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REVIEW ARTICLE

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The human brain and its neural stem cells postmortem: From dead brains to live therapy

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Abstract Contrary to the traditional dogma of being a relatively invariable and quiescent organ lacking the capability to regenerate, there is now widespread evidence that the human brain harbors multipotent neural stem cells, possibly throughout senescence. These cells can divide and give rise to neuroectodermal progeny in vivo and are now regarded as powerful prospective candidates for repairing or enhancing the functional capability of neural tissue in trauma or diseases associated with degeneration or malperfusion. Hopes primarily rest upon techniques to either recruit endogenous stem cells or to utilize exogenous donor-derived material for transplantation. In the search for suitable human cell sources, embryonic, fetal, and adult stem cells appear highly controversial, as they are accompanied by various still-unresolved moral and legal challenges. Fascinatingly, however, recent reports indicate the successful isolation and expansion of viable neural stem cells from the rodent and human brain within a considerable postmortem interval, suggesting that postmortem neural stem cells could potentially become an acceptable alternative cellular resource. This article will provide a brief overview about neural stem cells, their prominent features, and prospects for a cellular therapy, and will furthermore illuminate the cells in particular with respect to their newly discovered postmortem provenience, their advantage as a potential cell source, and several unfolding forensic considerations. Also, important ethical, social, and legal implications arising from this hitherto unpracticed cellular harvest of brain tissue from the deceased are outlined.

Keywords Neural stem cell · Human brain · Postmortem · Therapeutic applications · Ethical and legal requisites

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Introduction

In recent years, the role of neural stem cells from the central nervous system (CNS) of mammals including humans became more prominent. As biologically fascinating entities, neural stem cells have become milestones in theoretical and applied medical research and stand in the forefront of scientific efforts to utilize hitherto unthinkable cellular techniques in therapy. They are ascribed to the so-called tissue stem cells and their most commonly accepted defining features [73, 87]—(1) to remain in an undifferentiated state without a determined phenotype under certain conditions, (2) to be able to divide and proliferate, and (3) to be capable of de novo neurogenesis generating differentiated offspring such as neurons, astroglia, and oligodendroglia upon induction (multipotency)—make neural stem cells ideal candidates for many longed-for therapeutic concepts that aim at repairing or enhancing the functional attributes of neural tissue in trauma or disease.

There is widespread evidence that neural stem cells persist in the adult CNS and continue to account for de novo neurogenesis in the mature brain throughout the entire life span. They represent specialized types of competent cells residing in "neurogenic" or "silent" regions of the brain that can generate neurons spontaneously and following induction via local signals. Neurogenesis requires specific constellations of particular signaling cues to be properly administered to such cells

by their microenvironment in a spatiotemporally concerted fashion. These signals may not only activate bona fide stem cells to make new neurons, but may also stimulate pre-existing neural progenitors, i.e., cells that are committed into a specific neural cell line but have still retained the ability to divide and proliferate, to engender "stem cell-like" cells upon demand. It is thought that injury alone may suffice to enliven neurogenesis. However, the capacity of brain tissue for neurogenic response does exhibit a significant regional heterogeneity. Thus, in silent regions the injury-activated factors and resulting alterations in local signaling (endogenous) may require additionally applied growth factors or grafts of competent cells (exogenous) to effectively result in a stimulated neurogenesis. Notwithstanding the fact that its precise regulatory mechanisms still remain mostly unknown, it is now clear that the adult brain is indeed capable of adding or replacing neurons that can assume functional roles in the tissue.

Neurogenesis and neural stem cells in adult mammals including humans

Bona fide neural stem cells have been shown to reside in the adult rodent and primate brain where they are thought to be located in the spontaneously neurogenic hippocampus and the subventricular zone [3, 4, 38, 94, 101]; however, stem cell-like cells and progenitors have been identified in various other areas [10, 11, 93, 95, 102, 129, 133]. As nonhuman primates exhibit a particular phylogenetic proximity to humans with long life spans and elaborate cognitive abilities, the conjecture suggested itself that neurogenesis may also be of significance in the adult human brain. And indeed, the human CNS, a tissue that was previously believed to be incapable of originating new neurons, does harbor multipotent neural stem cells and progenitors in various areas and developmental stages. And as many of the neurogenic areas of rodent and lower primate brain seem to find their complement in humans, the organ brain has apparently managed to preserve crucial germinal tissue properties throughout its adult state during human phylogenesis. Thus, neurogenesis in the adult human brain has first been discovered to take place in periventricular subependymal, cortical, and neocortical areas [52, 104], and the hippocampus [31], which is thought to harbor multipotent neural stem cells [59]. Stem cells probably also reside in the cortex [6], (sub)ventricular zone [49], and olfactory organ [72, 92]. Besides candidates with multipotent developmental competence, cell lineages with phenotypical restrictions have been found and isolated from the adult human brain including biased oligodendrocyte and neuron-restricted progenitors from the ventricle-lining parenchyma [113] and the hippocampus [114]. Taken together, all present reports and data concomitantly support the existence of neural stem cells and restricted progenitor cells with neurogenic and gliogenic potential in the adult human CNS throughout senescence.

Prospects for a cellular therapy

The fate of neural stem cells can be directed to develop into phenotypes of diverse tissue-specific target cells in vitro and in vivo whereby the cells have revealed a fascinating capacity to transdifferentiate into cell lineages not normally found in the organ or tissue of residence, including transgermal conversions into cells of different blastodermic layers. This unanticipated plasticity of neural stem cells was surprising as the CNS has always been regarded as being the most imperturbable among adult tissues in terms of proliferation and de novo generation of cells. Equally interesting for practical purposes may be the observations that neuroectodermal progeny can perhaps also be generated from other tissue stem cells such as the hematopoietic system [30], skeletal muscle [112], bone marrow [81, 116], adipose tissue [115], or umbilical cord [82]. These astonishing findings immediately encourage notions to apply stem cells in therapy, but are equally reminiscent of how vague our current knowledge of the mechanisms underlying the developmental formation of neural stem cells and progenitors still is. For investigations, neural stem cells from the rodent and human CNS can be isolated, expanded in serum-free culture, and propagated into continuous stem cell lines using mitogenic growth factors such as EGF and/or FGF-2. The as yet undifferentiated cells can be further differentiated in vitro and induced to generate neuroectodermal progeny, for example, via epigenetic factors added to the medium [1, 16, 78]. Separated cell fractions of such a suspension may then have significant therapeutic value. It has been shown, for example, that the neuronal progeny of adult neural stem cells can functionally reintegrate into existing networks and influence its functions [17, 108, 125, 132], and it is assumed that these adult generated neurons can replace other neurons of the same class that have died [89]. But except for, perhaps, in the adult olfactory bulb, where newly recruited neurons may serve to balance apoptosis, regenerate the tissue, and thus conserve its functional discrimination capacity for life [18, 41], it seems unlikely that a neuronal replacement strategy via endogenous de novo neurogenesis in other adult brain regions such as the hippocampus has evolved in response to a normal wear and tear. Instead, it may have rather emerged to rejuvenate key brain circuits as they are necessary to maintain certain forms of learning and memory [51, 63, 75, 89, 90, 122]. Here, several reports suggest that newly adult generated neurons can enhance synaptic plasticity in the adult hippocampus [118, 124], which is thought to directly affect learning and memory [64].

Thus, therapies that could utilize the features of neural stem cells to proliferate, divide, and differentiate along with their capacity to reconstruct neuronal circuitry hold much promise for pathologies of the nervous system that are characterized by dysfunction or loss of neurons or glia cells. As (adult) neural stem cells can be genetically modified and manipulated [26, 33], the envisioned ex-

ploitation of their therapeutic potential now seems to be imaginable and leaves hope for the cell's subsequent functional integration and contribution to cell replacement and the repair or enhancement of tissue function. In addition to their functions as neuronal network enhancers, grafted cells could also be tailored to secrete neurochemically active substances such as growth factors, tissue hormones, neuromodulators, transmitters, antibodies, or even to remyelinate axons. An alternative approach to grafts of exogenous cells may be the recruitment of endogenous stem cells or progenitors [58, 103]. Various insults such as ischemia, stroke, or trauma can stimulate their proliferation in known neurogenic or nonneurogenic sites [20, 37, 43, 47, 98, 128, 139, 141]. Fascinatingly, the cells and/or their progeny may then also migrate to affected lesion sites and subsequently contribute to replenishing the defective tissue in situ [7, 34, 48, 56, 85].

Eligible neural stem cell types for clinical applications

The roadmap to translating neural stem cells into clinical settings, however, still remains to be a complex and challenging task that must be cautiously prepared and performed [69, 123]. Before a perennial transfer of neural stem cells into clinical settings can be carried out, other perhaps more principal problems relating to the provenience of the cellular material to be used will have to be addressed: the use of xenogenic cells from animals, for example, remains problematic in humans. Although the human brain appears to be an immunopriviledged site [13, 74] in which grafts may potentially survive longer than in other organs and tissues, enough is still not known about its long-term response as a host when accepting xenografts and xenotransplants. Even larger than the issues of histocompatibility appear to be the concerns about possibly transmittable xenozoonoses as they are thought to originate from persistent pathogens such as viruses [45, 100]. It is presently not fully understood, for example, if and how endogenous retroviruses or other unknown and potentially harmful classes constitute a health risk to humans. Furthermore, the still-discussed controversy about whether grafts from a nonhuman donor species from the animal kingdom into the human CNS will entail an alteration of a patient's personality [44, 88] is additionally conducive to the present difficulty of accurately assessing the general risk and degree of severity of a postxenograft impairment of the patient's quality of life. An advantage of neural stem cells gained from animals would certainly be the high number of usable harvest sources, the quantity of accessible material for transplantation, as well as their immediate availability. However, on the side of graft cells with a *human* origin, such as pluripotent human embryonic stem cells, difficulties do likewise prevail as research with them remains to be subject to considerable ethical controversy and numerous international statutory restrictions [5, 79], which will continue to exert delay on their therapeutic application, even in the long run. On the other hand, autologous sources of stem cells from the adult human brain can not easily be well established from CNS locations except for perhaps the olfactory neuroepithelium [60], because of the expected serious consequences of an operative invasion and the possible permanent damage to the donor. The inherent complexities of standardizing the

Table 1 Comparison of different stem cell types with respect to their potential application in CNS therapy

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Xenogenic SC	High number of usable harvest sources	Host immune-rejection
	High quantity of accessible material for transplantation	Xenozoonoses
	Immediate availability	Possible alteration of patient's personality (esp. if used in brain)
hESC	Pluripotency	Teratoma formation
	Therapeutic cloning	Ethically denounced
	Unlimited self-renewal in vitro	Subject to international statutory restrictions
hNSC, adult, autologous	Immune-compatibility	Isolation impractical from CNS without damage to donor
	Age dependency	
hUCB-SC	Not ethically stigmatized	Mesenchymal origin
	Easy and standardized acquisition	
hNSC, postmortem	Not ethically stigmatized	Short PMI desired
	Readily accessible	Significant education of donors and recipients
	Vast harvest source	Religious provisos
	Easy and standardized acquisition	

Listed are some of the most prominent assets and drawbacks. Clearly, however, none of the candidates have yet reached the final stage of clinical approval for humans, despite considerable success in research and development with them. For hNSC postmortem, time will tell what can be expected from them and how they will meet their demands

SC Stem cell, *hESC* human embryonic stem cell, *hNSC* human neural stem cell, *hUCB-SC* human umbilical cord blood stem cell, *PMI* postmortem interval

acquisition of neural stem cells, their treatment in vitro, and the methods of transplantation to be employed have therefore fuelled an ongoing quest for alternative cellular sources that are not ethically denounced, well accessible, and can readily be standardized. Yet, only a few sources of human stem cells may be in agreement with these requirements. New examples are human umbilical cord blood stem cells, which are recently being debated [36, 86], as well as neural stem cells/progenitors from the human brain after death. Representing a hitherto unheeded potential human stem cell source, these latter cells may likewise be consistent with the above demands and defy the aforementioned difficulties. They could thus spur additional hopes for new avenues in neural stem cell therapy. Table 1 summarizes the most important presently discussed stem cell types with respect to their advantages and disadvantages in application.

Neural stem cells/progenitors from the human brain postmortem

Fascinatingly, neural stem cells and progenitors can be extracted from diverse locations in the brain of deceased mammals after death. In the past few years, their isolation has been reported from the striatum, the forebrain subependymal zone, and the spinal chord of deceased rodents [61, 62, 138], as well as the postmortem hippocampus, SVZ [97], cortex [119], olfactory neuroepithelium [84, 109], and retina [54] of humans. Cells from brains of various ages and both genders have been successfully transferred into viable cultures with a postmortem delay of up to 140 h. They can express various cell markers that include nestin, vimentin, GFAP, DCX, Sox2, Ki-67, nucleostemin or CD133 [54, 119], which allow their well-defined identification. Interestingly, they can then be propagated as neurospheres or as adherent monolayers in vitro, clonally expanded [62, 119, 138], and induced to differentiate in multipotency yielding the known neuroectodermal lineages, whereby the cultured ratio of emerging neurons, astroglia, and oligodendrocytes has been observed to change with the donor age [97, 138]. The principal avenue to sustaining cells extracted from dead brain tissue in vitro may not seem overly novel, as other human postmortem derived cells have previously been shown to obey culturing well [80], but for neural stem cells from the brains of deceased rats it has unexpectedly been observed that they exhibit the same properties with respect to the formation of neurospheres, cellular proliferation, and differentiation as those from living animals, if the initiation of culture occurs within a certain postmortem interval, in the case of adult rats 48 h. Within that window, distinct differences in the density or distribution of nestin-positive postmortem stem cells could not be observed around the neurogenic lateral ventricle, suggesting that the stem cells in that area may have survived and retained their cellular properties within that time interval [138]. A storage condition of 4C, rather than room temperature, was also shown to dramatically prolong the neurogenic potential of postmortem stem cells [61]. For applications at a later time, human postmortem progenitors can be cryopreserved and recultured for up to 30 population doublings with only moderate losses in cell regeneration [97, 119].

With respect to a comparison of cultures from fetal, neonatal, postnatal, and adult neural stem cells at identical postmortem delays, differences in cellular response and dynamics can be observed, whereupon fetal viz. the "youngest" cells clearly show a higher proliferative capacity [97, 119, 138]. It was also reported that it took longer for cultures to reach their highest neurosphere density if they had been derived from older animals [138]. Findings in human cultures confirm these observations, suggesting that donor age is likely a determinant factor for the proliferative as well as the developmental potential of cultured postmortem progenitors [54, 97]. Electrophysiological measurements on postmortem cortical progenitors from in particular premature infants indicted to differentiate in vitro suggested that their progeny, although from dead tissue but apparently immature enough, can even give rise to functional neurons under specific differentiation conditions [119]. The responsibilities for these changes in the cellular dynamics with age are proposed to partly lie within the inherent differences in the neural stem cell's capacity for self-renewal, their length of telomeres and their ability to respond to growth factors [138]. All of the above results may imply that, although cultures have been established from cells with a postmortem delay of up to 140 h (rodents) as well as from human donor corpses with an age well beyond 90 years [61, 109], best culture viability and thus optimal requisites for therapeutic applications are likely to be achieved with brain tissue specimens as *young* as possible and postmortem intervals as *short* as possible. Here, all neonatal and postneonatal infant deaths represent a substantial reservoir that could become a valuable resource for therapeutic applications in the future. For practical purposes, novel forensic techniques are available to estimate the postmortem interval at the time of tissue harvest [117, 127].

The finding that tissue age and cell age and hence the age of the donor emerge as clearly a dominant quality parameter for cultivation, plasticity, and therapeutic success with postmortem neural stem cells, besides the health status of the donor, does not come unexpected. There is evidence of increasing replicative impairment in stem cells with progressing age yielding a decline in their pools, changes in normal function, and the occurrence of increased chances of differentiation or malignant transformations [99]. Including epigenetic modifications (reviewed in [9, 110]), cellular DNA is under ongoing bout by endogenous and exogenous genotoxic stress resulting in a transient and accumulated damage of its integrity. Under normal conditions, the stem cell as well as other cells counteract against these incidents. With age, however, that defense potency may decline [99]. Aging is then thought to have negative effects on maturation, regenerative potential, homing, and engraftment of various types of nonneural stem cells [25, 29, 67, 131], and, most importantly, to influence the behavior of neural stem cells and their

progeny in the aging hippocampus [107], the potentially most important cellular source of neural stem cells post-mortem. Full comprehension of the potential causal connection between age and genomic instability, however, is only emerging these days [14, 39], but additionally stimulated by the astonishing finding that embryonic stem cells seem to exhibit a remarkable resistance against genomic wobbling [57].

Neural stem cell survival in the dying brain?

The concept of culture viability after considerable time intervals following the bodily demise appears quite intriguing, even more so as processes of global ischemia are known to cause irrevocable pathological lesions such as necrosis, apoptosis, or severe inflammatory response in brain tissue already immediately after onset [8, 28, 71]. The reported data suggest, however, that neural stem cells and progenitors specifically possess a strong survival potential and a particular cellular resistance to ischemic and oxidative stress conditions in the tissue as compared to neurons. It has been shown that stem cells in particular are well adapted to proliferating in a low-O_2 environment. Decreased oxygen accounts for distinct trophic and proliferative effects in rodent central nervous precursors, neural crest stem cells, as well as in human hematopoietic stem cells in vitro [27, 83, 126]. Lowered oxygen cultures clearly favor cell proliferation and survival, and result in a significant increase in total cell numbers. Furthermore, they appear to be necessary to maintain full pluripotency in human embryonic stem cells [32]. In mouse hematopoietic stem cells, various subsets are differently affected by oxygen tension as well as differently selected in vitro by hypoxia [22]. This may lead to the conclusion that stem cells and progenitors respond differently to hypoxic conditions, which was recently confirmed for human stem cells [27], and that stem cells may exhibit a conserved response toward reduced levels of oxygen around them [83, 126]. Strikingly, also neurogenesis of adult neural stem cells and synaptic plasticity are significantly potentiated by global ischemic conditions in the brain [15, 121]. Yet, how precisely and to what extent neural stem cells manage to survive the quickly progressing conditions of irreversible oxygen deficiency in the postmortem brain is presently not fully understood. Several coexisting mechanisms may have relevance here: First of all, stem cells and progenitors, defined as being relatively quiescent and proliferating cells, are lacking a highly active and sophisticated biochemical apparatus as they are only awaiting molecular instructions from their microenvironment to route them into dedicated developmental fates such as division, migration, differentiation, or apoptosis. This relative dormancy and low metabolic rate may contribute to their survivability and defer the cell's demise during cessation of oxygen and nutrient input upon death of the brain. Then, neural stem cells from certain areas of the brain may also rely on anaerobic metabolism and exhibit a particular resistance to apoptotic cell death [111]. In cultured CNS stem cells, it was suggested that a reduced apoptosis could be reflecting upon the impact of lowered O_2 levels [126]. It has further been conjectured that stem cells in the brain can be surrounded by specific other cells that can provide a microenvironmental niche and can serve the stem cells as aides for their maintenance [68, 96, 105]. Here, the rich vasculature observed in neurogenic regions is thought to act as such a niche by providing the stem cells with easier access to the nutrients and other factors, and could thus help to prolong their survival [96, 140]. Furthermore, the effects of oxygen-dependent gene expression mechanisms may offer a contribution. It is known that hypoxic conditions can lead to changes in gene expression regulated via the so-called hypoxia-inducible transcription factors (HIF), which are usually not present in normoxic cells but emerge upon a lack of oxygen sensed by the cell itself [76, 134]. Further downstream, these factors can induce transcriptional pathways that can promote survival and proliferation and thus make it tempting to speculate about the existence of a pathway that specifically facilitates these in stem cells [83]. Support for this hypothesis comes from observations that the induced target genes are involved in energy metabolism, apoptosis, erythropoiesis, as well as angiogenesis [50, 106, 120, 134], and that specifically factors and receptors of the latter appear to play a critical role in the regulation of survival and self-renewal of stem cells. In human hematopoietic stem cells, for example, an increase in the secretion of the vascular endothelial growth factor (VEGF) was shown under hypoxia [27]. VEGF is believed to act on proliferation and survival in endothelial cells via molecular pathways such as ERK, MAPK, PKB, PKC, NOS, Akt, and FAK, as well as on an enhancement of the cell's responsiveness to angiogenic factors via upregulation of its downstream signaling receptors. In addition, VEGF has been shown to exert signaling and maintenance functions in central nervous system neurons [91] and neural stem cells under 24-h anoxia [77]. Interestingly, hypoxia-induced VEGF levels of the brain were also proposed to serve as an estimate basis for the postmortem interval [127]. The above findings that the balance between their survival, self-renewal, and differentiation may be tightly regulated by intrinsic molecular oxygen sensing mechanisms in various types of stem cells give reason to the belief that similar events also rescue neural stem cells in the dying brain whereby the locally emerging tissue anoxia induces a balance shift toward their survival and conservation of viability postmortem, at least for some time. This assumption can probably still be upheld although the in vitro oxygen reduction in most of the above reports comprised a decreasing range from ambient 21% to more "physiological" tissue conditions of somewhere between 1 and 5%, which only inaccurately resemble the true developing conditions of O_2 deficiency in the postmortem brain. And last, but not least, neural stem cells from the adult hippocampus and the SVZ of rats were recently shown to express hemoglobin [35], which leads to the hypothesis that neural stem cells in the brain may benefit from, if not specifically utilize, its known physiological role in oxygen transport and detoxification of

reactive oxygen species in their attempt to increase the oxygen bioavailability in the dying tissue. More detailed studies are expected in the near future to shed more light on the molecular behavior of neural stem cells and progenitors when struggling to survive the hostile conditions as they arise in the dying brain.

The above reports concordantly show that the human brain after death represents an interesting and valuable potential source of neural stem cells for intervals of up to 2 days after cessation of bodily life signs that could meet the above demands and eliminate the dependence on fetal or embryonic sources and thus avoid serious ethical issues. Until lead-off trials to introduce them into practice can be made, however, more insights into the details of their cellular behavior and specifically their survivability after death, proliferative potential, cell-lineage dynamics, potential to be directed toward neuronal phenotypes in a controlled manner, and response to grafting conditions are necessary. The latter must also include experience with the functional recovery of animals in various models of CNS injury and disease following the administration of the cells. More proficiency and knowledge is also required with respect to the assessment of a potential tissue donor's medical status. Although medical reports are usually on hand in hospital settings or pathology to reveal important information about the donor's clinical antecedent, existing drug therapies, or the cause of death, other variables—such as the donor's genetic and developmental background, and the nature and extent of possibly underlying but unknown diseases, in particular infectious or psychiatric processes at or near the time of death—can neither be excluded easily, if at all controlled. But they will likely exert a major influence on the condition and "quality" of the harvested cells and thus require further detailed investigation.

Forensic relevance of neural stem cells postmortem

With these still unresolved issues in mind, numerous questions with forensic relevance open up. It appears very probable, for example, that the tissue conditions evolving in the postmortem brain will exert influence on the biochemical machinery of its cells and thereby affect its molecular units—transcriptome, proteome, or physiome. Most recent data indicate that protein synthesis as well as posttranslational protein modifications can be extremely sensitive to the duration of the postmortem interval [19, 65, 66], which again may influence the intrinsic properties and behavior of the cells. Furthermore, a postmortem delay has been discovered to influence the outcome of brain cell-marker detection via ongoing catabolic processes [46]. An unswayed and reliable cell-marker detection, however, will become imperative for the forensic investigation of postmortem tissue damages existing prior to a cell harvest [53]. Yet, what consequences may these findings have for forensics aside from influencing cell-marker reliability? It may be speculated that the above clearly postmortem induced molecular changes in brain cells may also entail similar effects in neural stem cells including in those that still remain viable upon death at least for some time. Moreover, it is conceivable that these changes could vary specifically with the cause of death and perhaps even establish a coherent causal interrelation with the latter. The surviving stem cells may thereby "conserve" the tissue pathology developing during death, whereas other non-surviving cells are doomed to perish via autolysis over the course of time. At a later time point then, this molecular engram may possibly provide valuable insights into the cellular history and tissue processes as they occurred during the previous postmortem interval or during death. Certainly, though, long postmortem intervals will slowly wash out any existing incident-related cell feature specificity, and all cellular events will sooner or later terminate in the common final path of tissue decay, yet it appears fascinating that such specific cellular signature, if timely and carefully measured, could potentially reflect ongoing physiological, pharmacological, or pathological tissue events in conjunction with the bodily demise. These would probably include psychiatric processes at or near the time of death, agonal or emotional events surrounding the death [42], as much as preexisting diseases, trauma, or the mentioned nature of death (intoxication, suffocation, etc.). This again could shed more light on the molecular determinants of the cause of death and time of death, two prominent forensic issues of utmost importance for which there presently exists, to our knowledge, no comparable routine in forensic science. In addition, it would be ideal for laboratory purposes to devise normalized cell culture protocols as a methodological basis whereby the neural stem cell's pathology transfer from its time in the vital organ into the postmortem viability could be read out in culture and associated with novel forensic questions. Here, challenging problems may include, for example, whether duration and dynamics of agonal events as well as the onset of death could specifically be observed in culture and if so, what effects their different forms may have; how long stressors may have been in effect in the vital phase preceding death; how long molecular changes could be observed before being superposed by autolytic processes; or whether the antecedent of hypoxia in the brain such as during emerging suffocation could be reconstructed and derived from the observed mechanisms. In addition to this, potential postmortem neural stem cell culture assays for quality control may become essential for the cell's therapeutic applications in the future.

Ethical and legal implications and prerequisites for the use of neural stem cells from the human brain postmortem

The plans to utilize postmortem neural stem cells for research and therapy will also enforce the consideration and discussion of the ethical and legal conditions under

which the brain as a whole or the desired confined brain tissue areas can be removed from a donor. Several novel problems and questions occur here to the forensic scientist and pathologist that will require urgent clarification. Three of them shall be considered here.

First, as the human brain is widely and most comfortably agreed upon to provide the best organic representative of a domicile, if any, of human soul and spirit as well as the origin of human dignity, it may necessitate a reformulation of the ethical guidelines and legal provisions in society that must underlie its planned application in therapy, taking the existing diversity of religious and spiritual ideas into account. This may be of importance for potential donors as well as for the recipients of human brain-derived cells. For "traditional" donors of organs and tissues, only the donation and utilization of heart and eyes has sometimes been seen as problematic so far. They have otherwise not often denied their informed consent for giving organs such as kidneys, liver, lungs, pancreas, skin, or meningeal and cartilage tissues. The association of the brain with the distinguishing features of the human entity and cognition, however, may lead to a denial of consent for its donation. As it could be argued that the decision to donate brain tissue for transplantation may practically be influenced by the exertion of constitutional rights for religious freedom and practice, this has never been debated and dealt with profoundly in society in the past, making such reservations comprehensible. They will need to be addressed via novel discussion and information initiatives on a broad scale that must involve societal, political, and religious interest communities as well as include the offering of individual counseling. At this time, these debates appear to be the exclusive means to bridge between individual decision conflicts of donors or their families and the practical approaches toward utilizing the hitherto unknown therapeutic value of the postmortem brain.

Second, it is presently unclear in many countries to what extent brain tissue extraction for research and potential applications will require approval from the donors at all. Similar to any other organ donated for transplantation, the conservation of the complete organ to be removed from the cranium will undoubtedly demand the donor's voluntary disposition [40, 135, 136]. Whether the same also holds true for small and only narrowly circumscribed singular areas of parenchyma, such as the hippocampus, the olfactory bulb, or the subventricular zone, has not yet been satisfactorily resolved. German advocates of a liberal standpoint argue that tissue (and organ) extraction from the dead can be performed devoid of consent from the donors (in their lifetime) or their families (after their death) because a higher object of legal protection ("Rechtsgut") such as the reconstitution of organ function or maintenance of human life does principally prevail over a lower one, the personal right for inviolacy and integrity of the dead [130] (chapter 22, sect. 131, VIII, sect. 12, lines 1–8 and footnote 20, p 1152). Furthermore, the utilization of specifically marginal amounts of tissue material from a human corpse on behalf of progress in medicine may be socially adequate in a welfare state society, especially since the corpse is often subject to autopsy through a forensic scientist or pathologist anyway. In fact, it is part of the daily routine of such investigators (in Germany) to take tissue specimens and samples for further examination. But to our knowledge, acceptable normative magnitudes have not yet been established here. On the contrary, opponents counter that an informed consent is always a prerequisite for corpse probe sampling for any purpose as, unless required by law, the right for self-determination and the postmortem personal rights of the decedents and their families including the percept for inviolacy and integrity of the dead oppose the illegitimate "coercive organ extraction" and functionalization of the defunct as a freely available biological resource [130] (chapter 22, sect. 131, VIII, sect. 12, lines 9–17 and footnote 21, p 1152). And this may even conjoin more importance for the organ brain, as outlined above. Should one agree the brain tissue specimen in question and the research procedures applied to them to be in conformity with the definitions set forth in the World Medical Association Declaration of Helsinki and the Council of Europe's Convention on Human Rights and Biomedicine, then the obtainment of consent would be in accordance with their provisions [24, 137]. Specifically for Germany, the execution of such rights has been defended as being a justifiable and thus preferred method [70]; the same is true for the United Kingdom [12] and the United States [2, 21]. In practice, however, it must clearly be determined how the demanded attempts to request permission for tissue sampling from the deceased's families can obey good ethical standards, especially during times when the relatives can still be in bereavement [23, 55]. With that in mind, the relatively short postmortem interval for a workable brain tissue extraction further complicates the situation, especially for newborns and infants.

Third (and last), it is presently unclear (e.g. in Germany) if even approaching relatives to inquire about a permission itself is not legally problematic due to possibly occurring direct conflicts with existing data protection laws (Bundesdatenschutzgesetz BDSG, 2003, in Germany). In contrast to the pathologist, who may often be in contact with the family and relatives, the forensic investigator mostly receives his investigation orders from the state prosecuting attorney's office and may not necessarily have to do with the relatives at all. But even if he does, he may not legally be allowed to use or transmit contact data for the purpose of requesting information on issues that are neither authorized nor covered by his work assignment. The data are thus protected by the law, emphasize data protection activists. Proponents, on the other hand, argue that a clear-cut adjudication on whether data protection laws even come into play at this point is still pending. Their objections are that the data which the requesting forensic investigator will instrumentalize to establish contact certainly used to be personal data but is now, in the case of a deceased, not attributable to a living person any more. But exactly the living person is the foundation of data protection laws and the justifying subject under its protection. Dead persons are not considered persons any more and thus cannot be protected.

Yet, even under the existing (German) legal regulations, personal data may eventually become legally transmittable (for other purposes) insofar as the transmission and usage will be essential to ensure the legitimate interests of the responsible party (forensics) and does not interfere with the interests of the involved relatives worth protecting. Legitimate interests of the forensic scientist, even being those of the general public, could be, for example, the conduction of research and subsequent development of therapeutic applications from the harvested tissue specimen in question. In addition, transplantation laws (Transplantationsgesetz TPG, 1997 in Germany) and the code of criminal procedure (Strafprozessordnung StPO, 2001 in Germany) may allow the transmission of personal data and the contacting of the relatives in Germany, to wit, if it can be assumed that the deceased possesses transplantable tissues or organs that may be of relevance in medicine or for the purpose of research. At this point, also the differentiation between various types of interests such as commercial, research, or therapeutic kinds may become relevant. Interests of the involved families, however, include the basic right of informational self-determination, the protection against violation of reverence as part of a constitutional personal right, or the safeguard from infringement of the quietude of the dead. In legal disputes, these complex and opposing interest configurations will have to be assessed and weighed against one another. Presently, however, it appears that the statutory situation in various countries including members of the European Union (including Germany) or the United States are not yet fully adapted to these novel conditions and will therefore require urgent legislative handling and clarification.

All of the above outlined issues, to our knowledge, have not yet been profoundly addressed and resolved, especially not with respect to the organ brain after death, which will only now be seen as exhibiting a hitherto unknown therapeutic value. Considering the rapid pace of progress in worldwide biomedical science, a prompt pursuit is thus strongly advised.

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