CRYOPSERVATION OF THE BRAIN: A STEP TO IMMORTALITY OR A USELESS EXPERIMENT?

Medical sciences
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Introduction. The prospect of prolonged anabiosis is still viewed with skepticism by the cryobiological research community, as it is not yet possible to reversibly cryopreserve large organs such as the heart or brain, let alone the whole body [1]. Although reversible suspended animation is not currently an option, structural preservation of the body is a possible alternative with the aim of preserving the molecular components of the human body sufficiently intact for future recovery, as resuscitation procedures can be developed along with technologies for the treatment of acutely lethal conditions, including trauma, ischemic injury, and chronic diseases such as biological aging [2].

The aim of this work was to review works devoted to the development of a method of long-term, including postmortem, storage of the brain with the possibility of subsequent restoration of consciousness and psychological features.

Materials and methods: literary sources on the specified topic for the period 2020-2025.

Results and Discussion. It is widely accepted that a person's personal identity is maintained by their psychological properties that define their personal identity [3]. Continuous neural activity is not essential for maintaining the psychological properties of the brain, as evidenced by three lines of evidence. First, studies of rabbit hippocampal slices show that biological time can be suspended by cryopreservation without loss of correlates of long-term memory, suggesting that key aspects of cognitive function can be preserved despite the temporary cessation of molecular motion. [4] Second, during the surgical procedure of deep hypothermic circulatory

arrest, the electrical activity of the brain is temporarily stopped without significant effects on long-term memory or personality [5]. Finally, cases of hypothermic cardiac arrest, such as those in avalanche survivors, further demonstrate that prolonged periods without blood flow to the brain, while temporarily halting electrical activity, do not necessarily result in the loss of long-term memory or personality traits [6]. Information stored in stable brain structures is important because brain function can be temporarily halted and then restored [7]. Only structures associated with long-term memory and personality traits are likely to be preserved. These structures relate to both individual biomolecules and their spatially organized interactions, which determine morphological characteristics that can be studied using microscopic methods.

A growing body of evidence suggests that the only neural process that can trigger long-term memory retrieval is the rapid electrochemical flow of ions through the connectome, a complete map of brain cell connections [8]. While the connectome provides a morphological framework, it is highly likely that certain biomolecules, such as ion channels, ion pumps, and neurotransmitter receptors, also play critical roles in mediating memory retrieval and other cognitive functions. Thus, the extent to which the biomolecule-annotated connectome is preserved is the most meaningful metric for assessing the quality of a brain preservation procedure.

100 years of storage may be a reasonable starting point. Four categories of methods are currently being considered that could potentially preserve the brain for this length of time, each with its own advantages and disadvantages.

Cryopreservation without cryoprotectants (i.e., “unprotected” cryopreservation) is a widely available and easy-to-perform method, but it results in ice formation, which not only causes morphological damage but also significant conformational changes in biomolecules, including changes in their arrangement and disruption of the hydrated shell of proteins, as the mechanical impact of ice can rupture cell membranes, leading to leakage of intracellular contents [9, 10]. In cryonics, researchers typically perfuse cryoprotectants, which causes the brain to transition to a vitreous or vitrified state [11]. To avoid osmotic damage, cryoprotectants must be

administered gradually, following a multi-step procedure that involves precise adjustment of cooling and warming temperatures, as well as careful adjustment of cryoprotectant concentration gradients [12].

The use of fixation followed by cryopreservation combines two powerful preservation techniques that can be useful for maintaining structural stability over long periods of time if one of them fails. For example, perfusion with the chemical preservative glutaraldehyde and the blood-brain barrier modifier sodium dodecyl sulfate is followed by perfusion with the cryoprotectant ethylene glycol (ASC) [13]. In 2018, this technique was found to preserve intact porcine brains with acceptable levels of connectome preservation as assessed by electron microscopy. However, achieving the same level of complete connectome preservation in human brains using this approach has not yet been demonstrated. The mechanism by which aldehyde fixation reduces structural damage during cryopreservation with cryoprotectants likely involves: (a) stabilization of cell membranes to reduce damage caused by dehydration and osmotic processes; (b) strengthening the vascular structure, which improves perfusion by cryoprotectants; and/or (c) increasing the permeability of cells to cryoprotectants. From a biochemical point of view, glutaraldehyde fixation rapidly causes cross-linking of biomolecules, which allows for the stabilization of most cytoplasmic proteins in a matter of minutes. At the same time, such fixation is extremely toxic to cells, and given current viability criteria, their complete death is expected. The change in the distribution of small molecules is a common limitation of all methods of perfusion preservation of the brain.

In the polymer embedding method, fixation is performed and the brain is then processed for embedding in a material that can harden. [14]. These include traditional paraffin embedding, more specialized methods with epoxy or acrylic resins commonly used for electron microscopy, and embedding agents commonly used for plastination, such as silicone, epoxy, or polyester [15]. Although embedding in polymers can provide high-quality morphological preservation, this process typically requires lipid extraction and is technically challenging and prone to distortion when performed on human brain-sized specimens. Due to limited incubation times, some

resins require prior sectioning of the tissue into smaller pieces, which can cause damage at the edges of the sections. Alternative techniques, such as plastination, have shown potential for processing larger specimens, but brain tissue is typically sectioned into thin layers before embedding. The level of ultrastructural preservation achieved using plastination remains uncertain, especially for brain tissue, given the potential for damage caused by water crystallization during dehydration at temperatures below -25°C [16]. The main advantage of polymer embedding methods is their potential for long-term preservation without the need for ongoing maintenance. Given the significant variability in the level of ultrastructural and biomolecular preservation between different polymer methods, it is appropriate to initiate further study of this topic to determine the effectiveness of such approaches in practical applications. [17]

Regardless of the chosen method of preserving brain structure, two main approaches to its possible restoration are considered: methods based on molecular nanotechnology and the concept of full brain emulation. Regarding the first approach, the initial stage will most likely be high-precision molecular visualization and modeling, which will allow using computer analysis to reconstruct the probable initial biomolecular states and guide the restoration process. It is worth noting that the leading concepts of nanotechnological restoration after cryopreservation involve the possibility of removing aldehyde crosslinks - similar to other molecular damage that occurs during brain preservation [18]. To do this, future nanodevices should not only detect chemical bonds created by fixing agents, but also recognize the context of their formation in order to distinguish artificial bonds from natural ones formed in a living organism. After that, the crosslinks can be broken and the corresponding aldehydes can be removed.

Another widely discussed strategy is whole-brain emulation. In one version of this approach, brain tissue is pre-processed, segmented, and subjected to detailed molecular scanning [19]. Special software then reconstructs the brain’s original state prior to damage caused by cell death and preservation processes. The result is a possible “rebirth” of the individual, either as a robotic body in the real world or as a

digital avatar in an artificial environment. The main ethical and practical challenges of this approach are concerns about losing control over bodily autonomy, as well as the risk of being placed in an unwanted or even hostile virtual environment.

Conclusion: Despite significant technological advances in cryonics, there are currently no methods that guarantee the complete and reversible preservation of all brain functions, including consciousness and personality. Although experiments with vitrification, aldehyde stabilization, and polymer embedding demonstrate promising results at the morphological level, they are still far from practical application in clinical human preservation.

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