Rules for the Brain Preservation Technology Prize

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Prize purse as of June 12, 2010: *$100,000 US*

Brief overview and motivation behind the prize

The nonprofit Brain Preservation Foundation (BPF) hereby officially announces a cash prize for the first individual or team to rigorously demonstrate a surgical technique capable of inexpensively and completely preserving an entire human brain for long-term (>100 years) storage with such fidelity that the structure of every neuronal process and every synaptic connection remains intact and traceable using today's electron microscopic (EM) imaging techniques. Such perfect "ultrastructure" preservation has currently only been demonstrated on small pieces of brain tissue no larger than a cubic millimeter; however, existing research suggests that extending such preservation to an entire human brain is a readily achievable goal, albeit a technically challenging one. We at the BPF consider the development of a whole brain ultrastructure preservation procedure to be of the utmost importance and we believe that once it is developed and fully demonstrated (i.e. once the cash prize is awarded) that the technique should be made available as a widely affordable and accessible elective emergency surgical procedure in hospitals to all who desire it.

Why would a person want to preserve one's brain in a static form after death? Perhaps the most compelling near-term reason is to allow for brain donation to scientific research. Several institutions are currently developing the technology necessary to map the precise connectivity of relatively large volumes of brain tissue with the eventual goal of creating a connection-level map of an entire human brain. Such a map will radically expand our understanding of, and inquiry into brain function, in much the same way mapping of the human genome has for human biology. Development of a whole brain ultrastructure preservation procedure, as called for by this prize, is a *necessary precursor* to any such scientific endeavor. Better maps of our "human connectome" may also advance the field of artificial intelligence, by allowing us to create significantly more biologically-inspired computers, capable of more useful and complex tasks. Another, more personal, reason one may desire high-quality brain preservation is to leave behind *memories and experiences* for one's loved ones and for future generations. Neuroscientists are just beginning to reconstruct mental images and memories from electrophysiological and fMRI recorded brain activity (Kay, Naselaris, Prenger, & Gallant, 2008; Quiroga, Reddy, Kreiman, Koch, & Fried, 2005; Stanley, Li, & Dan, 1999) but future techniques are likely to be able to extract whole memories and experiences from the static connectivity of the brain alone.

The most exciting longer-term possibility that high-quality brain preservation potentially allows for is the possibility to "reverse" one's death using future technology. It is the overwhelming consensus view of the scientific community that the essence of who we are as individuals is directly written into the unique wiring of the hundred billion neurons in our brain. All of our conscious experiences, emotions, and thoughts arise as a result of computations performed by our brain's circuits. Further, we know that a person's brain activity can be completely halted (as occurs for up to an hour in the surgical procedure of Profound Hypothermia and Circulatory Arrest) and then restarted with no long-term loss of memory or identity (Sullivan, Sekhar, Duong, Mergner, & Alyano, 1999). These facts imply that a surgical procedure for preserving a whole human brain at the ultrastructure level should be viewed as a procedure for putting the patient into a state of "suspended animation" where they can wait unconscious and unchanging for decades until future advanced technology allows them to be brought back to life – either by molecular-level repair and reintegration of the preserved brain into a future biological or robotic body (Klein & Sethe, 2004), or by high-resolution scanning of the preserved brain followed by computer emulation and uploading into a robotic or virtual body (Sandberg & Bostrom, 2008). As science and technology continue their rapid advance, other restorative techniques we have not yet envisioned will surely also emerge.

In short, this is a prize for developing the first surgical procedure which can offer a validated, scientifically sound alternative to death. The surgical research team which eventually wins this prize will gain much more than the cash purse; they will be rightly revered for having made one of the greatest medical breakthroughs in history. They will bring hope to thousands of terminally ill and elderly patients worldwide and will bring relief and a new found sense of optimism and expectation of future progress to all those who currently labor under the knowledge of their biological mortality. We encourage all medical research teams having the necessary expertise to join in this competition, and we encourage all individuals and institutions which have monetary and other relevant resources to assist the efforts of those medical research teams which decide to compete.

Rules and Structure of the Prize

This prize competition is structured into two stages: Stage#1 - Preservation of an entire mouse brain (or similar small mammalian brain) using a technique that is applicable to a laboratory environment. Stage#2 - Preservation of a large mammalian brain (a pig for example) using a surgical technique meeting all the medical standards necessary for it to be applied (as an elective procedure) to a human patient in a hospital setting, and using a procedure that, with minor modifications, might potentially be offered for less than US$20,000 by appropriately trained medical professionals. The first group to complete stage#1's requirements will win 1⁄4 of the total prize purse accumulated up to that date. The first group to complete stage#2's requirements will win the remaining prize purse, or the entire prize purse if no one has previously met stage#1's requirements. A third stage of the competition (having a separate prize purse) is anticipated to promote the legal, accessible, and relatively inexpensive medical application of a human brain preservation procedure in particular countries around the world, and may involve a prize for the first surgical team to perform a successful and legal procedure in a particular country. This current rules document does not cover this additional stage.

The quality of neuronal circuit preservation in the animal brains will be judged by a statistically rigorous but sparse survey of the entire brain at the electron microscope level and the resulting images will be compared against "gold standard" electron micrographs of brain tissue in the existing scientific literature. The preserved brain block will be horizontally sectioned at 1mm intervals from the top of the cortex to the base of the brain stem, and each resulting block face will be electron imaged to verify the quality of ultrastructure preservation throughout (thus 5-10 block faces would be imaged to cover a full mouse brain and 30-50 would be imaged to cover a pig brain). The technique of hot knife microtomy (McGee-Russell, De Bruijn, & Gosztonyi, 1990) is one way this thick section removal could be accomplished which would in addition allow for the intact retrieval of small sub blocks to be used for later 3D volume electron imaging (see below). As each 1mm section is removed, the resulting block surface will be polished with a diamond knife and put into a scanning electron microscope for block face imaging (Denk & Horstmann, 2004). Each block face will be scanned in its entirety at a resolution sufficient to detect gross anatomical damage (e.g. cracks, voids, etc.) then a sufficient number of high-resolution (5nm pixel size) micrographs will be taken to provide a convincing case that the entire surface shows good ultrastructure preservation.

Electron microscopy of small volumes of brain tissue has been performed for decades in this and other manners and there is consensus in the community about what constitutes "well preserved ultrastructure". As a general rule, cell membranes should be intact and clearly visible, cellular components and organelles should be present in their typical locations and amounts, and synapses should be intact and contain clearly visible vesicles and synaptic densities. Please consult the following reference texts for more information on this definition of "well preserved ultrastructure" (Hayat, 2000; Peters, Palay, & Webster, 1991) along with additional images on the www.brainpreservation.org website. Each horizontal block face brain slice (at 1mm intervals) must pass this test to verify that ultrastructure has been preserved across the entire brain.

During horizontal sectioning of the preserved brain block, at least three sub blocks will be removed from various points in the brain for 3D volume electron imaging. This 3D volume imaging should have a lateral resolution of better than 10nm and can be performed using either traditional ultrathin serial sectioning for transmission electron microscopy (TEM) (Harris et al., 2006), tape-collected ultrathin serial sectioning for scanning electron microscopy (SEM) (Hayworth, 2008), Serial Block Face SEM (Denk & Horstmann, 2004), Focused Ion Beam SEM (Knott, Marchman, Wall, & Lich, 2008), or some equivalent technique that can trace the exact synaptic connectivity of brain tissue. Each imaged volume will be at least 10x10x10microns in size and must demonstrate that all processes and synaptic connections can in principle be traced throughout the volume. This dual requirement of a full brain survey at 1mm increments and selected volume reconstructions of traced neural connectivity is designed to provide irrefutable proof that the technique does indeed preserve the precise connectivity of the entire brain.

To win the prize, a team must demonstrate this level of preservation on at least two brains. For the first brain the surgical procedure and imaging can be performed without direct oversight by the BPF. The resulting images will then be sent to the BPF for evaluation. If these are found acceptable the team will be invited to preserve a second brain by the same protocol while a BPF representative provides oversight and documentation of the process. The resulting intact brain block will then be taken by the BPF representative and will be transferred to independent imaging facilities for evaluation by BPF. The BPF has been founded by an expert in large volume electron microscopic imaging of neural tissue and, as such, the BPF will make every effort to assist in the logistics (and costs if possible) associated with imaging the small and large mammalian brains. The BPF also pledges to be equitable in providing this technical and financial imaging assistance to competing teams. However, due to the cost and time involved in providing this assistance the BPF cannot be obligated to do so - the final burden of imaging costs and techniques lies with the competing teams.

In addition to this basic demonstration of ultrastructure preservation and traceability, the team must also make a convincing case that there are relatively inexpensive storage conditions under which the brain block would remain suitable for such imaging for >100 years. This may involve separate experiments in which a piece of the preserved brain is subjected to an accelerated aging environment (e.g. mild vibration and temperature swings). They must also make the case that the preservation technique is reasonably repeatable – for stage #2 (pig brain, hospital-like setting) more than one failed attempt between the first brain and the second brain will nullify the first brain's passing score and thus require a redemonstration from scratch.

Finally, to win the prize the team must successfully publish a complete description of the technique and imaging results in an open peer-reviewed scientific journal. Any additional micrographs that are not included in the resulting publication must be made openly available by the team for public scrutiny.

The above rules cover both stage#1 (mouse brain, laboratory setting) and stage#2 (pig brain, hospital-like setting). For stage#2 there are additional requirements for winning. For stage #2 the surgical

protocol demonstrated to the BPF representative must be virtually identical to what would be expected or reasonable in a hospital setting for a human patient who has elected to undergo this procedure either prior to or shortly after declaration of legal death. The surgical procedure should include non-destructive tests for quality which can be applied during the surgical procedure (e.g. the ability to detect if a large part of the brain's vasculature is blocked or has ruptured) and should allow for potential workarounds if such a problem situation occurs (e.g. an open skull surgical intervention to repair the vascular damage). To restate, one should be able (in anticipation of this procedure's eventual application on human patients) to determine with reasonable certainty if a particular preservation procedure was a success *without* the need for destructive testing, even though destructive testing (as described above) will be used for final determination of success for awarding the prize.

Again, as this preservation procedure is meant to eventually become a widely accessible medical procedure, a case must be made that it could eventually be implemented inexpensively enough that it might become broadly adopted by all who might choose it. Finally, all procedures must conform to strict safety regulations and must be absolutely safe for the personnel involved. This is not a minor point since any brain preservation procedure will likely involve the pressure perfusion of large quantities of highly toxic chemicals, even the vapors of which could cause severe damage to unprotected surgical staff.

These rules for winning the prize (both stage#1 and stage#2) are meant as guidelines. The final decision rests with the BPF and we reserve the right to modify these rules to fit unforeseen circumstances. For example, if it proves impossible to perform the 1mm sectioning and block face imaging on a brain that was preserved in a particular manner, we will work with the team to design another imaging protocol which can provide absolute proof that ultrastructure has been preserved across the entire brain.

Additional rules regarding contestant registration, time limits, purse payout, etc.

Contestant groups (and individuals) are required to file an official registration form with the BPF at least six months prior to sending the BPF brain slice images for prize consideration. The BPF may require additional legal documents to be signed by contestants as part of registering for the prize. Registration with the BPF is important as it ensures time for a full discussion with BPF representatives of the requirements for winning the prize and allows us to work with individual teams to overcome any potential roadblocks to a proper and complete evaluation of their attempts. Registration also allows for contestant groups to be listed on the BPF website, which will potentially assist the groups in obtaining additional funding for their research and which may assist the BPF in general promotion of the Brain Preservation Technology Prize in the media. Alternatively, registering teams may wish to remain anonymous until and unless they win the prize. The BPF reserves the right to eliminate contestants for violation of rules, safety, inappropriate media statements pertaining to the prize, clear inability to win the prize, etc.

Further, the prize purse will be paid out only to the individual or group that is designated in the official registration form, and any attempt at winning the prize will be assigned to an existing registration account. In this way, the BPF can ensure ahead of any potentially-winning demonstration who precisely the prize will be paid to if the attempt is successful. Of course, it is the responsibility of the contestants themselves (not the BPF) to decide on how to disburse the prize winnings among the various entities that assisted in the successful demonstration (e.g. the institution the research was performed at, the investors who funded the research, the individual scientists who performed the research, etc.)

Currently the majority of the purse of the Brain Preservation Technology Prize is funded by a wealthy individual who is contractually obligated to pay the prize purse, in a single lump sum within 30 days, to the individual or group designated by the BPF when the above criteria for winning the prize are met as determined by the BPF. The BPF will make every effort to ensure that this contract is fulfilled and that payment to the winner happens in a timely manner.

We at the BPF anticipate that this prize (stage#1 and stage#2) will be won in five years or less. If the prize is not won within 10 years of the first announcement (by June 12, 2020) then the prize will be void unless the BPF and purse donors agree to extend the prize offering beyond this date. The BPF reserves the right to revoke the prize offering at any time if a non-contestant clearly accomplishes the goals outlined above for whole brain ultrastructure preservation demonstration. The BPF also reserves the right to revoke the prize if it is deemed to not be advancing research toward the goals outlined above – in which case the BPF will post on its website a notification that the prize offering will be terminated within three (3) months if no registered contestant group objects. The BPF also reserves the right to significantly modify the structure and rules of this prize in the advent that an additional donor(s) requires it to do so as a prerequisite for increasing the total prize purse available.

Additional Background Information

The above sections cover the official rules of the prize. Remaining sections below are meant only as background information and to provide clarifying examples.

What will the winning technique likely look like?

The above criteria for winning the Brain Preservation Technology Prize have been written to be relatively neutral with respect to different preservation technologies – what is important is that the technique be capable of demonstrating (via today's electron microscopic imaging techniques) quality ultrastructure preservation across an entire brain, be capable of long-term (>100years) storage, and be reliable enough to be applied to humans in a hospital environment with a high chance of success. Having said that, it is important to layout here as clear a picture as we can what the winning technology is *likely* to look like. This is to help make the prize structure concrete and to help focus attention on the types of technological research most needed.

The two general classes of preservation technology which exist today are cryonic preservation (perfusion of an individual's vascular system with cryoprotective chemicals followed by long-term storage at near liquid nitrogen temperature) and chemical preservation (perfusion of an individual's vascular system with chemical fixative agents, e.g. glutaraldehyde and osmium tetroxide, followed by plastic resin infiltration and curing for long-term storage at room temperature). Both techniques have already been successfully demonstrated on small pieces of brain tissue, but to date neither has demonstrated ultrastructure preservation across an entire brain. (See www.brainpreservation.org for an accessible technical overview of the existing state-of-the-art in cryonic and chemical brain preservation techniques.)

There are potential advantages and disadvantages to both of these techniques. Cryonic preservation has the potential to be reversible using simple rewarming, thus it makes fewer assumptions about the future technological progress necessary to revive a preserved brain. In practice however, cryonic preservation is not reversible today nor is it likely to be in the next few decades. Further, it is difficult in practice to prevent local ice formation (which destroys ultrastructure) during cryonic preservation; however, much progress has been made in recent years toward developing cryoprotectants and protocols which can vitrify (i.e. solidify without ice formation) brain tissue (Pichugin, Fahy, & Morin, 2006). An additional problem with cryonic preservation is that tissue cannot be directly imaged in an electron microscope to verify quality of ultrastructure preservation. In fact, to image the tissue it must typically be rewarmed and put through a chemical fixation and plastic embedding process to allow ultrathin sectioning and imaging in the electron microscope. Additional problems with cryonic preservation are crack formation (due to thermal stresses) and long-term storage issues (due to the ever-present possibility of thermal variations over decades of storage).

Unlike cryonics, chemical fixation and plastic embedding (i.e. chemopreservation) has to date escaped public attention as a possible means of providing suspended animation to reach future medical technology (although this idea was clearly put forward in the scientific press as early as 1988 (Olson, 1988)). Nevertheless, neuroscientists engaged in brain mapping projects around the world are today using, improving, and verifying chemopreservation technologies for small pieces of neural tissue. Furthermore, chemical fixation and plastic embedding is the "gold standard" laboratory technique used for virtually all studies of neuronal circuits today. The standard procedure for preparing animal brain tissue for electron microscopic imaging starts with perfusing the animal's heart with a solution of glutaraldehyde – a toxic chemical which "crosslinks" all of the proteins in every cell fixing them in place and preventing their reactivity which would otherwise lead to decay. Other deadly chemicals (e.g. osmium tetroxide) are used to fix lipid molecules in place, then all the water is removed from the tissue and replaced with a plastic resin which can be cured into a solid block. The result is a hard plastic block containing brain tissue in which all the water has been removed from every nook and cranny of intra and extracellular space and has been replaced with hardened plastic. The structure of the neural circuits is perfectly preserved in this plastic matrix creating, in essence, a perfect fossil which preserves every synaptic connection in great detail.

If such a chemical preservation and plastic embedding technique could be applied to a whole mammalian brain, and conceivably be delivered to human patients at a cost equal to or less than US$20,000 by a surgical team, it would satisfy the requirements of the Brain Preservation Technology Prize. Unfortunately to date this technique has only been successfully applied to much smaller volumes of tissue. The primary technical challenge is that it is difficult for fixation chemicals to diffuse long distances through neural tissue. The solution seems likely to lie in developing protocols for perfusion of the vascular system which can evenly and rapidly distribute fixatives throughout the entire capillary network of the brain.

Because it is inherently more difficult to successfully distribute cryoprotectant chemicals to all parts of a brain while simultaneously lowering its temperature to prevent ischemic damage, we believe that a chemical preservation technique will most likely be the winner of the prize. (Nevertheless, we welcome teams to use a variety of low temperature and cryonics protocols if those seem best suited to achieving success.) A likely winning technique may therefore look like this:

1.) Open heart surgery is performed on the animal and tubes are inserted into the major vessels of the heart to allow direct perfusion and active withdrawal of chemical fixatives via a device similar to a cardiopulmonary bypass machine.

2.) Anticoagulant and vasodilation medication is administered to the animal to prevent blockages of the brain's vascular network.

3.) Glutaraldehyde is rapidly perfused into the vascular system such that it quickly reaches all parts of the brain stopping decay processes and rigidifying the vascular network in an open state. Steps after this are less time critical and the animal may be transferred to a more specialized facility to perform

all following steps. (In the eventual medical application of the technique, only this “emergency glutaraldehyde perfusion” would be performed in a hospital setting.)

4.) Further perfusions of buffer solutions, osmium tetroxide (to fix lipids), uranyl acetate etc. are used to further fix the brain tissue and to provide the contrast necessary to allow for eventual imaging of ultrastructure in the electron microscope.

5.) Small holes are drilled in the skull to allow access for tubes to be inserted into the ventricular and subdural spaces of the brain. These tubes are hooked up to another machine capable of providing direct perfusion and active withdrawal to the brain’s ventricular spaces.

6.) A series of increasingly concentrated ethanol solutions is perfused simultaneously into the vascular system and the ventricular spaces. This removes all water from the brain tissue.

7.) A series of increasingly concentrated organic solvent (in ethanol) solutions is perfused simultaneously into the vascular system and the ventricular spaces. This removes all ethanol from the tissue.

8.) A series of increasingly concentrated plastic resin (dissolved in organic solvent) solutions is perfused simultaneously into the vascular system and the ventricular spaces. With increasing concentration this mixture becomes too viscous to be further perfused through the vascular system but it is continued via the ventricular circuit until all brain tissue has been infiltrated with resin.

9.) The animal is then placed in a curing oven until the plastic resin is fully solidified. For the purposes of prize verification (a step unnecessary in human brain preservation), skull and dura mater is then removed to reveal an osmium blackened brain encased in solid plastic.

10.) The plastic-embedded brain is then reembedded in a block of plastic (roughly 1 cm cubed for a mouse, 5 cm cubed for a pig) and this “brain block” is then mounted on a special sectioning device which uses a heated knife to remove horizontal sections of chemopreserved brain at 1mm intervals, from the top of the cortex to the bottom of the brain stem (roughly 5-10 sections for a mouse brain, 30-50 sections for a pig brain). After each heated section is removed, a diamond knife is used to polish the surface of the brain block so that it is perfectly smooth to allow for SEM block face imaging (Denk & Horstmann, 2004). The brain block is then mounted inside the chamber of an SEM and the freshly polished face is imaged in total at low resolution and select images are taken at high resolution (5nm pixel size). This process is continued until the entire brain has been SEM imaged at 1mm intervals. The resulting images are evaluated to verify that all regions have well preserved ultrastructure.

11.) In the process of 1mm sectioning, at least three small samples are removed from three different thick sections that were prepared by the heated knife procedure (McGee-Russell et al., 1990) described above. These are chosen to include important brain regions such as cortex, thalamus, etc. These samples are then further sectioned or ion ablated in much finer increments (tens of nanometers),

and volume imaged using, for example, the FIBSEM technique (Knott et al., 2008). The resulting volume images are evaluated to verify that all neuronal processes and synaptic connections are traceable.

Concluding remarks:

The idea of placing a terminally ill patient into a state of suspended animation so that she can reach the future medical technology necessary to revive and cure her has been around since at least the early 1960's. This idea, because it is in principle scientifically sound, initially attracted much interest from both scientists and laypersons and gave rise to the practice of low-temperature (cryonic) preservation. However, even the best cryonic techniques of the 1960's produced gross, extensive damage to brain tissue as seen by light and electron microscopic examination. All reasonable scientists looking at this pervasive damage to neural connectivity rightly concluded that cryonic suspension using existing techniques was insufficient to the task. People selling cryonic suspensions to individuals were labeled as naive, or worse, quacks and charlatans, and the vast majority of scientists, especially cryobiologists, quickly distanced themselves from the enterprise. With the advent of vitrification (Fahy et al., 2004), cryonics has made significant technical advances in recent decades, but preservation of neural ultrastructure with the level of rigor described in this prize remains undemonstrated. Today, almost everyone has heard of cryonics but many assume the entire concept is fundamentally flawed, and almost all assume that no significantly simpler, less expensive, and more verified preservation options (like chemopreservation) are on the horizon. Cryonics as a concept is not flawed, but it will require further research and technology development to assess it for the circuit-level preservation it has so far not demonstrated. The same is true for whole brain chemopreservation (fixation and plastic embedding). This prize is meant to help marshal the resources and spur the interest necessary to bring about this scientific reevaluation of these (and perhaps other) brain preservation technologies.

The goal of whole brain ultrastructure preservation set by this prize is simultaneously achievable (existing literature suggests how it might be performed) and meaningful (existing neuroscience and cognitive science models suggest brain ultrastructure defines a person's unique memories and identity and thus is potentially sufficient to allow future technologies to bring the preserved person back to life). Winning the prize should be seen as the beginning of a new science of long-term medical preservation, one that continually researches how to achieve the best and most reliable preservation and which dovetails into the research necessary to revive preserved persons.

In summary, our technological ability and our knowledge of how the brain works have advanced almost immeasurably over the last 50 years. It is now high time to make a fresh scientific reevaluation of the current state of brain preservation technology. The Brain Preservation Technology Prize sets a target goal of demonstrating inexpensive and reliable whole brain ultrastructure preservation, a goal we believe is imminently achievable. It is our sincerest hope that innovation leaders in the scientific and medical communities will embrace the challenge laid out here with an open mind and engage it with the vision, care, and earnestness that it is due.

NOTE: Please see the document "Background Information Pertaining to the Brain Preservation Technology Prize" (www.brainpreservation.org) for a discussion of the motivation behind the prize, a brief history of the idea of brain preservation as a medical procedure, an accessible technical overview of the existing state-of-the-art in cryonic and chemical brain preservation techniques, and an overview of the electron microscopic imaging techniques that will be employed to evaluate the quality of whole brain preservation attempts.

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