproduce the functional microarchitecture of the corresponding brain regions.

96 Rifes et al. (2020).

97 Cederquist et al. (2019).

Common brain organoid systems are also subject to a fundamental limitation: the production of brain organoids is based on the ability of human cells to self-organise, i.e., their ability to arrange and connect themselves in a certain way without any external influence. This ability is astonishingly well developed, but it also has its limitations. For example, although the nerve cells in the brain organoid initially arrange themselves roughly in the layered architecture[98] typical of the brain, this architecture is lost over time.[99] In other words, although individual cells develop in the same way as in the foetal brain,[100] the ordered structure of a human brain cannot be achieved in brain organoids.

Even though great strides have been made in brain organoid research, it is not yet conceivable to develop methods supporting the formation of functional brain areas. Brain organoids thus remain—for the time being—a reductionist brain model, which can map certain properties of the brain but not others. However, a complete representation of all developmental processes by brain organoids is not necessary for research into certain neurological and psychiatric diseases.

3.2 Interaction with the environment

Neuronal networks require interaction with the environment and processing of environmental stimuli in functional units for successful neuronal specification and maturation, and ultimately development of consciousness (see Chapter 4.2.2). In the future, brain organoids could be imparted with multiple sensory receptors, for example visual or pain-sensitive (nociceptive) receptors, as well as motor effectors. Initial studies have already shown the possibility of interconnecting brain organoids with photoreceptors[101] and motor neurons.[102] A recent study has shown the establishment of eye systems during brain organoid development, further demonstrating that the sensory cells of these eye systems respond to light stimuli, convert these stimuli into neuronal electrical activity, and transmit these electrical signals to other neurons (see Figure 6).[103]

98 Lancaster et al. (2013).

99 Bhaduri et al. (2020).

100 Gordon et al. (2021).

101 Quadrato et al. (2017).

102 Giandomenico et al. (2019).

103 Gabriel et al. (2021).

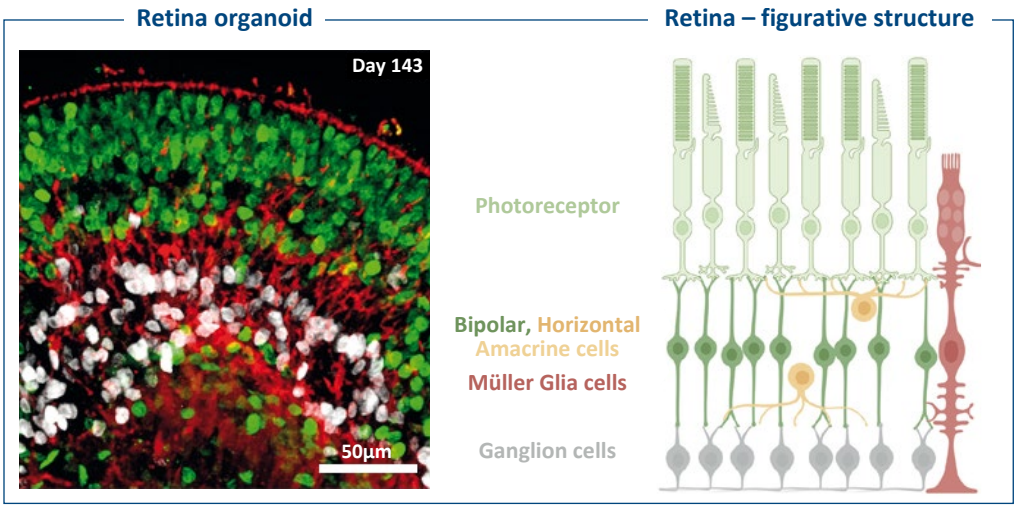


Figure 6: Microscopic image of a section of a retinal organoid and a sketch of the cellular structure of the retina.[104]

These examples show that, in theory, brain organoids can receive information from the environment and process it to some extent. Corresponding interfaces with the environment may increase the size of brain organoids, change their structure, and lead to a greater differentiation of their structure and neuronal activity patterns, particularly via intrinsic plasticity mechanisms, though at present it is difficult to estimate the degree of brain organoid complexity that could be achieved.

However, questions regarding which stimuli brain organoids could receive and how they process these stimuli must remain separate topics of research in the future. For example, the sensation of pain is a complex process that involves different brain areas. Even if such receptors are conceivable in future brain organoids, this does not mean that these organoids could then actually feel pain. Brain organoids are expected to lack the corresponding prerequisites, especially the totality and interconnection of the brain areas involved in such a process. The same applies, for example, to visual stimuli—vision is a complex process that requires areas of the cerebral cortex to communicate with each other and interpret and link the received stimuli in a meaningful way. The formation of certain receptors and the activation of connected nerve cells is therefore only a very small component of the complex processes and activities taking place in the human brain. Therefore, although brain organoids may receive suitable sensory stimulation, their structural and functional limitations—which will not readily be resolved—preclude the ordered specification of brain areas that would support complex brain activities.

3.3 Vascularisation of brain organoids

A significant limitation to the formation of very mature cell types and complex neuronal circuits relates to the cultivation conditions *in vitro*. The lack of blood vessels restricts the supply of nutrients, particularly inside the brain organoids, limiting their growth. Brain organoids cultivated over a long period therefore increasingly lose their three-dimensional organisation, i.e., the regular topographical arrangement of different cell types. Later stages of brain development, and pathological processes in brain organoids, which are produced from cells of patients with psychiatric or neuro-

104 According to Menuchin-Lasowski et al. (2022).

degenerative diseases, are currently difficult to study *in vitro*. However, human brain organoids can be integrated into different host organisms at different stages of development, providing a natural developmental environment with better nutrient supply and support for more extensive organoid growth with the help of the host organism's vascular system. The specific effects of such a developmental environment on the structural and functional organisation of brain organoids and their integration into the nervous system are currently hard to assess. On the one hand, species-specific differences between the host environment and brain organoid may limit the latter's developmental potential. On the other hand, connecting the systems of different species could provide synergistic opportunities for growth that cannot currently be predicted with present-day knowledge.

The transplantation of human brain organoids into a living organism has so far been carried out on various mammals (mainly mice, but also rats and macaques) (see Chapter 2.1.3).[105] However, inherent limitations with such experiments include the limited available space for the expansion of a human brain organoid within the body of a rodent without damaging the host tissue; and the limited time span of observation of brain organoids transplanted into adult mice of only a few months, as the lifespan of mice is 3 years.[106]

Nevertheless, there are opportunities to overcome these limitations. One such scenario would entail the transplantation of a brain organoid into the brain of a large, long-living organism—for example, a domestic pig or a primate—allowing for the three-dimensional expansion of brain organoids over many years. Although such experiments would allow for the complete vascularisation of brain organoids as well as the formation of intensive neuronal connections between the host and transplant, it is uncertain whether they would allow for a better structural and functional formation of the organoids and for the requisite complexity and appropriate degree of maturity of the cells and circuits. Another experimental scenario relates to the transplantation of the organoid into the host organism at a very early stage of development, for example, an intrauterine transplant into a foetal animal brain, an approach that could lead to much stronger integration of the human cells into functional circuits of the animal brain. However, both experimental approaches raise different ethical issues that need to be considered when weighing appropriate procedures (see Chapter 4.2.3 and Box 3).

Research is currently underway to produce vascularisation *in vitro*, circumventing such issues. One approach involves integrating mechanical pump systems into assembloids of brain and blood vessel organoids. Another method entails the production of blood vessels *in vitro* and their combination with brain organoids, but this has not yet resulted in the successful integration of a completely closed functioning blood vessel system into a brain organoid.[107]

105 Mansour et al. (2018); Kitahara et al. (2020); Wang et al. (2020).

106 Mansour et al. (2018).

107 Cakir et al. (2019).

4 Moral and legal claims for protection

4.1 Substantiation of claims for protection

When dealing with brain organoids, the main ethical issue is whether we ourselves might have any moral obligations towards them. This topic is usually debated in bioethics using the keyword "moral status". For the public, the mere creation of brain organoids is sometimes described as the unnatural handling of human cells and is therefore considered problematic. Even though we do not share such concerns and do not consider such arguments to be valid, we would like to address them briefly here.

4.1.1 Naturalness versus artificiality

In addition to concerns about possible claims for data protection or problematic research objectives, there may be concerns about the "unnaturalness" of the research objects in question, particularly considering that organoids originate from humans.

Firstly, it is necessary to clarify the terms "nature" and "naturalness", which are subject to such criticism.[108] "Nature" relates to the state of *everything* that exists while "naturalness" relates to the essence of what exists. Regarding the latter sense, there are different understandings of which needs, characteristics, capabilities, or lifeforms are constitutive and typical—according to their *nature*. Lastly, a third concept, prominent in bioethical contexts, is "procedural naturalness" and relates to the contrast between naturalness and the control humans have on certain biological processes and phenomena—for example, between the natural inheritance of biological properties and the technological manipulation of the inheriting process. In the example of conventional plant cultivation compared to gene editing, such a demarcation is difficult in purely descriptive terms and falls behind today's great green and red genetic engineering controversies in evaluative terms.

A contrasting term to naturalness is artificiality, which is of particular relevance in this context. The classification of characteristics as natural or artificial, and more so their evaluation, often depends on the context of the assessment. Compared to robots, androids, or cyborg machinery, in one's own brain, the human being is *nature*, not only as part of exterior nature, but also as the essence of being—which is frequently regarded as worthy of protection. In other contexts, however, our superiority over nature is emphasised. An illuminating example of this is the widespread idea that our capacity for *free will* (fortunately) removes us from the natural-and-causal constraints. There is no abstract-general criterion for classifying such qualities. Therefore, in individual cases, it is often done by contrasting comparisons: *the natural versus the divine*—here humans belong to nature; in other comparisons such as between natural and intellectual (creative, spiritual) or between natural and cultural, such qualities are positioned outside of nature.

108 Birnbacher (2006).

There is a broad spectrum of different positions between these concepts of nature and their logical references. It is indisputable that brain organoids have no designated place in the existing order of existence, are new entities with new human-like characteristics, and must be artificially produced. But what does this mean for ethics?

Contrary to what the great metaphysical concepts of nature and naturalness suggest, it is difficult to derive blanket arguments from them. On the one hand, it is certainly true that the containment of "cosmic dangers" of the human environment has already been one of the self-evident tasks of human activity: combatting natural disasters is frequently cited in this regard as a large part of medical activity. Furthermore, an interest in the self-transformation of human nature through physical intervention, education, and training is obviously part of this nature itself. On the other hand, it is indisputably advisable to apply a "heuristic of fear"[109] to all interventions in external and internal nature—and always exercise caution and scepticism, as their potential for disastrous consequences may be difficult to assess at the present time. Such potential for negative consequences may include effects on the social dimensions of justice, freedom, and recognition, as they are controversially discussed, for example, in the context of the medical-technical improvement of the human being.[110] Lastly, references to nature and naturalness can likely also offer intuitive and argumentative relief. But overall, it is true that prohibitions of action cannot be justified coherently and therefore plausibly by mere references to the cosmic order, the nature of human beings, or the naturalness of the processes. At best, these concepts may serve as guidelines from which deviation may require justification, with the degree of justification determined on a case-by-case basis. In this sense, the production of brain organoids as a research instrument is not fundamentally more questionable than the development of artificial models of human organs or the imitation of human mental or physical abilities.

Another aspect of potential intuitive misgivings about research on brain organoids could be reduced to the "symbolic value" of the physiological (human) origin of such entities or to their biological proximity to human brains.[111] On closer inspection, however, it is not clear why these aspects should have a moral significance, which we do not attribute, with good reason, to the production and research use of other human cell and tissue cultures.

4.1.2 Moral status: general and specific

Over the past decade, in bioethics, moral status has become a key concept when discussing the ethically legitimate treatment of various entities. It usually marks the point (albeit often controversial) at which attribution and systematic justification of moral rights are granted to these entities based on their own intrinsic value—the standard wording is: "for their own sake".[112] Previous controversies about status have related mainly to human embryos and animals.[113] In recent research, however, status considera-

109 Jonas (1984).

110 Buchanan (2011); Habermas (2001); Haraway (1991); Merkel et al. (2007); Schöne-Seifert & Talbot (2009).

111 Likewise critical Koplin et al. (2021), p. 261 ff.

112 For example, Jaworska & Tannenbaum (2021), p. 1: "An entity has moral status if and only if it or its interests morally matter to some degree for the entity's own sake." Occasionally, status attributions are also justified by non-intrinsic qualities of the respective entity: in the case of embryos, for example, by the social relations to their donors of future parents: see Warren (1997). For brain organoids, this aspect is not invoked as far as we can see.

113 Damschen & Schönecker (2003); Steinbock (2007); DeGrazia (2008).

tions have increasingly been applied to novel entities such as chimeras (see Box 3), hypothetical posthumans, artificial intelligence of the future,[114] or even human brain organoids.

Moral status is generally understood to be a characteristic that is fully attributed to born humans and not to inanimate things or products. What lies in between humans and inanimate objects is assigned a widely varying value, and the presumed status is justified in different ways accordingly. Divergence begins with the question of whether moral status is a threshold or a gradual concept and continues with the question of the justification of status.[115]

Early human embryos are a clear example of the diversity of status perceptions:[116] while the Catholic Church, for example, grants them full moral status from the outset—the right to dignity and the protection of life of a born human being—others give them weaker claims for protection, which increase however over the course of their development. Still others wish to grant them at most symbolic respect but no claim to protection of their existence. These positions are justified with arguments as diverse as divine or natural law, belonging to the human species, and especially the current or future possession of certain abilities such as subjective sentience, self-awareness rationality, morality, or autonomy.

A widespread general understanding of moral status and what follows therefrom links this status to the possession of subjective interests of the corresponding entity, with increasing weight attributed to increasingly complex interests.[117] According to this, a being that is sensitive to pain, for example, has a subjective interest and thus a corresponding right to avoid pain, which may only be infringed for more weighty counter reasons. From this perspective, more complex interests justify a correspondingly weightier protection of interests. As controversial as the individual evaluations and considerations may be—for example, the conflict between human and animal interests in the context of animal experiments—representatives of this view unanimously regard sentience as a necessary minimum condition for any moral status for artificial entities as well as for non-human natural beings. Regarding human beings, on the other hand, according to widespread opinion, sentience is not, or only subordinately, regarded as decisive, both at the end and the beginning of life. That is, those who believe brain death is not the death of a human in the ethical and/or legal sense often declare the irreversible extinction of the previously existing sentience to be irrelevant. Instead, for example, the memory of the deceased, which is also of importance for the legal concept of post-mortem personal rights, plays an important role. Mainly however, as already mentioned, human embryos are frequently ascribed full moral status long *before* they become sentient. Admittedly, this lies within the controversial potentiality argument,[118] which has no significance for brain organoids from the outset due to their lack of corresponding developmental capacity.

114 See corresponding chapter in Clarke, Zohny & Savulescu (2021); Müller (2021).

115 See for example Buchanan (2009); Clarke & Savulescu (2021); Jaworska & Tannenbaum (2021).

116 See Damschen & Schönecker (2003).

117 DeGrazia (2008, 2021).

118 See for example Damschen & Schönecker (2003); McMahan (2002) p. 302-306; Merkel (2002) p. 161-178; Stier & Schöne-Seifert (2013).

Regardless of these differences, there is widespread agreement on the moral status of brain organoids [119] that justifies claims for protection which could and should only be attributed to them if they exceed the threshold of a minimal capacity for subjective positive or negative feelings (*sentience*) (see Chapter 4.2). Only with the presence of this capacity could brain organoids plausibly be considered to have their own interests and thus their own well-being, which could be impaired for example by the infliction of pain. It is not uncommon at this point to refer to the broader criterion of subjective *perceptiveness*, which is also referred to as "phenomenal consciousness" or "qualia consciousness" (see Chapter 4.2). However, it seems more convincing—although not discussed in detail—to consider subjective perception without an accompanying capacity for feeling (*valence*) (as it can be postulated at least in thought experiments) as irrelevant to status.[120]

Based on this, the present statement assumes that brain organoids should be attributed a moral status if and only if they become sentient. It is widely agreed that brain organoids, as produced according to the current state of research, are far from this threshold. At the same time, a question that is becoming increasingly important is how to determine whether brain organoids will be able to feel or have sensitivity to pain at some point in the future[121]. Some ethicists are discussing the application of a precautionary principle to future advances in brain organoid research, instead of entering a grey area in which sensitivity can no longer be ruled out.[122]

However, in light of the possibility of producing sentient brain organoids, which cannot be ruled out in the future, questions arise about the hypothetical consequences for research. Here, a minority proposes to completely dispense with the production and use of such entities, but it is most commonly proposed to regulate this research in the same way as animal experiments, where the infliction of pain has to be avoided or reduced to a minimum—an ethical issue on which, as is well known, different positions are held.[123] Finally, a constructive, but perhaps presently utopian strategy, is to genetically manipulate brain organoids in such a way that they would be incapable of feeling.[124]

To accomplish this, attempts have been made to supply brain organoids with blood vessels and enable them to develop for longer time periods, as explained above (see Chapter 3.3), via implantation into the brains of experimental animals, creating "chimeras", if one uses a broad definition of this term (see Box 3). The production and use of such human-animal chimeras raises fundamental normative questions about the limits and

119 Review of publications on the moral status of brain organoids in Lavazza (2021a) and Koplin et al (2021).

120 Also Koplin et al. (2021), p. 255 ff.

121 Neuroethicist Cheshire writes: "How many neurons would it take to generate a human thought is a question that no mathematical formula can adequately answer. In what configuration or at what stage of development a cerebral organoid would begin to have limited sentience might be detectable with future technology, but probably not in advance of the threshold being reached and the problem already upon us. Ethical decisions to guide cerebral organoid research cannot wait for these questions to be answered definitively. There may be no more difficult question in current neuroethics than what to do with wondrous wisps of grey matter that want to become brains." Cheshire (2020), p. 33.

122 Birch & Browning (2021); Żuradzki (2021).

123 For example, Ach & Borchers (2018), Hostiuc et al. (2019), Koplin & Savulescu (2019).

124 Also Koplin et al. (2021).

consequences of "humanising" recipient animals; however, these chimeras will not be pursued further in this statement because according to current knowledge, in the foreseeable future, brain organoid transplants will not cause any relevant changes in the animal host brain. The special cognitive functions of the human brain are not due to the special structural and functional properties of human neurons, but to the unique functional architecture of the brain as a whole.

Here it is also not necessary to address the ethical questions that arise for a special field of research, not discussed further, which includes the practice of implanting human nerve (precursor) cells (and not brain organoids). In theory, however, it's possible that such developmental implants could to some degree change the brain-based abilities of the recipient animals, so that status debates may be applicable here. Corresponding consequences are being discussed, in particular, for the implantation of human nerve cells into primate brains.[125]

4.2 Consciousness as the basis of ethical and legal evaluations

As already explained in Chapter 4.1.1, the attribution of a mental inner life (consciousness) is usually considered an important or even a more decisive aspect of whether and to what extent an entity is worth protecting for its own sake. The varying degree of attributions proposed is reflected in historical concepts of a *scala naturae* as well as in the gradations of protection commonly made between humans and animals, between more or less evolved animals, or between animals and plants and inanimate nature. Even if this basic premise is criticised by some, we presuppose this here.

4.2.1 Consciousness: approaches and questions

Experiences of consciousness, for example, the subjective perception of a flower scent, the feeling of sadness, or the conviction that a certain fact is true, are constitutive components of human existence and provide manifold reasons for human action. Conscious information processing involves storing what we have experienced in our memory and being able to recall it, combining what we know with new sensory impressions and making it usable for upcoming decisions and actions, linking information from the various sensory systems with each other, and weighing the expected consequences of an undertaking. At first glance, it is true that for humans the contents that penetrate into consciousness can be expressed and reported in language, in contrast to unconscious processes.

However, this description falls short, as neither humans who have not yet developed their linguistic ability or have lost it nor animals that have only limited communication would be denied the ability to consciously process stimuli for this reason alone. Although it is unclear or disputed in which variants and to what extent different animal species possess a mental inner life, experiences of consciousness as we know it are undoubtedly phenomena of the natural world and as such objects of neuroscientific and psychological research. At the same time, they are the subject of philosophical theories, because as mental and moreover genuinely private experiences, they can only be understood and classified subjectively by means of introspection or indirectly through

125 See Hyun et al. (2020); Lavazza (2021a); Taupitz (2021); German Ethics Council (2011), p. 110 ff.; National Academies of Sciences, Engineering, and Medicine & Committee on Ethical, Legal, and Regulatory Issues Associated with Neural Chimeras and organoid (2021).

communication and behaviour. Furthermore, they are closely connected in a theoretical sense with fundamental philosophical controversies, for example, about the self, freedom of will, or the moral significance of the different mental constitutions of living beings.

Despite much progress in identifying the necessary neurobiological conditions of consciousness, there are profound intra- and interdisciplinary gaps in our knowledge and disagreements including (but not limited to) the following:

- how different states of consciousness should best be classified and labelled;
- whether consciousness (in its different variants) is ultimately brought about by certain causally effective anatomical brain structures[126] or by certain brain functions;[127]
- in which way artificial information processing devices can generate phenomena resembling subjective perception, or whether both types of states seem to belong to different categories of being;[128]
- what is the presumed evolutionary advantage of conscious over non-conscious perception;
- and how conscious mental states "cooperate" with non-conscious mental states.

None of these problems can be dealt with in detail here but taken together they indicate the large body of knowledge that is expected from a large and significant future research in the field of consciousness. It is important to consider, however, that for all the ambiguities in the scientific community there is at least widespread agreement on the following:

- convincing (and philosophical) theories of consciousness have to be at least in a weak sense naturalising, i.e., compatible with basic scientific convictions and empirical findings.[129] This applies, for example, to findings from the treatment of patients with brain injuries, from anaesthesia research, neurophysiology, or from human and non-human developmental biology, and from behavioural research;
- (i) qualitative experience (phenomenal or qualia consciousness, which also includes the ability to distinguish subjectively positive or negative perceptions) is intrinsically different from (ii) consciousness of thought or decision content, (iii) ego consciousness, and (iv) wakefulness;[130]
- consciousness can be realised gradually;
- phenomenal consciousness, associated with feelings, is morally relevant (in particular pain and suffering are states from which we should protect ourselves and others on moral grounds);
- in everyday morality, just as in ethics, the capacity for certain states of consciousness plays an important role in the attribution of an intrinsic value which then raises claims to protection, or dignity ("moral status") of the referred entities.

126 For example, the currently prominent Integrated Information Theory (IIT) and the Higher Order Theory, belong to the camp of these views, see Singer (2019).

127 The known Global Workspace Theory (GWS) fits here; see Baars (1988).

128 This is the problem of the brain-spirit "explanation gap"; see Levine (1983).

129 Carruthers & Gennaro (2020).

130 For a known classification proposal see Block (2002).

4.2.2 Neurobiological preconditions for consciousness

According to present-day knowledge, consciousness requires the cooperation of numerous, functionally specialised brain centres and the precisely coordinated dynamics of interacting nerve cells. In order to generate functions to which we assign the attribute "conscious", the biological structures involved need to be of sufficient size, complexity, and differentiation.

All hypotheses about the neuronal correlates of consciousness assume that conscious states are based on the integration of information provided by processes in different regions of the brain. The assumption is that this requires particular motives of connectivity. These are found in the cerebral cortex, but are also found in modified form in the brains of species that do not have a cerebral cortex, such as birds and reptiles. Therefore, the prerequisites for conscious states are also met in these species. There is also agreement that the neural networks responsible for generating conscious processes have to be kept in a critical, very finely regulated state of excitation. In all brains that are considered capable of consciousness-generating information processing, this task is performed by special structures that control the excitability of the entire brain and that are responsible for, among other things, controlling the states of sleep and wakefulness. If these structures fail, conscious processes cannot take place. However, the integration of information required for conscious processing does not take place in these excitability controlling structures.

The search for a single area of the cerebral cortex, which could be responsible for the integration of all the contents that appear in consciousness, has so far been unsuccessful. Therefore, preferred theories currently postulate that the necessary integration of contents is realised via dynamic interactions between a great many brain regions. Methodological advances have made it possible to establish close correlations between conscious processes and highly complex, temporally, and spatially structured activity patterns. These patterns involve large areas of the cerebral cortex, exhibit a particularly high degree of temporal coordination, and reflect the special features of the functional architecture of the neural networks that produce them. In their individual expression, they reflect the totality of genetically and epigenetically determined characteristics of the respective brain.

While the blueprint of the connection architecture of brains is genetically determined, it is further shaped through interaction with the environment to serve as substrate for higher cognitive functions. The neuronal networks therefore have to be connected to the environment of the organism via sensory organs and be able to interact with the environment to influence them via "effectors". The cascades of self-organising developmental states taking place in the organism and its nervous system, respectively, are thus finely tuned to each other and mutually dependent.

Brains only acquire the abilities that we assume as prerequisites for conscious processing if they are embedded in the organism that supports interactions with the environment. For the development of the special characteristics of human consciousness, it is further indispensable that brains can differentiate their functions in interaction with a social and cultural environment.

4.2.3 Can or could brain organoids develop consciousness?

The question of whether brain organoids can develop consciousness has to be answered depending on whether it refers to brain organoids (i) under present and currently foreseeable conditions of generation or (ii) under hypothetical circumstances that might arise in the future. In any case, for the present and the foreseeable future, the emergence of consciousness in brain organoids is considered impossible by experts involved in this debate.[131] This applies irrespective of the partial functional similarities with foetal brains described above. Brain organoids lack the neurobiological prerequisites for consciousness, as outlined above, in three respects:

- they do not contain sufficient nerve cells for enabling the complex performance of conscious processes;[132]
- they do not contain the various types of differentiated cells required for this process;[133]
- nerve cell networks of brain organoids lack the structural and functional differentiation that is only made possible via interactions with a simultaneously developing organism.

This makes the self-organisation of brain organoids fundamentally different from the development of the foetal brain. It is not conceivable that in the foreseeable future brain organoids will form circuits that come even close to reproducing the extremely differentiated functional architecture of the foetal brain. As such, nerve cells will form organ-specific connections to each other and develop spontaneous activity. When connected to sensory organs that can also be developed *in vitro*, they will also elicit responses to environmental stimuli. Therefore, the resulting activity patterns share certain basic features with those exhibited by normally developed brains. And they could do this to a greater extent with the improvement of culture conditions. However, these activities will lack all those attributes that are considered a minimal prerequisite for the meaningful processing of sensory information. Thus, in the foreseeable future this rules out the possibility that brain organoids will develop functions that could enable sensations, feelings, intentions, or other attributes of consciousness.

It cannot be answered based on current knowledge of whether this could be different if one day larger and more complex brain organoids could be produced, for example through successful vascularisation.

The concern that brain organoids, as they are currently used and according to current knowledge, could develop a mental inner life—even if only rudimentary—is clearly unjustified on the basis of the preceding considerations. Nevertheless, such concerns are understandable from a layman's point of view. They are fuelled by the misleading popular scientific term "mini brain" as well as by the existing gaps in knowledge and disagreements between experts in explaining consciousness (see Chapter 4.2.1). It is therefore important to emphasise at this point that there is broad scientific consensus on the minimum biological conditions of consciousness and that brain organoids cannot meet these conditions at present or in the foreseeable future. Possible claims for protection can therefore not be justified on this basis.

131 National Academies of Sciences, Engineering, and Medicine & Committee on Ethical, Legal, and Regulatory Issues Associated with Neural Chimeras and Organoids (2021).

132 Brain organoids consist of approximately 1 millionth the number of cells of a fully developed human brain.

133 So far, they form only neurons and astrocytes.

Nevertheless, possible demands that might arise in the future still need to be considered.[134] This is particularly relevant, as the production and use of increasingly complex brain organoids is certainly in the interests of research. For example, there is pressure to support research into the possible detection of neurophysiological correlates of consciousness, i.e., the discovery of externally recognisable signs of consciousness.[135] However, this endeavour is currently proving to be of little use with regard to brain organoids. The diagnosis of the presence of a conscious state requires the analysis of behavioural performance and, ideally, reports from the organism in question regarding its internal state. As long as brain organoids represent self-contained systems that cannot communicate with the environment via sensory organs and effectors, such be haviour-based diagnostic methods are not available. While it is possible to determine electrophysiological correlates of conscious brain states, their validation requires behaviour-based clarification of the corresponding states of consciousness. And the converse conclusion that certain activity patterns of neural networks would indicate the presence of a conscious state is not valid. Should one day in the distant future, brain organoids exhibit activity cycles that follow a circadian rhythm, i.e., a 24-hour sequence, then it cannot be concluded that they fluctuate between stages of unconscious sleep and conscious wakefulness. It would only mean that the circadian oscillators present in all the cells are also effective in the cells of brain organoids. In the same way, it would not be possible to conclude that the reactions of cells in brain organoids to electrical and chemical stimuli correlate with sensations. Incidentally, this also applies to reactions of simple organisms that are not able to express their inner states in a way that can be interpreted as an indication of sensation. Mere reactions to stimuli, which also occur in nerve muscle preparations, are not indicators of the presence of sensations or even consciousness.

Nevertheless, it should be considered at least hypothetically what the ethical consequences would be if brain organoids were to have a mental inner life in the distant future. The views published on this so far diverge: while some draw a red line where the emergence of even minimal consciousness lies, which would prohibit any further research on such hypothetical entities, others take a very cautious but less restrictive position:[136] they argue for a possible existing duty to stop pain, but no fundamental ban on research. At this point, there are at least distance parallels to the ethical assessment of animal experiments. There too, it is a question of assessing the stress on organisms that are attributed sentience. The question of consciousness, on the other hand, is secondary according to current legal and ethical criteria. At most, assessments of a possible differentiation of conscious states are included in the assessment of reasonableness. This applies, for example, to the question of whether the organism under consideration has the capacity to feel fear and the pain of separation or be aware of its own finite nature.

A third group of discourse participants does not commit itself but calls for a thorough debate.[137] In this debate, a distinction will have to be made between different variants and degrees of consciousness—not unlike the debate on how to deal with the hypothetical consciousness of artificial intelligence (see Box 5).

134 Baertschi et al. (2020); Lavazza & Massimini (2018b); National Academies of Sciences, Engineering, and Medicine & Committee on Ethical, Legal, and Regulatory Issues Associated with Neural Chimeras and organoid (2021).

135 Lavazza & Massimini (2018b).

136 Restrictive in this sense Lavazza (2021b); less restrictive Koplin & Savulescu (2019).

137 Greely (2021); Lavazza & Massimini (2018b).

Box 5: Consciousness in the context of artificial intelligence

In the context of artificial intelligence (AI), philosophy and AI experts have intensively discussed for decades whether and under what conditions computers or robots can develop states of consciousness and what the ethical consequences of this would be. Since the turn of the millennium, researchers and engineers have also explicitly strived to give information technology systems consciousness—on the one hand, to better understand what consciousness actually is, but on the other hand, to raise the interaction of humans with such systems to a new level.[138]

As non-biological systems, computers and robots are categorically not entities for which we have so far granted moral claims for protection. This would change in certain circumstances if AI systems were to be attributed consciousness. In this respect, there are parallels with the corresponding debates on brain organoids. In both cases the issue is the characterisation, attribution, and evaluation of subjective states of perception in novel entities. For AI systems, the following four increasingly demanding criteria are discussed regarding the presence of differently understood consciousness:

(i) Sensors that allow a system to perceive information about its environment, for analysis and use—possibly by means of interactive control. This extraordinarily weak criterion is already met by temperature controllers with simple feedback loops and more so by a wide variety of prediction systems based on machine learning. Most authors refer in this case to intelligence instead of consciousness, and on this basis probably no one would ascribe intrinsic value and thus moral status to systems.

(ii) Behaviours of the system that are also otherwise typically attributed to consciousness. These include the communication of sensations but perhaps also the ability to distinguish between self and the external world, and between one's own present and past. Criticism of this behaviouristic criterion points, among other things, to the notorious gap between assumed and real subjective perception of a system with the "Chinese room" argument.[139]

(iii) Internal structures of the system, which correspond to or explain the capabilities in section (ii).[140] Here, theories of consciousness that consider only structural, and not genuinely biological, components in biological systems to be essential prerequisites allow for "optimism" regarding the possibility of AI consciousness. However, even the optimists would probably have to commit to the following criterion (iv), if they agree to the primacy of the mental for moral evaluation.

138 Franklin et al. (2012); Haikonen (2011); Takayama & Takeno (2017).

139 This refers to a thought experiment developed by John Searle (1984), p. 31 ff., in which a person in a closed room communicates with the outside world only via written notes. The person is asked questions in Chinese, which they answer with the help of instructions given in their mother tongue with written notes, which are also described in Chinese. Outside the room, the false impression is given that the person can gather the meaning of the questions asked in Chinese. Walach (2013). However, quite a few philosophers question the conclusiveness of this argument.

140 Krauss & Maier (2020); Reggia et al. (2016); Sloman & Chrisley (2003).

(iv) Phenomenal consciousness, i.e., subjective-qualitative perception where the nature and extent of corresponding states can still remain completely undetermined. Many authors believe, either provisionally or on the basis of fundamental considerations, that all digital systems do not possess this ability.[141] Others believe that certain structural components evoke phenomenal consciousness in artificial *as well* as biological systems.[142] For the attribution of a potential intrinsic value to artificial systems, this position alone seems plausible. In any case, it is to be expected that if artificial intelligence were to develop phenomenal consciousness, if at all, it is at some distant time in the future.

4.3 Legal claims for protection

German law is anthropocentric[143] and as a result makes a distinction between legal subjects and legal objects. The human being as a legal subject can be the bearer of rights and obligations. Legal objects, for example things, can be assigned to humans by the legal system, and rights can therefore relate to them.[144]

Animals are legal objects within the meaning of the German Civil Code (BGB). They are not things in the sense of Section 90a Clause 1 BGB, but according to Section 90a Clause 3 BGB, the provisions for things are applied to them accordingly, unless otherwise stipulated. In addition, they are protected by special laws—primarily the German Animal Protection Act (TierSchG).

Consequently, the legal classification of new entities also has to take place according to the dichotomy of legal subject/legal object. A dichotomy between humans and animals, for example in the case of chimeras, is not possible under the current law.[145] To state whether a living being is entitled to protective rights or it falls under animal protection, it is necessary to first make a classification of the being as a "human" or as an "animal". The German Constitution (GG) does not provide any indication of the criteria to be used for this.[146]

The classification of an entity to the category of legal object does not mean that this entity would not enjoy any legal protection. For example, Article 20a GG explicitly includes animals and the environment in a constitutional requirement for their protection, without at the same time granting them their own rights.[147] "There can be no 'bilateral legal relations' between humans and objects of nature, including animals, which would logically presuppose on both sides their own personality and thus their own legal subjectivity."[148]

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141 Haladjian & Montemayor (2015, 2016); Hildt (2019); Reggia (2013).

142 Sloman & Chrisley (2003).

143 Herdegen in: Maunz et al. (2020), Art. 1 para. 3 side note 6.

144 The legal of capacity of legal persons only represents a superficial break with this juxtaposition. The granting of legal capacity to an organisation, in which or with which natural persons exercise their fundamental freedom, also aims to strengthen their individual freedom. See Remmert in: Maunz et al. (2020), Art. 19 para. 3 GG side note. 37; it is also an expression of a personal element Goldhammer & Sieber (2018), p. 22 f.

145 German Ethics Council (2011), p. 34 f. In ethical terms the assessment may be different, see German Ethics Council (2011), p. 67 f.

146 German Ethics Council (2011), p. 35 f.

147 For more details (also on the dispute about the anthropocentric direction of Art. 20a German Constitution GG, Schulze-Fielitz in: Dreier (2015), Art. 20a German Constitution (GG) side note 29 ff., 56; Epiney in: Mangoldt et al. (2018), Art. 20a German Constitution (GG) side note 24 ff., 88.

148 Scholz in: Maunz et al. (2020), Art. 20a German Constitution (GG) side note 75.

In addition, it should be noted that the classification as a legal subject or a legal object as such does not yet include a conclusion on the scope of legal protection associated therewith. The German Animal Protection Act, for example, contains provisions of varying strictness for vertebrates (which are themselves included in the protection in a differentiated manner[149]) (inter alia Sections 4, 5, 6 German Animal Protection Act, TierSchG), warm-blooded animals (inter alia Section 4a TierSchG), and cold-blooded animals (inter alia Section 4b TierSchG). For humans, i.e., legal subjects, different protections, for example for born and unborn humans, are also permitted according to the law of the Federal Constitutional Court and are also contained in the applicable law.[150]

To answer the question of which possible claims for protection can be asserted for human brain organoids, the applicable claims for the protection of human embryos are outlined in the following: the legal situation is by no means clear with respect to the early stages of indisputably human life for the development of born humans. This will be followed by a discussion of possible claims for the protection of artificially created entities. Lastly, it will be explained to what extent those human beings whose cells are used for the production of brain organoids have a right to co-determination in their production.

4.3.1 Embryos *in vitro*

In the international literature, it is occasionally argued that well-developed brain organoids should be granted comparable protection to embryos. If the incipient development of the nervous system is regarded as decisive for the protection of embryos,[151] it follows from this point of view that well-developed brain organoids should be equated to embryos. As a result, the question is which entities like human embryos *in vitro* enjoy the protection of fundamental rights or at least protection under objective law. This question is hotly disputed:[152] the Federal Constitutional Court has so far only dealt with constitutionally required protection in connection with abortion, i.e., in relation to embryos and foetuses *in utero*, and in this respect has affirmed a certain duty of the state to protect the foetus.[153] The Court has emphasised very clearly for the developmental phase after nidation (implantation of the embryo in the uterus): "The legislature is in principle not obliged to take the same measures of a criminal law nature to protect unborn life, as it considers appropriate and necessary to safeguard born life".[154] And the Court expressly left open whether "human life already comes into being with the fusion of the egg and sperm cell".[155] Consequently, the literature rightly points out that the legislature has very broad regulatory discretion as to which entities outside the human body it considers worthy of protection and in what way.[156]

149 For example Section 4 para. 3 (TierSchG) on dogs, cats and primates.

150 See Dederer (2020b), p. 61.

151 See more Taupitz (2021); Taupitz (2022), p. 100 f.

152 Extensive evidence in Dederer (2020b), p. 62; Merkel (2002).

153 However, it is unclear whether the court considered the *nasciturus*, i.e., the embryo from the time of its implantation in the uterus (later foetus), to be protected in its capacity as a bearer of fundamental rights or only from the objective-legal content of the fundamental right norms. In the first pregnancy judgment, the court expressly left this open; Federal Constitutional Court (BVerfGE) 39, 1 (41 f.). In the second pregnancy judgment (BVerfGE 88, 203 ff.), the court did not take a clear position on this question, see Dederer (2020b), p. 54.

154 BVerfGE 39, 1 (45). More on the freedom of the legislator Dederer (2020b), p. 63 ff.

155 Federal Constitutional Court (BVerGE) 88, 202 ff. side note 151.

156 Dederer (2020b), p. 63 ff.

At the level of simple law, i.e., below the constitution, the German Embryo Protection Act (ESchG) protects entities like human embryos that have been created by fertilisation from the so-called nuclear fusion process (Section 8 para. 1 alt. 1 ESchG), i.e., from the dissolution of the membranes of the pronuclei. In addition, every totipotent cell removed from such an embryo is considered an embryo in the legal sense of the ESchG (Section 8 para. 1 alt. 2 ESchG),[157] i.e., every cell that is able to divide and develop into an individual if all other necessary conditions are met. It is being disputed whether entities that are not created by fertilisation and cells removed from them, such as entities after cell nuclear transfer, are also covered by the German Embryo Protection Act.[158] According to prevailing opinion, the criminal law nature of the ESchG rejects their protection, prohibiting an interpretation beyond the wording of the law to the detriment of a possible perpetrator.[159] The same applies to the prohibition of cloning pursuant to Section 6 ESchG.[160] According to prevailing opinion, it is also necessary that a holistic organism develop from the respective entity at least up to the expression of the so-called primitive streak.[161]

4.3.2 Embryoids

In light of the dispute about whether and in what way constitutional protection extends to human embryos *in vitro*, no clear statement can be made about entities that are different from human embryos created by fertilisation. Embryoids, which according to recent findings can be cultivated up to blastoids, i.e., artificially produced structures resembling blastocysts,[162] are in any case not explicitly addressed at the level of simple law, particularly the German Embryo Protection Act. According to prevailing opinion, they are not covered by the law because they are not formed via fertilisation and no holistic organism can develop from them up to the formation of the primitive streak (see above).[163]

4.3.3 Brain organoids

The living human being is a legal subject; the body of a human being is not a legal object—it is not capable of being owned. However, when separated from the body, a now independent (not totipotent) body substance is subject to the property law of the German Civil Code (BGB).[164] Ownership can exist therein. If "a new movable thing" has been produced "by processing or transforming one or more substances [original cell]", the researcher producing it acquires ownership according to Section 950 German Civil

157 "For the purposes of this Act 'embryo' means the fertilised viable human ovum from the time of nuclear fusion as well as any totipotent cell derived from an embryo which is capable of dividing and developing into an individual if other necessary conditions are met."

158 On dispute Taupitz in: Günther et al. (2014), C. II. Section 8 side note 49 ff.; Kersten (2004), p. 36 ff.; Gassner/Opper in: Opper et al. (2020), p. 255 ff., 272 f. See further the references in the following note.

159 Dederer (2020b), p. 56 f., with further evidence; Faltus (2021), p. 128; Müller-Terpitz (2017).

160 Faltus (2021), p. 128.

161 Taupitz (2021), p. 409; specifically on embryoids Faltus (2021), p. 128.

162 Liu et al. (2021); Yu et al. (2021); see also Faltus (2021), p. 125 ff.

163 See specially on embryoids Faltus (2021), p. 128.

164 The disputed question of how the emergence of ownership at the time of separation from the body can be substantiated does not need to be discussed here, see Schreiber (2019), p. 41 ff.

Code BGB (at this moment at the very latest[165]).[166] This also applies if the new thing is an organoid, i.e., a three-dimensional cell structure cultivated *in vitro* from stem cells, which resembles an organ in terms of cell types, structure, and function.[167]

Human brain organoids consist of human cells. The comments above regarding the cells of human origin and organoids therefore apply in general. Unlike embryos, brain organoids cannot develop into a whole organism[168] or even a human being,[169] thus similar protection afforded to embryos in the ESchG cannot be derived from current law and is not constitutionally required.[170] Such strong legal protection would not be provided because (i) of the comparatively different method of production, which avoids fertilisation; (ii) the creation occurs in a different context than fertilisation and not for the purpose of generating an offspring;[171] (iii) the generated entity has a completely different form;[172] and (iv) the entity does not have the abilities of a fully developed human brain to perform central integration, regulation, and coordination services for an organism.[173] In the foreseeable future, brain organoids will not be able to develop higher brain activities or even consciousness (see Chapter 2.1). Brain organoids *in vitro* can probably at best reproduce the function of individual centres. Even if this changes in the future, a human being cannot be reduced to individual characteristics such as pain perception or consciousness and a constitutional mandate for protection cannot therefore be linked to individual characteristics of this kind.[174] Overall, brain organoids should therefore not be ascribed comparable status to embryos *in vitro*. This also applies to very advanced brain organoids—as they are not legal subjects,[175] but legal objects.

4.3.4 Further entities: animals and chimeras

As already presented in the introduction, animals and their handling are covered in a differentiated manner by the German Animal Protection Act. However, there are no specific regulations for the transplantation of (human) brain organoids into living animals. Therefore, for the production and use of human-animal chimeras, various regulations are recommended to supplement the existing law. In particular, it is recommended that such research projects be evaluated by specialised, interdisciplinary ethics committees. [176] This also seems to be very sensible.

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165 Who (initially) acquires ownership of the bodily substances at the time of separation from the body is disputed, see Schreiber (2019), p. 42 ff.

166 Schreiber (2019), p. 42 ff.; Zech (2007), p. 99 ff.

167 Bartfeld et al. (2020).

168 Faltus (2021), p. 133.

169 See from the ethical discussion: Schicktanz (2020), p. 200; Koplin & Savulescu (2019), p. 762.

170 Taupitz (2021); Taupitz (2022); in this sense also Dederer (2020a), p. 43: "It is clear at this point [...], that brain organoids are not to be classified as humans."

171 See Taupitz (2001), p. 3440 on such aspects for the assessment of artificially produced entities: similarly, later German Ethics Council (2011), p. 100; further evidence on corresponding considerations in Anglo-Saxon literature with regard to the moral status of early embryos in Hostiuc et al.

172 On the importance of likeness for the recognition of an entity as a "human being" in the sense of the human dignity guarantee: Dederer (2020b), p. 74.

173 From the ethical debate: Baertschi et al. (2020), p. 14; Hyun et al. (2020), p. 5; Koplin & Savulescu (2019), p. 761.

174 Taupitz (2021); Taupitz (2022).

175 Whereby the classification as a legal subject is controversial, even with regard to embryos *in vitro*, see above 4.3.1.

176 Taupitz (2021); German Ethics Council (2011), p. 119 ff.; Taupitz & Weschka (2009), p. 435 ff., 455 ff.

4.3.5 The right of donors to determine the use of cell material

Persons whose cells are to be used for producing a new entity have a right to determine, or at least co-determine, the use of the cells. This is the case for human gametes used for artificial insemination and thus for the creation of a child. For this, the German Embryo Protection Act (ESchG) requires the explicit consent of the gamete donors (Section 4 ESchG).

However, the following applies to other body cells: if body substances are separated from (living) human bodies, the general right of personality of the original carrier continues, at least if genetic material is present in it.[177] There may then be two rights to the body material with different scopes. If the researcher has acquired ownership of it, this right enables them to exclude others from using and doing with it as seems fit, but only, as the law itself adds in Section 903 BGB to the "powers of the owner", "insofar as the rights of third parties are not in conflict".

Such a right is the general personality right of the donor of the body material. Whether this right is infringed by the use of the body material is determined on the basis of a comprehensive balancing of interests. Specifically, on the part of the researcher, the freedom of research comes into play in the present context; on the part of the donor, it is a question of how strongly they are affected by the use. For example, if the donor is identifiable and conclusions could be drawn about them from the body material, their right to informed self-determination is affected and consent is therefore required.

But also, in other cases where the donor is affected, especially in socially controversial or ethically disputed research cases, to which the donor may not wish to contribute their material, the donor must be adequately informed about the use and give their consent. Because of the special, not least anthropological, significance attributed to human brain cells, this also applies to the production of brain organoids.[178] It is necessary to outline the basic feature and goals of research with brain organoids, including the expected characteristics of these entities, before the donor of the primary cells can give their informed consent. As genetic information about the donors can be derived from the cells used, it is necessary to use appropriate data protection measures and, if possible, anonymisation or at least pseudonymisation and safe storage of the key.

177 Schröder & Taupitz (1991), p. 42 ff. For a detailed overview of the nuanced differences in opinion, see Schreiber (2019), p. 41 ff.

178 Taupitz (2021), p. 411; Taupitz (2022), p. 104 ff.

5 Conclusions

The research on and with brain organoids offers new possibilities for obtaining a better understanding of individual processes and functions of the human brain. In addition, this research allows us to address specific, application-oriented questions related to the emergence of early developmental disorders that can lead to neurological and psychiatric diseases, the mechanism of action of viral infections, or the onset of neurodegenerative processes. On this basis, it is expected that new therapeutic approaches could one day be made possible. As a simplified model system for developing human brain structures, brain organoids enable experimental access to investigations not possible with other model systems.

Nevertheless, brain organoids that can be created with current technologies do not represent the human brain as a whole, but only individual subfunctions, structures, and processes. For the foreseeable future, it is not expected that brain organoids will be able to develop a sensation of pain or other, be it only rudimentary, states of consciousness. However, brain organoid research represents a highly dynamic field with substantial progress in recent years and high expectations for future work.

With this in mind, the authors of this statement have come to the following conclusions:

1. Research on and with brain organoids *in vitro* does not require any additional specific regulation at the moment or in the near future. There are also sufficient regulations regarding the conditions in which human cells may be used to generate brain organoids.

2. Research *in vivo*, in which brain organoids from human neuronal cells are transplanted into animals, is regulated by the German Animal Protection Act. The ethics committees established by law should have expertise in the field of brain research for the evaluation of the research discussed here.

3. As this is a dynamic area of research, it is possible that the current limits of the functional potential of brain organoids could shift in the future. Such developmental possibilities and their ethical, legal, and social relevance must be (i) continuously and realistically assessed and (ii) regulated at an early stage if necessary. Regarding the first aspect, only the procedures of science-internal and science-public discussion can be held responsible. Regarding the second aspect, regulatory and supervisory activities may become necessary one day, and need to be assigned to corresponding bodies such as the Central Ethics Committee for Stem Cell Research (ZES).

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