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CRYONICS INSTITUTE NEWSLETTER

Cryonics insights and information for members and friends of the Cryonics Institute

cryonics.org · info@cryonics.org · 1 (866) 288-2796

CI PRESIDENT'S REPORT

Hello everyone and greetings.

I'm pleased to report our 2023 Annual General Meeting was another success, including some specal guests visiting us all the way from Mexico where they plan to start up a new Cryonics Support Group. Keynote speakers included Chuck Bartl discussing his Minnesota Cryonics Rapid Response Group, Nikki Olsen covering Standby Apps and Aschwin DeWolf who spoke as a guest of the Immortalst Society meeting directy following CI's meeting. Photos from the AGM are in this issue and for those who would like to view the entire presentation you can watch it online **here**.

Speaking of standby apps, please take a moment to consider installing the CI Check-In app on your Android device for an extra layer of security in the event of a cryonics emergency. Check-In is also useful for anyone who lives alone or spends a lot of time alone in remote locations like hikers, campers or hunters

The core code has been updated to better comply with modern standards and hopefuly we will have an updated version 2.0 available sometime next year with further improvements and features. Kudos to Nikki Olsen for contributing her considerable professional experience to this project.

The CI Check-In App is free and can be downloaded **here**. .

The membership has spoken and cast their votes in the Special Election for Directorship which ended Nov 27. This election was conducted to officially fill the two-year remainder of Jim Broughton's unfinished term.

Candidacy was open to all eligible CI members, and two candidates ran for the positon, including Interim Director Dr. Don Kleinsek and CI Member Nicholas Lacombe.

The results are as follows:

Dr Don Kleinsek 116

Nicholas Lacombe 35

I would like to congratulate Dr Kleinsek on his win and sincerely thank both candidates for putting forth their candidacies. I would also remind aspiring candidates for future elections that there are many more ways to serve CI than serving on the board as a director. The work involved in making CI what it is and in keeping it going steady and strong is never-ending and we are always looking for talented people with time and donations to help advance our mission. Again, thank you both Don and Nicholas for your efforts and continued willingness to contribute to make CI better for all of us.

I have also been busy getting the word out about cryonics and about CI, having conducted a number of interviews over the past few months with different news and media outlets from around the world, from smaller blogs to national publications. It's very rewarding to see interest growing in what we do and I am grateful and honered to be able to serve as your spokesman and President.

In closing I would ask that those of you who are CI members to please take a moment to review your suspension and proof-of-funding funding documents, consider your standby arrangements and do everything you can to ensure a successful suspension when the time comes. And if you have questions, don't hesitate to contact us - we're here to help.

Sincerely

Dennis Kowalski
President - Cryonics Institute

CRYONICS INSTITUTE MAGAZINE

The digital newsletter of the Cryonics Institute
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ARTICLE SUBMISSIONS

Cryonics Institute or cryonics-related articles are welcome. Submissions: **dg@cryonics.org**

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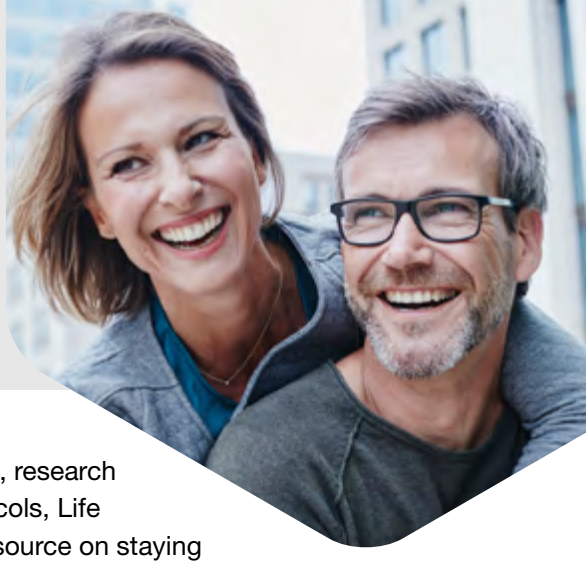

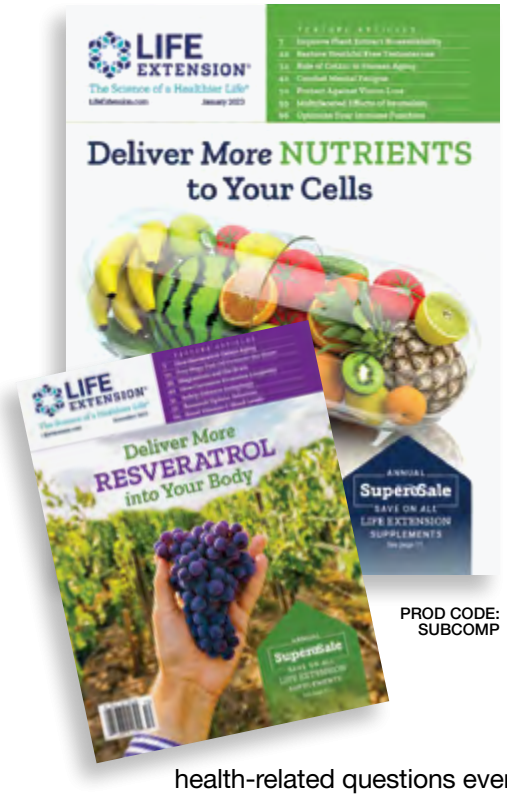

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Membership Benefits

Why join the Cryonics Institute?

The choice is clear: Irreversible physical death, dissolution and decay, or the possibility of a vibrant and joyful renewed life. Don't you want that chance for yourself, your spouse, parents and children?

1) A Second Chance at Life

Membership qualifies you to arrange and fund a vitrification (anti-crystallization) perfusion and cooling upon legal death, followed by long-term storage in liquid nitrogen. Instead of certain death, you and your loved ones could have a chance at rejuvenated, healthy physical revival through cryopreservation.

2) Affordable Cryopreservation

The Cryonics Institute (CI) offers full-body cryopreservation for as little as $28,000.

3) Affordable Membership

Become a Lifetime Member for a one-time payment of only $1,250, with no dues to pay. Or join as a Yearly Member with a $75 inititation fee and dues of just $120 per year, payable by check, credit card or PayPal.

4) Lower Prices for Spouses and Children

The cost of a Lifetime Membership for a spouse of a Lifetime Member is half-price and minor children of a Lifetime Member receive membership free of charge.

5) Quality of Treatment

CI employed a Ph.D level cryobiologist to develop CI-VM-1, CI's vitrification mixture which can help prevent crystalline formation at cryogenic temperatures.

6) Standby Options and Assistance

CI's use of Locally-Trained Funeral Directors means that our members can get knowledgeable, licensed care. Or members can arrange for professional cryonics standby and transport by subcontracting with **Suspended Animation, Inc** or **International Cryomedicine Experts** (I.C.E.) Ci also offers Standby Training Materials and Kits for members who choose to perform Local Standby.

7) Affordable Funding Options

Cryopreservation with CI can be funded through life insurance policies issued in the USA or other countries. Prepayment and other options for funding are also available to CI members.

8) Cutting-Edge Cryonics Information

Members receive a free e-subscription to the Cryonics Institute Newsletter, as well as access to our Facebook page, Twitter feed, YouTube channel and an official members-only forum.

9) Helpful, Professional Support

CI's professional staff is available to answer any questions and address any concerns you may have about CI, your membership or Cryopreservation.

10) Additional Preservation Services

CI offers a sampling kit, shipping and long-term liquid nitrogen storage of tissues and DNA from members, their families or pets for just $98.

11) Support Education and Research

Membership fees help CI to fund important cryonics research and public outreach, education and information programs to advance the science of cryonics.

12) Member Ownership and Control

CI Members are the ultimate authority in the organization and own all CI assets. They elect the Board of Directors, from whom are chosen our officers. CI members also can change the Bylaws of the organization (except for corporate purposes).

CRYONICS INSTITUTE
Technology for Life

To get started, contact us at:

(586) 791-5961 • email: info@cryonics.org

Visit us online at www.cryonics.org

CI NEWS

What's happening at the Cryonics Institute

PHOTO GALLERY

2023 Annual General Meeting of the Cryonics Institute

View the full meeting online ***HERE***


cryonics.org


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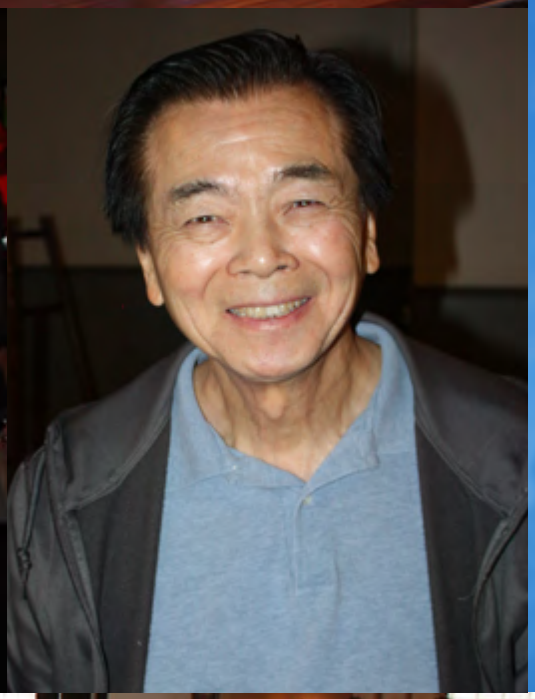


CRYONICS INSTITUTE
Technology for Life

Member Readiness Checklist

You've signed up for cryonics - what are the next steps?

Welcome Aboard! You have taken the first critical step in preparing for the future and possibly ensuring your own survival. Now what should you do? People often ask "What can I do to make sure I have an optimal suspension?" Here's a checklist of important steps to consider.

- ☐ Become a fully funded member through life insurance or easy pre-payments

  Some members use term life and invest or pay off the difference at regular intervals. Some use whole life or just prepay the costs outright. You have to decide what is best for you, but it is best to act sooner rather then later as insurance prices tend to rise as you get older and some people become uninsurable because of unforeseen health issues. You may even consider making CI the owner of your life insurance policy.

- ☐ Keep CI informed on a regular basis about your health status or address changes. Make sure your CI paperwork and funding are always up to date. CI cannot help you if we do not know you need help.

- ☐ Keep your family and friends up to date on your wishes to be cryopreserved. Being reclusive about cryonics can be costly and cause catastrophic results.

- ☐ Keep your doctor, lawyer, and funeral director up to date on your wishes to be cryopreserved. The right approach to the right professionals can be an asset.

- ☐ Prepare and execute a Living Will and Power of Attorney for Health Care that reflects your cryonics-related wishes. Make sure that CI is updated at regular intervals as well.

- ☐ Review the CI Standby Manual and other materials designed to help you with you Standby Planning. Also, consider joining or forming a local standby group to support your cryonics wishes. This may be one of the most important decisions you can make after you are fully funded. As they say-"Failing to plan is planning to fail".

- ☐ Always wear your cryonics bracelet or necklace identifying your wishes should you become incapacitated. Keep a wallet card as well. If you aren't around people who support your wishes and you can't speak for yourself a medical bracelet can help save you.

- ☐ Get involved! If you can, donate time and money. Cryonics is not a turnkey operation. Pay attention and look for further tips and advice to make both your personal arrangements and cryonics as a whole a success. The stronger our organization is, the stronger your chances of success.

- ☐ Keep your records, contact information and contracts up to date. It is recommended you review your relevant information annually at a minimum. One way is to schedule time to review all your materials at the same time you submit your required Annual Proof of Funding to CI. Also, Be especially aware of easy to forget things like a new email, phone number or address. Remember, you can also contact us at any time to ask if you have any outstanding paperwork or other info that needs to be updated.

  The online CI Members' Information Form is a great resource for updating your current information on file.

CI NEWS

What's happening at the Cryonics Institute

Pet Cryosuspension Services Available

Did you know CI Members can take advantage of our cryonic suspension services for their pets? Instead of burial or cremation, you can give a loyal and beloved pet the same second chance at life that we have through cryopreservation.

Many members who have preserved their pets say it's a comforting thought that their longtime animal companions now have the same chance to live again in a better future. Ci currently has nearly 200 pets in cryosuspension.

Cryopreservation of pets is only available to Lifetime and Yearly Members of the Cryonics Institute. Excluding the cost of Membership, the typical cost of cryopreserving a cat or dog is $5,800 up to 15 pounds in weight plus $150 per pound for every pound above 15 for dogs. This does not include shipping and veterinarian expenses. CI will also preserve other types of pets and pricing is similarly by the size and weight scale for dogs. Please contact us to inquire about specific pricing and procedures for pet patients, or visit cryonics.org for more complete details.

CI NEWS

What's happening at the Cryonics Institute

Visiting Hours For Family Members of CI Patients

Monday:	2:00pm - 4:00pm
Tuesday	2:00om - 4:00pm
Wednesday	2:00pm - 4:00pm
Thursday	2:00pm - 4:00pm

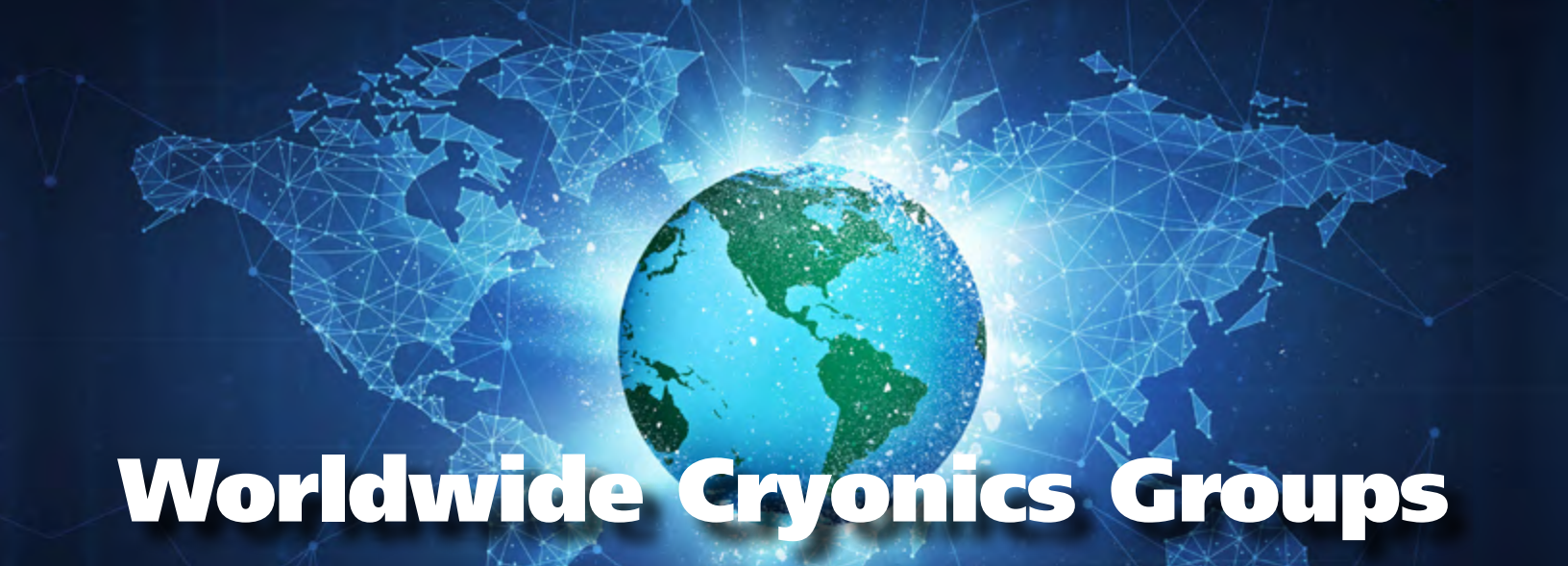
We ask that visitors kindly give us at least **one month advance notice** to ensure there are no scheduling conflicts. We cannot guarantee that the facility will be accessible to visitors who have not scheduled their visit in advance.

**** These visiting hours ar subject to change without notice due to patient or pet emergencies. ****

These requiirements have been established for multiple reasons, but most importantly for protecting our patients, members and facility.

Questions regarding visitation can be directed to Andy Zawacki, Facility Manager at info@cryonics.org or 1-586-791-5961.

Thank you!



Worldwide Cryonics Groups

AUSTRALIA: The Cryonics Association of Australasia offers support and information for Australia & nearby countries. **caalist@prix.pricom.com.au.** Their Public Relations Officer is Philip Rhoades. **phil@pricom.com.au** GPO Box 3411, Sydney, NSW 2001 Australia. Phone: +6128001 6204 (office) or +61 2 99226979 (home.)

BELGIUM: Cryonics Belgium is an organisation that exists to inform interested parties and, if desired, can assist with handling the paperwork for a cryonic suspension. The website can be found at **www.cryonicsbelgium.com**. To get in touch, please send an email to **info@cryonicsbelgium.com**.

BHUTAN: Can help Cryonics Institute Members who need help for the transport & hospital explanation about the cryonics procedure to the Dr and authorities in Thimphou & Paro. Contacts : Jamyang Palden & Tenzin Rabgay / Emails : **palde002@umn.edu** or **jamgarnett@hotmail.co** Phones : Jamyang / 975-2-32-66-50 & Tenzin / 975-2-77-21-01-87

CANADA: This is a very active group that participated in Toronto's first cryopreservation. President, Christine Gaspar; Vice President, Gary Tripp. Visit them at: **http://www.cryocdn.org/**. There is a subgroup called the Toronto Local Group. Meeting dates and other conversations are held via the Yahoo group. This is a closed group. To join write: **csc5@cryocdn.org**

BRITISH COLUMBIA: The Lifespan Society advocates for radical life extension. They also organize conferences and educational outreach events on life extension issues. Lifespan welcomes all Canadians as members, although voting in the society is open to BC residents. Contact Carrie Radomski, President at **carrie@lifespanbc.ca** Web site **www.lifespansociety.com**

QUEBEC: Contact: Stephan Beauregard, C.I. Director & Official Administrator of the Cryonics Institute Facebook Page. Information about Cryonics & perfusion services in Montreal for all cryonicicts. Services available in French & English: **stephan@cryonics.org**

CHILE: Community oriented to provide reliable information on human cryopreservation, as far as technical scientific as well as other practical aspects. Dissemination, awareness and education on issues related to the extension of life in general and cryonics in particular. Contact José Luis Galdames via **galdamesh.jl@gmail.com.**

FINLAND: The Finnish Cryonics Society, (KRYOFIN) was established in 2008 and is an organization collaborating with all nearby groups and organizations. Contact them at: **kryoniikka.fi** Their President is Ville Salmensuu **ville@salmensuu.fi**

FRANCE: SOCIETE CRYONICS DE FRANCE is a non profit French organization working closely with European cryonics groups. For more information: J.Roland Missionnier: phone: 33 (0) 6 64 90 98 41 or email: **cryonicsnews.inpi@gmail.com** • **Facebook group**

https://www.francecryonics.fr/a-propos/ Vivien Gruss, member of Cryonics Institute, has opened a web site for the information of persons interested in cryonic suspension.

GERMANY: DGAB There are a number of Cryonicists in Germany. Their Organization is called "Deutsche Gesellschaft für Angewandte Biostase e.V.", or short "DGAB". More information on their homepage at **www.biostase.de**. If there are further questions, contact their Board at **vorstand@biostase.de**

GERMANY: CRYONICS-GERMANY is an active group providing cryonics support, including a special 8-member Standby Response Team. Members from Germany or Internationally are welcome to join. at **http://cryonics-germany.org**. Direct inquiries to **contact@cryonics-germany.org**.

INDIA: Can help Cryonics Institute Members who need help for the transport & hospital explication about the cryonics procedure to the Dr and authority in Bangalore & Vellore Area. Contacts : Br Sankeerth & Bioster Vignesh / Email : **vicky23101994@gmail.com** Phones : Bioster / 918148049058 & Br Sankeerth / 917795115939

ITALY: The Italian Cryonics Group (inside the Life Extension Research Group (LIFEXT Research Group)) **www.lifext.org** and relative forum: **forum.lifext.org**. Contact Giovanni Ranzo at: **giovanni1410@gmail.com**

Kriorus Italy: Representative Filippo Polistena, email: filippopolistena45@gmail.com. phone: +39 334 298 9378

JAPAN: Hikaru Midorikawa is President Japan Cryonics Association. Formed in 1998, our goals are to disseminate cryonics information in Japan, to provide cryonics services in Japan, and eventually, to allow cryonics to take root in the Japanese society. Contact **mid_hikaru@yahoo.co.jp** or **http://www.cryonics.jp/**

NEPAL: Can help Cryonics Institute Members who need help for the transport & hospital explanation about the cryonics procedure to the Dr and authorities in Kathmandu. Contact : Suresh K. Shrestha / Email : **toursuresh@gmail.com** Phone : 977-985-1071364 / PO Box 14480 Kathmandu.

THE NETHERLANDS: Dutch Cryonics Organization is the local support group since 2002 and able to provide advice, standby, perfusion and shipment 24/7, in case of need. We are an active group utilizing the latest equipment. New members from The Netherlands welcome.

E-mail: **info@cryonisme.nl**
website: **http://www.cryonisme.nl**

NORWAY : Can help Cryonics Institute Members who need help for the transport & hospital explication about the cryonics procedure to the Dr, funeral home and authority at Sandvika. Contacts : Gunnar Hammersmark Sandvika Begegravelsesbyraa / Phones : 011-47-2279-7736

RUSSIA: KrioRus is a Russian cryonics organization operating in Russia, CIS and Eastern Europe that exists to help arrange cryopreservation and longterm suspension locally, or with CI or Alcor. Please contact **kriorus@gmail.com** for additional information or visit **http://www.kriorus.ru**. Phone: +7 962 947-50-79

SWEDEN: **www.kryonik.se** or Facebook: Svenska Kryonikföreningen. Initially, the society will focus on providing information and assistance to those who wish to sign up for cryonics. Eventually, we also hope to provide practical assistance in cases, possibly in collaboration with other European groups.

SWITZERLAND: **www.cryosuisse.ch**

CRYOSUISSE The Swiss Society for Cryonics is an active group with over 30 members. To join, **email info@cryosuisse.ch**

UNITED STATES:

Minnesota: Minnesota Cryonics Rapid Response (MCRR) is a nonprofit standby, stabilization and transport group based in Minneapolis, Minnesota. We have a strong, longstanding working relationship with local funeral directors, and have successfully participated in significantly more-timely suspension efforts in Minnesota in cooperation with both Alcor and the Cryonics Institute. Contact: President, Chuck Bartl, **chuckbartl@yahoo.com**.

Washington DC Metro Region: Life Extension Society (LES) is a nonprofit organization of area cryonicists dedicated to enhancing local capabilities for standby, stabilization and transport. Members from both Alcor and Cryonics Institute are welcome. Contact: Mark Mugler, **mugsim2@gmail.com**.

UNITED KINGDOM: Cryonics UK is a nonprofit UK based standby group. **www.cryonics-uk.org** Cryonics UK can be contacted via the following people: Tim Gibson: phone: 07905 371495, email: **tim.gibson@cryonics-uk.org**. Victoria Stevens: phone: 01287 669201, email: **vicstevens@hotmail.co.uk**. Graham Hipkiss: phone: 0115 8492179 / 07752 251 564, email: **ghipkiss@hotmail.com**. Alan Sinclair: phone: 01273 587 660 / 07719 820715, email: **cryoservices@yahoo.co.uk**

Can help Cryonics Institute Members who need help, funeral home, transport at London. Contact : F.A. Albin & Sons / Arthur Stanley House Phone : 020-7237-3637

INTERNATIONAL: The Cryonics Society is a global cryonics advocacy organization. **www.CryonicsSociety.org**. They publish an e-newsletter *FutureNews*. Phone: 1-585-643-1167.

HELP US STAY UP-TO-DATE!

Please send any corrections or changes to the address below. If you know of, or are considering starting a support, standby or other cryonics-related group in your area, please send details to

dg@cryonics.org.

Please note, this list is provided as an information resource only. Inclusion on the list does not constitute an endorsement by the Cryonics Institute or our affiliated organizations. We urge our readers to use this list as a starting point to research groups that may meet their own individual needs. We further note that readers should always use their own informed judgment and a reasonable amount of caution in dealing with any organization and/or individual listed.

CI MEMBERSHIP

DECEMBER 2023

Members	1,975	Pets	253
Patients	247	DNA/Tissue	345
		SA	350

TOTAL **2,222**

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Scientists discover clues to aging and healing from a squishy sea creature

A relative of jellyfish and corals regrows its entire body with help from "aging" cells.

Hydractinia symbiolongicarpus, a small, tube-shaped animal that lives on the shells of hermit crabs.Christy Schnitzler, Ph.D., Whitney Marine Labs

Insights into healing and aging were discovered by National Institutes of Health researchers and their collaborators, who studied how a tiny sea creature regenerates an entire new body from only its mouth. The researchers sequenced RNA from Hydractinia symbiolongicarpus, a small, tube-shaped animal that lives on the shells of hermit crabs. Just as the Hydractinia were beginning to regenerate new bodies, the researchers detected a molecular signature associated with the biological process of aging, also known as senescence. According to the study published in Cell Reports, Hydractinia demonstrates that the fundamental biological processes of healing and aging are intertwined, providing new perspective on how aging evolved.

"Studies like this that explore the biology of unusual organisms reveal both how universal many biological processes are and how much we have yet to understand about their functions, relationships and evolution," said Charles Rotimi, Ph.D., director of the Intramural Research Program at the National Human Genome Research Institute (NHGRI), part of NIH. "Such findings have great potential for providing novel insights into human biology."

Untangling the evolutionary origins of fundamental biological processes, such as aging and healing, is essential to understanding human health and disease. Humans have some capacity to regenerate, like healing a broken bone or even regrowing a damaged liver. Some other animals, such as salamanders and zebrafish, can replace entire limbs and replenish a variety of organs. However, animals with simple bodies, like Hydractinia, often have the most extreme regenerative abilities, such as growing a whole new body from a tissue fragment.

A regenerative role for senescence stands in contrast to findings in human cells. "Most studies on senescence are related to chronic inflammation, cancer and age-related diseases," said Andy Baxevanis, Ph.D., senior scientist at NHGRI and an author of the study. "Typically, in humans, senescent cells stay senescent, and these cells cause chronic inflammation and induce aging in adjacent cells. From animals like Hydractinia, we can learn about how senescence can be beneficial and expand our understanding of aging and healing."

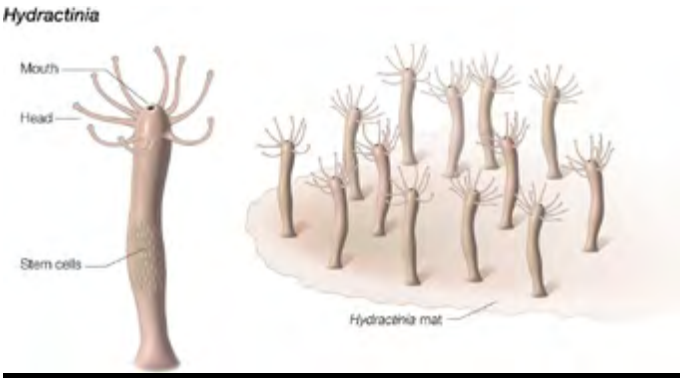

Diagram showing the composition of the Hydractinia symbiolongicarpusDarryl Leja, National Human Genome Research Institute

Previously, researchers found that Hydractinia has a special group of stem cells for regeneration. Stem cells can transform into other types of cells, and are therefore useful for creating new body parts. In humans, stem cells mainly act in development, but highly regenerative organisms like Hydractinia use stem cells throughout their lifetimes. Hydractinia stores its regeneration-driving stem cells in the lower trunk of its body. However, when the researchers remove the mouth — a part far from where the stem cells reside — the mouth grows a new body. Unlike human cells, which are locked in their fates, the adult cells of some highly regenerative organisms can revert into stem cells when the organism is wounded, though this process is not well understood. The researchers therefore theorized that Hydractinia must generate new stem cells and searched for molecular signals that could be directing this process.

When RNA sequencing pointed to senescence, the researchers scanned the genome of Hydractinia for sequences like those of senescence-related genes in humans. Of the three genes they identified, one was "turned on" in cells near the site where the animal was cut. When the researchers deleted this gene, the animals' ability to develop senescent cells was blocked, and without the senescent cells, the animals did not develop new stem cells and could not regenerate.

The researchers tracked the senescent cells in Hydractinia to find how this animal circumvents the harmful effects of senescence. Unexpectedly, the animals ejected the senescent cells out of their mouths. While humans can't get rid of aging cells that easily, the roles of senescence-related genes in Hydractinia suggest how the process of aging evolved.

We humans last shared an ancestor with Hydractinia — and its close relatives, jellyfish and corals — over 600 million years ago, and these animals don't age at all. Because of these factors, Hydractinia can provide crucial insights about our earliest animal ancestors. Therefore, the researchers theorize that regeneration may have been the original function of senescence in the first animals.

"We still don't understand how senescent cells trigger regeneration or how widespread this process is in the animal kingdom," said Dr. Baxevanis. "Fortunately, by studying some of our most distant animal relatives, we can start to unravel some of the secrets of regeneration and aging — secrets that may ultimately advance the field of regenerative medicine and the study of age-related diseases as well."

The National Human Genome Research Institute (NHGRI) is one of the 27 institutes and centers at the NIH, an agency of the Department of Health and Human Services. The NHGRI Division of Intramural Research develops and implements technology to understand, diagnose and treat genomic and genetic diseases. Additional information about NHGRI can be found at: https://www.genome.gov.

About the National Institutes of Health (NIH): NIH, the nation's medical research agency, includes 27 Institutes and Centers and is a component of the U.S. Department of Health and Human Services. NIH is the primary federal agency conducting and supporting basic, clinical, and translational medical research, and is investigating the causes, treatments, and cures for both common and rare diseases. For more information about NIH and its programs, visit www.nih.gov.

Reference

Miguel Salinas-Saavedra, Febrimarsa, Gabriel Krasovec, Helen R. Horkan, Andreas D. Baxevanis, Uri Frank. Senescence-induced cellular reprogramming drives cnidarian whole-body regeneration. Cell Reports, 2023, DOI: https://doi.org/10.1016/j.celrep.2023.112687(link is external).

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Vitrification and nanowarming enable long-term organ cryopreservation and life-sustaining kidney transplantation in a rat model

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Banking cryopreserved organs could transform transplantation into a planned procedure that more equitably reaches patients regardless of geographical and time constraints. Previous organ cryopreservation attempts have failed primarily due to ice formation, but a promising alternative is vitrification, or the rapid cooling of organs to a stable, ice-free, glass-like state. However, rewarming of vitrified organs can similarly fail due to ice crystallization if rewarming is too slow or cracking from thermal stress if rewarming is not uniform. Here we use "nanowarming," which employs alternating magnetic fields to heat nanoparticles within the organ vasculature, to achieve both rapid and uniform warming, after which the nanoparticles are removed by perfusion. We show that vitrified kidneys can be cryogenically stored (up to 100 days) and successfully recovered by nanowarming to allow transplantation and restore life-sustaining full renal function in nephrectomized recipients in a male rat model. Scaling this technology may one day enable organ banking for improved transplantation.

The ability to intentionally and reproducibly cryopreserve living biological systems, including cells, tissues, organs, and even whole organisms, began in 1949 with the preservation of fowl sperm using glycerol, a cryoprotective agent (CPA) that protected the sperm cells during freezing[1]. That work was followed by important proof-of-principle cryopreservation of mammalian blood and embryos with other CPAs[2,3]. These and other studies also demonstrated that injury from ice crystallization during freezing limited success, especially in larger systems[4–9]. Efforts to address this barrier led to "vitrification," an approach using higher concentrations of CPAs and faster rates of cooling that avoided crystallization entirely by forming a glassy state during cooling, as demonstrated in both embryos[10] and even whole rabbit kidneys[11]. By avoiding crystallization during both cooling and rewarming, mammalian embryo cryopreservation became a reality and transformed the field of reproductive technology. However, preventing crystallization during rewarming in larger bulk systems, like whole kidneys, remains elusive due to the inability of conventional convective rewarming (i.e., surface warming) to provide rapid and

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uniform heating rates across these larger scales. Indeed, vitrification followed by long-term transplant success with a kidney (or any organ) has never been reproducibly achieved.

Nevertheless, interest in organ cryopreservation has remained high due the short preservation limits for kidneys and other organs. Organ banking via cryopreservation would make organ transplantation an elective rather than an urgent/emergent procedure and revolutionize how organs are used to treat human disease. Better donor/recipient matching, improved equity in access, better patient preparation, better transplant tolerance protocols, increased organ utilization, and enhanced graft and patient survival could all be enabled by long-term organ banking.

The critical warming rates (CWR) needed to avoid ice crystallization during rewarming are typically an order of magnitude higher (10–1000 s °C/min) than the required critical cooling rates (CCR) (1–100 s °C/min), even with the aid of CPAs[12,13]. Further, temperature non-uniformity during rewarming produces thermal stress that can cause cracking. Thus, speed and uniformity of rewarming are the primary obstacles to success with vitrification.

To address these obstacles, we have developed "nanowarming," which achieves both objectives simultaneously by generating heat from within and throughout the organ rather than just at its surface[14]. In nanowarming, iron oxide nanoparticles (IONPs) are perfused throughout the organ vasculature along with CPA solutions. The organ is vitrified and then rewarmed on-demand by placing it in a radiofrequency (RF) coil that induces alternating magnetic fields from electric current flowing through the coil. The magnetic fields then generate an oscillatory response in the nanoparticles that generate heat throughout the system. Notably, nanowarming rates are not dependent on system size or boundary conditions since the RF frequencies used penetrate tissues without attenuation[14–16]. In addition, perfusion within the capillaries allows sufficiently uniform delivery of CPAs and IONPs regardless of organ size. Thus, nanowarming is intrinsically scalable to human-sized organs for clinical translation. Importantly, since the IONPs remain in the organ vasculature, they can be washed out during CPA perfusion unloading.

In the past, we and others have shown that nanowarming can rewarm vitrified organs (including kidneys) from animal models with physical success, but only partial biological recovery and no transplant data[17–20]. We found that CPA damage, not physical injury from vitrification and nanowarming, was the limiting step for biologic and functional recovery. We hypothesized that if we could overcome CPA injury, nanowarming would enable the recovery of viable and functional organs following vitrification.

Here we demonstrate, for the first time, both the physical and biological success of vitrifying and nanowarming an organ. By applying engineering principles, we optimized the CPA loading for low toxicity vitrification. That, combined with rapid and uniform nanowarming, enabled cryopreservation of kidneys for 1–100 days and on-demand rewarming. Recovery of organ function was demonstrated in vitro by normothermic machine perfusion and in vivo in a rat transplant model where both native kidneys were removed at the time of transplant. Full renal function was restored after nanowarming and transplantation, with the transplanted organs sustaining the life of the recipient animals.

Results

Overview of nanowarming

An overview of the kidney vitrification and nanowarming process is shown in Fig. 1. After recovery from the donor, the kidney is flushed with cold University of Wisconsin (UW) preservation solution, similar to clinical transplant practice, and connected to a pressure- and flow-controllable multithermic perfusion system. CPA is added to a carrier solution (LM5[21]) and then loaded by vascular perfusion. Loading concentration gradually increases to avoid osmotic stress due to the hypertonicity of these solutions. The duration of CPA loading balances the time needed for CPA transport from the vasculature to the extravascular (interstitial and cellular) space against CPA toxicity, which increases with longer exposure time. IONPs are perfused along with the final step of CPA loading. The IONPs used are silica and polyethylene glycol (PEG) coated, which increases stability in CPAs and provides for biocompatibility and organ washout[18,22]. Following CPA and IONP loading, the organ is placed in a controlled rate freezer and cooled at a rate faster than the CPA's CCR to below the glass transition temperature (T_g, ~ −128 °C), where the system enters a stable glassy state. The vitrified organ is then transferred to a −150 °C freezer for storage. When needed, the organ is removed from cryogenic storage, placed in an RF coil, and rewarmed at a rate exceeding the CPA's CWR. The CPA and IONPs are gradually unloaded, and the organ is ready for transplantation.

CPA loading and kidney vitrification

Our previous attempts at vitrification and nanowarming of rat kidneys were limited by damage from the CPA used (VS55)[18]. Here we changed to VMP as the CPA due to its reduced renal toxicity[21]. We used mass transport modeling to optimize the loading conditions for improved tissue CPA concentration for vitrification (see Supplementary Data and Supplementary Discussion). Figure 2a shows an example of arterial pressure, flow, and temperature during VMP perfusion with the modified protocol. We loaded IONPs at a concentration of 10 mg iron (Fe)/mL in VMP during the final 4.5 min at a constant flow of 0.5 mL/min, with a pressure of 44.9 ± 10.3 mmHg.

VMP-only treated kidneys (loading and unloading only but no nanoparticles or vitrification) were histologically similar to fresh control kidneys and much better than those treated with the prior CPA, VS55 (Fig. 3). VMP-only treated kidneys had normal glomeruli, Bowman's space, basement membranes, proximal convoluted tubules, distal convoluted tubules, collecting ducts, and vasculature. In contrast, VS55-only treated kidneys demonstrated increased Bowman's space, diffuse tubular necrosis, and hyaline changes (but with no vascular compromise). The histologic appearance confirmed that VMP

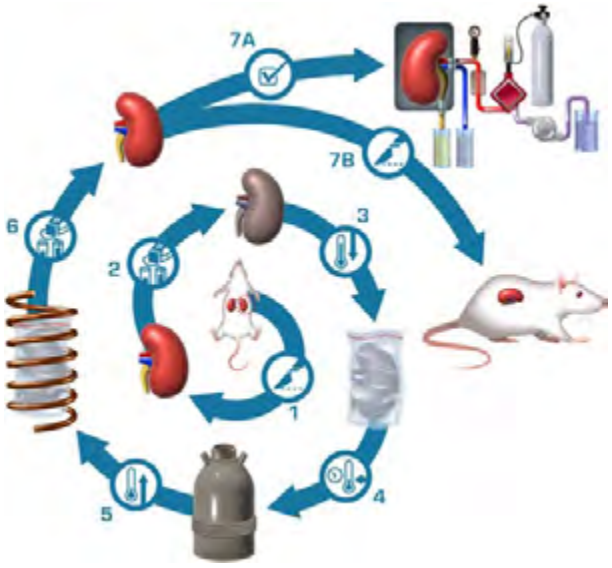

Fig. 1 | Overview of nanowarming. Depicted are the sequential steps of the nanowarming procedure as follows: (1) Kidney recovery from the donor; (2) Loading of cryoprotective agents (CPAs) and iron oxide nanoparticles (IONPs); (3) Rapid cooling to a vitrified state; (4) Storage at −150 °C until needed; (5) Rapid and uniform rewarming in radiofrequency (RF) alternating electromagnetic field; (6) Unloading of CPA and IONPs; and finally, organs are ready for (7A) assessment by normothermic machine perfusion or (7B) transplantation.

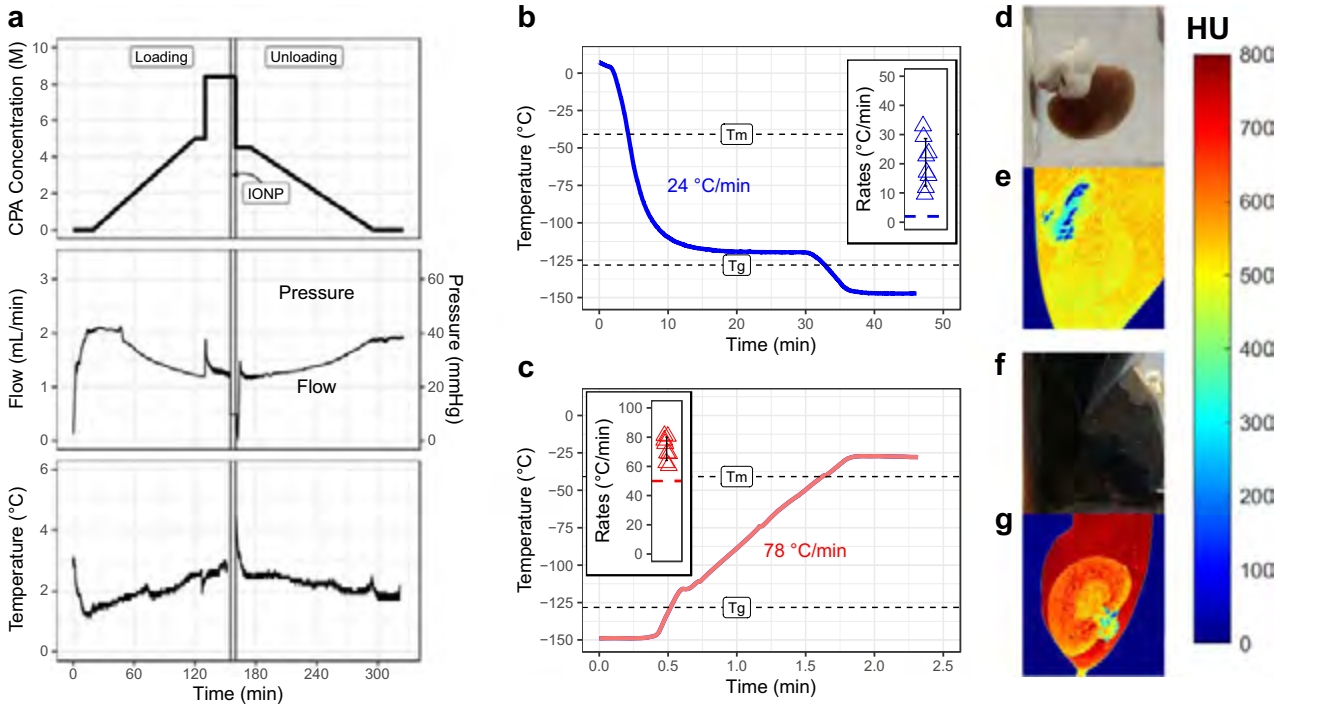

Fig. 2 | Vitrification and nanowarming of kidneys. a CPA concentration, perfusion pressure, arterial flow rate, and temperature profile during the loading and subsequent unloading of CPA and IONPs in rat kidneys. **b** Representative thermal profile during cooling from above the melting temperature (T_m = −40.8 °C) to an annealing step just above the glass transition temperature (T_g = −128.3 °C), and finally slower cooling into the glassy zone. The blue shaded area is the zone of risk for ice formation. Inset are cooling rates and the critical cooling rate (blue dashed line, CCR = 2 °C). n = 8. **c** Representative thermal history during nanowarming, rewarming rates, and the critical warming rate (red dashed line, CWR = 50 °C). n = 8. Representative photograph (**d**) and pseudocolor image acquired by micro-CT (**e**) of the vitrified VMP-loaded kidney. Representative photo (**f**) and pseudocolor image acquired by micro-CT (**g**) of the vitrified VMP + IONP-loaded kidney. The pseudocolor scale shows micro-CT image radiodensity (in Hounsfield units). Data are mean ± s.d. Source data are provided as a Source Data file. HU Hounsfield units; T_g glass transition temperature; T_m, melting temperature.

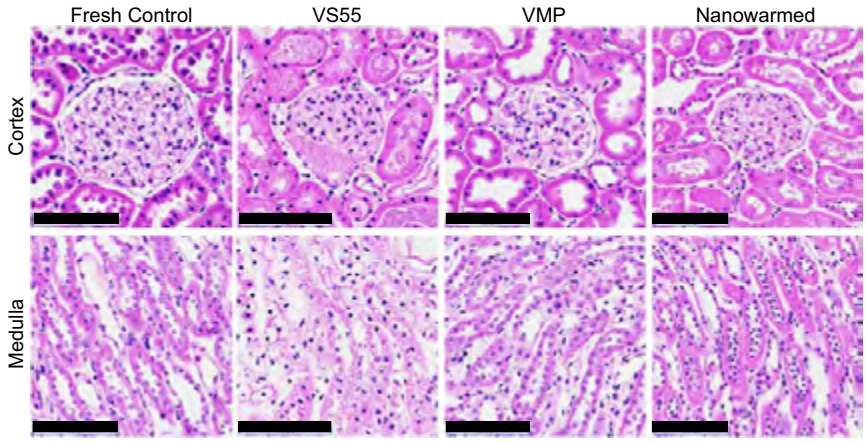

Fig. 3 | Histologic appearance of CPA-only treated and CPA plus nanowarmed kidneys. Histological changes (H&E) of renal cortex and medulla following treatment with CPA (VS55-only and VMP-only) and vitrification and nanowarming (N–W) compared to fresh untreated controls. Scale bars are 100 μm. Data are representative images from n = 6 (Fresh Control and Nanowarmed), n = 3 (VS55), and n = 4 (VMP) separate experiments for each condition. H&E, hematoxylin and eosin.

was less toxic than VS55, further assessed through ex vivo normothermic perfusion as discussed below. Further histologic details are in the Supplementary Information.

After CPA and IONP loading, the kidney was placed in a polyethylene bag containing 4 mg Fe/mL IONPs in full-strength VMP (8.4 M). A fiberoptic temperature probe was placed in the solution adjacent to the kidney to record the thermal history, and the organ was cooled to −150 °C in a controlled rate freezer and moved to a −150 °C freezer for storage. The mean cooling rate was 20.5 ± 8.1 °C/min (Fig. 2b), which was much faster than the 2 °C/min CCR of VMP in kidney tissue.

Successful vitrification was determined by direct visual inspection and micro-computed tomography (micro-CT). Because the opaque nanoparticles obscured direct visualization, some kidneys were vitrified without IONPs (Fig. 2d, e). The surrounding CPA was translucent and glassy in appearance, and the kidney lacked the changes seen

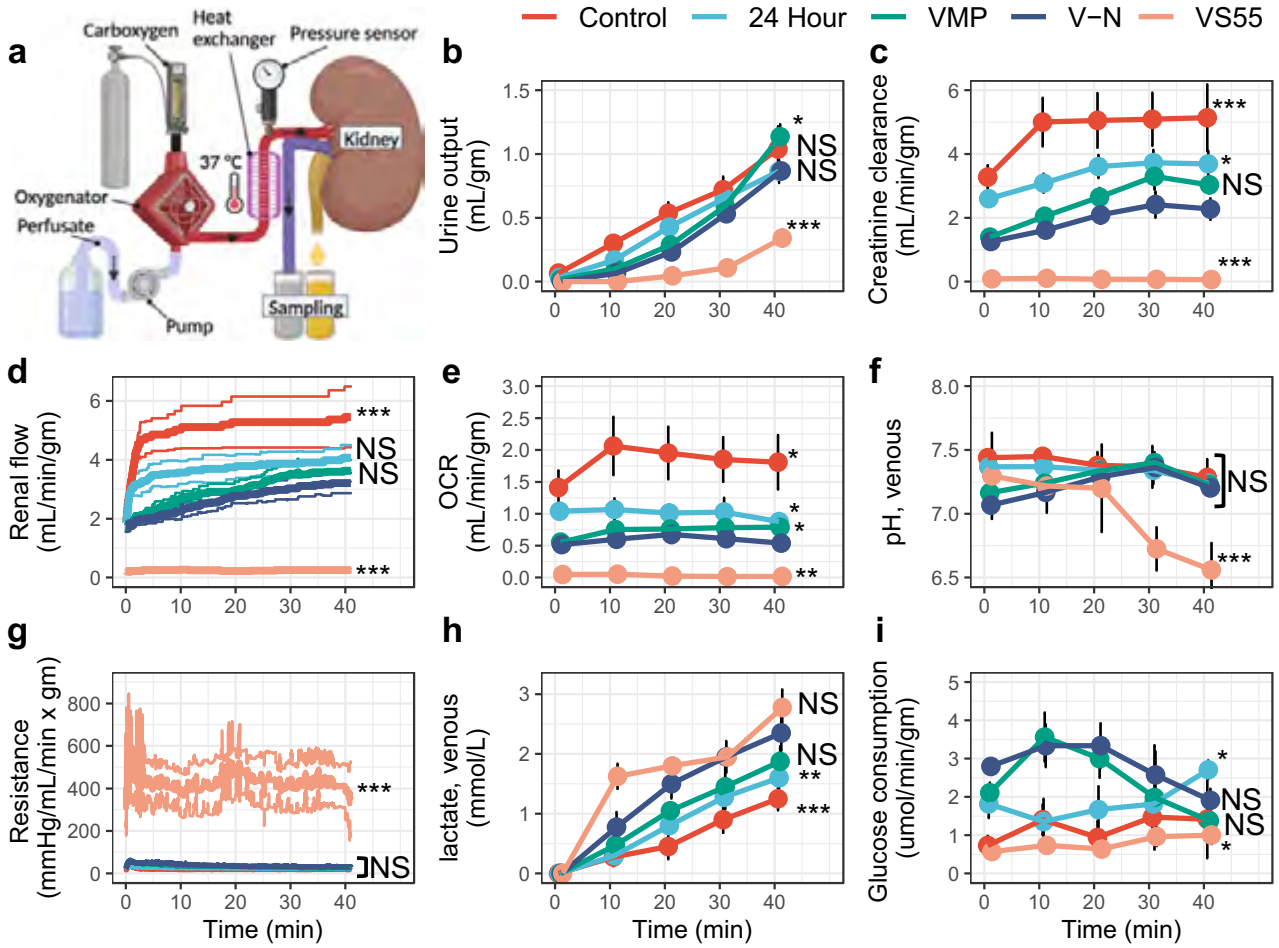

Fig. 4 | Normothermic machine perfusion of rat kidneys using acellular blood substitute. a Schematic of the normothermic perfusion system for diagnostic assessment of fresh untreated (control), 24-h UW preserved (24 h), VMP loaded-unloaded (VMP), VS55 loaded-unloaded (VS55), and vitrified and nanowarmed (V–N) rat kidneys. **b** Cumulative urine output over the course of perfusion. **c** Creatinine clearance. **d** Alterations in renal artery flow. **e** Oxygen consumption rate. **f** Venous pH. **g** Vascular resistance. **h** Lactate production. **i** Glucose consumption. Data are mean ± s.d. $n = 4$ per condition. Statistical comparison between nanowarmed kidney and other treatment groups at minute 40 using ANOVA and Tukey HSD (Games–Howell if unequal variance) post hoc tests. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; NS, not significant. Full statistical treatment in Supplementary materials. Source data are provided as a Source Data file. OCR oxygen consumption rate; V–N, vitrified and nanowarmed.

visually with freezing (color change to pale pink), indicating that the kidney and surrounding CPA appeared well vitrified. Some ice formed in the extrarenal adherent fat in the perihilar area, presumably due to hypoperfusion of that tissue and decreased CPA concentration. Micro-CT can discriminate between vitrified and frozen tissues based on radiodensity (Fig. 2e and Supplementary Data)[18,23]. The kidney extravascular space had a mean radiodensity of 507 ± 30 Hounsfield Units (HU), while the adherent fat outside the kidney was 267 ± 104 HU. These are consistent with successful vitrification of the kidney itself and ice present in the extrarenal fat.

For comparison, we also show a vitrified IONP-loaded kidney (Fig. 2f) where the opacity of IONPs obscured the visual appearance. Micro-CT showed successful vitrification of the CPA plus IONP-loaded kidneys, but the absolute radiodensity of vitrified vs. frozen tissue was shifted because of the IONPs (Fig. 2g). The solution, cortex, outer medulla, and inner medulla had mean HU values of 768 ± 20, 628 ± 15, 656 ± 20, and 560 ± 18, respectively. It is expected that these differences are due to regional variance in vascular density[21], leading to localized differences in IONP concentration as previously observed[18]. However, there were no areas of low HU attenuation that would suggest ice crystallization or linear transitions suggesting cracking (see Supplementary Data for micro-CT calibration of vitrified materials).

After vitrification, the kidneys were stored for 1–100 days at −150 °C.

Kidney nanowarming and post-nanowarming assessment

Kidney nanowarming was performed using a 15 kW RF coil at 63 kA/m and 180 kHz[14]. The measured warming rate adjacent to the kidney was 72.0 ± 8.0 °C/min (Fig. 2c), which is faster than the ~50 °C/min CWR of VMP in kidney tissue (Supplementary Methods).

Following nanowarming, CPA was unloaded by ramping from 4.2 M VMP (50% of full strength) plus 300 mM mannitol (to maintain osmotic balance) to 0 M CPA (LM5 carrier only) over 120 min (Fig. 2a). Venous and ureteral effluent CPA concentration at the end of unloading showed near complete CPA washout (Supplementary Data Fig. 2b).

Histologically, nanowarmed kidneys appeared similar to VMP-only treated ones and much better than VS55-only (Fig. 3). No extra "white space" was seen within the extravascular space of the nanowarmed kidneys, which would have been seen if ice had formed during the process[14].

To assess the function of nanowarmed kidneys, we utilized normothermic machine perfusion (NMP) of the kidneys with an oxygenated acellular blood substitute (Krebs–Henseleit Buffer) (Fig. 4a). During 40 min of NMP at 37 °C, hemodynamic parameters were

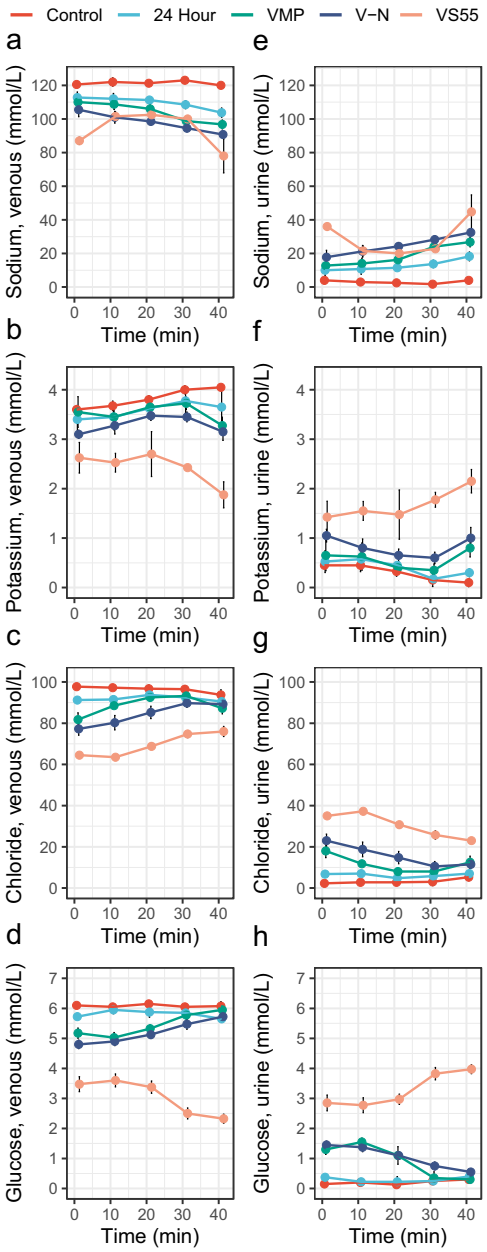

Fig. 5 | Electrolytes and glucose during normothermic perfusion of kidneys. **a**–**d** Venous effluent (left) and **e**–**h** Urine (right) effluent analysis of electrolytes and glucose during 40 min of normothermic machine perfusion of fresh control, 24-h cold stored, VMP-only, and VS55-only treated kidneys were compared to nanowarmed kidneys. Measured parameters include sodium (**a**, **e**), potassium (**b**, **f**), chloride (**c**, **g**), and glucose (**d**, **h**). Data are mean ± s.d. for $n = 4$ for each group and time point. Source data are provided as a Source Data file. V–N, vitrified and nanowarmed.

recorded, and the venous and ureter effluents were sampled. We compared nanowarmed kidneys to 4 other experimental groups: fresh control, 24-h cold stored (in UW solution at 4 °C), VMP-only (CPA load and unload without IONPs, vitrification, or nanowarming), and VS55-only. For each measure tested, the trend from best to worst was fresh control; followed by 24-h cold stored, VMP-only, and nanowarmed (all with statistically similar results); and then VS55-only, which performed the worst (Fig. 4).

Nanowarmed kidneys made urine immediately upon perfusion (Fig. 4b) and at rates that were not statistically different than fresh control or cold-stored kidneys and very similar to VMP-only treated organs, but much better than VS55-only. Creatinine clearance, a measure of overall renal function, was slightly reduced compared to fresh control and cold-stored kidneys, but similar to VMP-only (Fig. 4c). Nanowarmed kidney urine and venous electrolytes (Na+, K+, Cl−) were very similar to fresh control (Fig. 5). Urine glucose in nanowarmed kidneys normalized to <1 mmol/L, demonstrating an absence of glycosuria (Fig. 5 h).

Flow rate and vascular resistance at physiologic perfusion pressure (90–110 mmHg) provided an overall diagnostic assessment of the organs tested (Fig. 4d, g), as low vascular resistance during machine perfusion is associated with better post-transplantation performance[24]. The mean vascular resistance of nanowarmed kidneys after 40 min of perfusion was 31.3 ± 3.6 mmHg/mL/min × g, which was slightly higher but not statistically different than fresh control (18.8 ± 4.1, $P = 0.9$), 24-h cold stored (24.9 ± 2.6, $P = 1.0$) or VMP-only (27.5 ± 3.4, $P = 1.0$), but much lower than VS55-only (372 ± 131, $P < 0.001$).

Additionally, nanowarmed organs consumed oxygen and glucose during machine perfusion (Fig. 4e, i), indicating that they were metabolically active. Oxygen consumption by nanowarmed kidneys was slightly lower than fresh control but was very similar to 24-h cold stored or VMP-only. Initially, the venous pH of nanowarmed kidneys was somewhat acidic, suggesting anaerobic metabolism, but the pH normalized by 20 min of NMP (Fig. 4f). This likely reflected resumption of mitochondrial function and aerobic metabolism. Venous lactate, which correlates with organ quality in perfused organ systems[25,26], rose slowly for all groups, but the differences between groups were minor (Fig. 4h).

Transplantation of nanowarmed kidneys

As a final measure of organ function, we tested nanowarmed kidneys in a rat transplant model where the recipient's native kidneys were removed at the time of transplant. Thus, renal function and survival of the animal depended solely on the transplanted organ. We compared outcomes between fresh control kidney transplants and nanowarmed kidneys that had been vitrified for 1–100 days prior to rewarming and transplant. Intraoperatively, all nanowarmed kidneys reperfused rapidly and homogeneously upon restoration of blood flow and appeared similar to fresh control organ transplants (Fig. 6). Nanowarmed kidneys made urine within 40–45 min following reperfusion. In contrast, fresh control transplants made urine within a few minutes. We did not observe any vascular thrombosis.

Postoperatively, all fresh control and nanowarmed kidney transplants continued to produce urine, and all animals survived for the full 30-day study period. In syngeneic (Lewis to Lewis) nanowarmed kidney recipients, serum creatinine levels (a principal measure of renal function) were higher on postop day 1 than in the control transplants (Fig. 7b). Creatinine in the nanowarmed kidney recipients continued to rise, peaking between days 2–3, and then gradually declined to reach control levels over 2–3 weeks. From day 14 onward, the creatinine in nanowarmed recipients was not statistically different from that in control kidney recipients. The creatinine fell below 2.0 mg/dL by day 19 and into the normal range for healthy rats on day 23, remaining between 0.4 and 0.8 mg/dL until the end of follow-up.

During the first two postoperative weeks, nanowarmed kidney recipients also experienced more metabolic dysfunction than control transplants. Hyperkalemia peaked on days 2–3 and slowly declined after that (Fig. 7c). Partially compensated metabolic acidosis (low pH, low HCO3-, and low pCO2) was also resolved by day 15 (Fig. 7d–f). Serum lactate levels were slightly above the normal range but normalized by days 7-10 (Fig. 7g).

Following transplant, both control and nanowarmed kidney recipients increased body weight. Initially, nanowarmed organ recipients experienced greater weight gain, presumably due to hypervolemia. This ~10% excess weight gain resolved by days 10–12, after which body mass increased in parallel to control transplants (Fig. 7i). After an initial drop

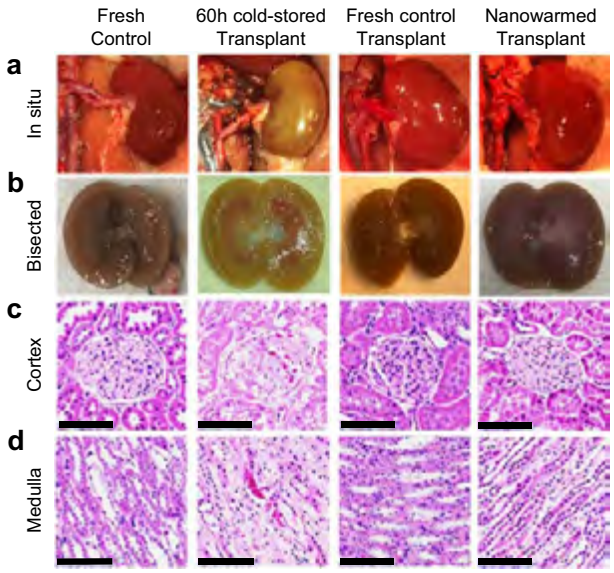

Fig. 6 | Gross and histologic appearance of nanowarmed and control kidney transplants. Representative appearance of fresh control kidney before cannulation and recovery ($n = 5$), 60-h cold-stored kidney at the time of intraoperative transplant organ failure ($n = 1$), fresh control kidney transplants at day 30 post transplant ($n = 5$), nanowarmed kidney transplants at day 30 post transplant ($n = 5$). **a** Gross images in situ. **b** Bisected kidneys following explant. **c** Histology of renal cortex (H&E). **d** Histology of renal medulla (H&E). Scale bars are 100 μm. H&E hematoxylin and eosin.

in hemoglobin in both groups due to surgical blood loss, hemoglobin rose steadily in the postop period, suggesting intact renal erythropoietin production and/or potentially hemoconcentration (Fig. 7h).

At the end of the planned posttransplant follow-up (postop day 30), animals were sacrificed for serum and urine analyses and histology. Both serum and urine laboratory parameters demonstrated statistically similar, and essentially normal, kidney function in both groups (Tables 1 and 2). Specifically, creatinine, blood urea nitrogen, and electrolytes were within normal limits for rats[27]. Urinalysis showed the absence of hematuria, pyuria, glycosuria, or proteinuria. The estimated glomerular filtration rate (eGFR) for nanowarmed kidney recipients was 2.2 ± 0.8 mL/min, which was not statistically different from control kidney transplant recipients (2.7 ± 0.7 mL/min, $P = 0.421$) and in the range for normal rats (median: 1.5 mL/min, interquartile range: 1.0–2.2 mL/min[28]). Normal liver function tests and creatinine kinase levels suggested a lack of systemic toxicity, but there was a mild elevation in serum amylase in the nanowarmed organ recipients. Of note, in these transplants there was no correlation of preservation time with peak Cr, time to normalization of Cr to <2.0, or terminal Cr (all $P > 0.5$, Kendall rank correlation).

For comparison, we performed limited testing of a long-term cold stored kidney (60 h in UW solution at 4 °C) transplant. The 60-h time point was chosen based on historical studies demonstrating a clear increase in kidney injury, with a decline in viability and graft function, occurring between 48 and 72 h of cold storage, depending on the preservative solution[29]. The 60-h cold stored kidney reperfused poorly with patchy ecchymosis over the surface and hilar congestion (Fig. 6). Failing to make urine, the rat was euthanized, and the gross appearance of the bisected kidney demonstrated congested medulla and ecchymosis in the cortex.

Microscopy of nanowarmed and transplanted kidneys

Nanowarmed and fresh control transplant kidneys were recovered at posttransplant day 30 and compared morphologically and histologically to untreated fresh control and 60-h cold stored and transplanted organs (Fig. 6). The transplanted nanowarmed and transplanted control organs were bisected. Both groups showed grossly intact architecture of the renal cortex, medulla, and pelvis (Fig. 6b). Histologic examination of transplanted nanowarmed kidneys demonstrated some focal tubular necrosis and hyaline change but intact basement membranes and vasculature (Fig. 6c, d). Microthrombi and infarcts were absent. There was a trace amount of yellowish-brown material in some of the thin-walled capillaries of most of the nanowarmed kidneys, best seen at the cortico-medullary junction and medulla but absent in glomeruli, which could represent a small amount of retained IONPs. Fresh control transplants also showed focal tubular necrosis in some areas with some hyaline change but otherwise normal histology. Large blood vessels and collecting ducts were normal in both nanowarmed and fresh control kidneys. The 60-h cold stored kidney transplants demonstrated mesangial hypercellularity and diffuse proximal convoluted tubule necrosis with congestion in the glomerulus and tubules in the medulla. Collecting ducts and larger blood vessels were normal. Further histologic details are in the Supplementary Information.

Discussion

Successful cryobanking of human organs prior to transplant would revolutionize how organs are recovered, allocated, and ultimately used to cure end-stage organ disease. Organ banking would improve donor/recipient matching, allow for better patient preparation and scheduled procedures, facilitate tolerance induction protocols in recipients, and increase organ utilization—all while supporting graft and patient survival. Here we show the first repeatable approach for successful cryopreservation of intact organs (rat kidneys) for up to 100 days prior to transplantation. Nanowarmed organs restored renal function and solely sustained the lives of nephrectomized transplant recipients for 30 days post transplant. These results show that prolonged organ banking for transplantation may finally be possible. While we demonstrate success in the rat kidney, our approach is translatable to other organs, and the intrinsic scalability of nanowarming suggests clinical translation is feasible.

In this study, we found an initial period of nanowarmed kidney graft dysfunction lasting 2–3 weeks, following which renal function normalized in all nanowarmed kidney transplant recipients. At the 30-day endpoint, renal function of nanowarmed kidney recipients was statistically similar to fresh control transplants, as measured by serum chemistries and urinalyses, and both groups were in the normal range for healthy control rats. Further, recovery of hemoglobin levels suggested intact renal erythropoietin production (and potentially hemoconcentration), and normal calcium levels suggested preserved renal conversion of vitamin D to its active form (1,25-dihydroxyvitamin D). The absence of glycosuria, hematuria, or proteinuria also supported the lack of renal damage. These data demonstrated physiologic renal recovery.

However, it is essential to consider the degree of initial graft dysfunction observed (i.e., peak and width of postoperative creatinine curve) and how this might predict long-term organ function. While we did not follow these recipients beyond 30 days, we can extrapolate potential long-term outcomes from clinical scenarios in human kidney transplantation. From the normothermic perfusion experiments, we found that nanowarmed kidneys produced the same amount of urine as fresh control kidneys (albeit with a slightly reduced creatinine clearance) and performed similarly to the 24-h cold stored control group for all measures. In the transplant experiments, nanowarmed kidneys had higher peak creatinine and took longer to achieve normal levels than fresh control transplants, but both groups performed much better than 60-h cold stored ones. Our nanowarmed kidney recipients had normal creatinine at the end of the 30-day follow-up, which in clinical transplantation predicts good long-term graft function in human kidney recipients[30]. Other predictors of long-term renal

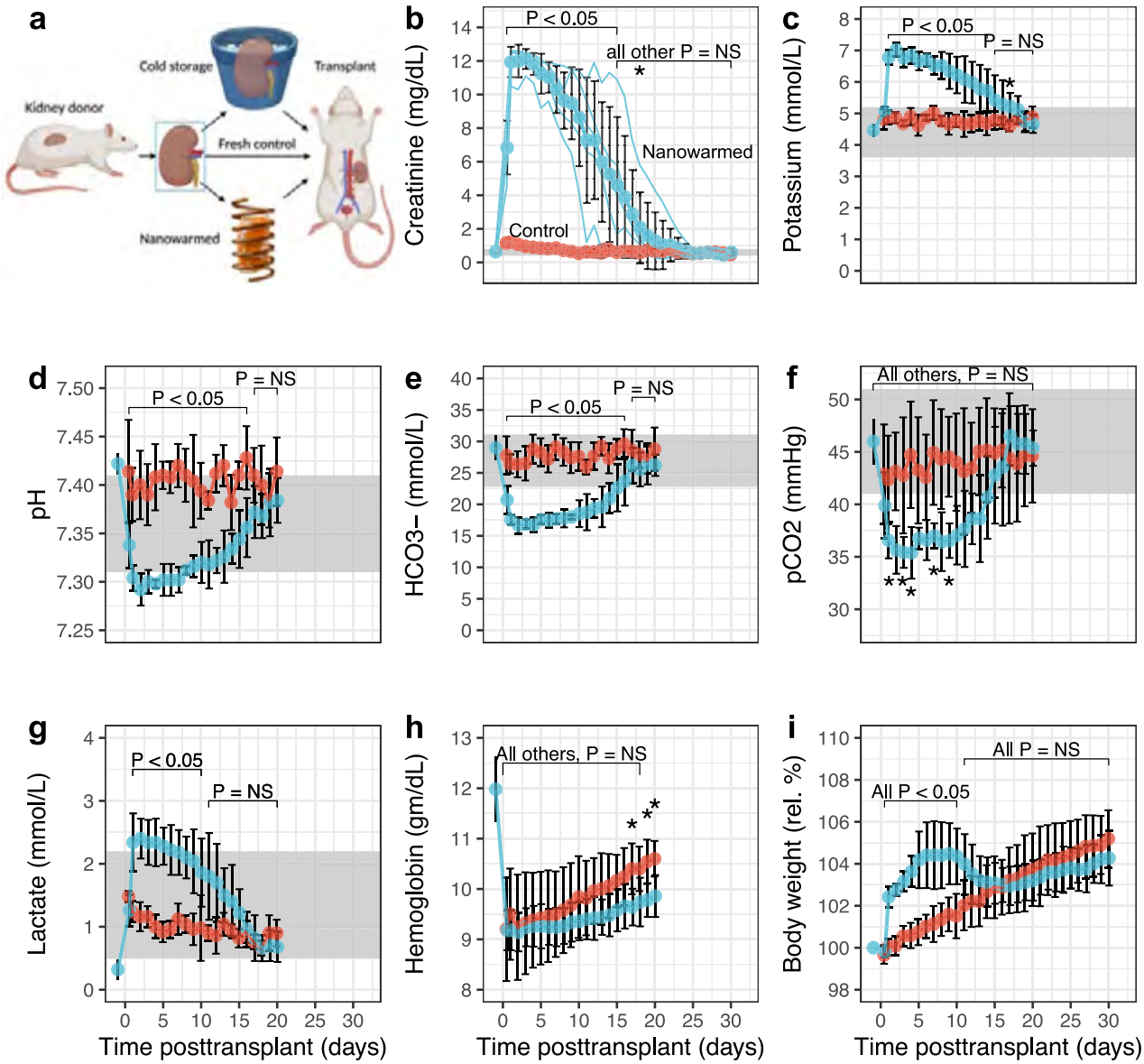

Fig. 7 | Transplant outcomes of nanowarmed and fresh control kidney transplants. a Depiction showing the transplant schema. Organs were recovered from donor rats and either immediately transplanted ($n = 5$); vitrified, nanowarmed and transplanted ($n = 5$); or stored for 60 h ($n = 1$) in UW solution prior to transplant. The 60-h cold stored kidney failed intraoperatively, and the recipient was sacrificed. Nanowarmed (blue) and fresh control kidney transplants (red) were followed for 30 days post syngeneic transplant with daily labs and assessments. **b** Serum creatinine with individual recipient curves and aggregate data shown; **c** Venous potassium; **d** Venous pH; **e** Venous bicarbonate (HCO3-); **f** Venous partial pressure of CO2 (pCO2); **g** Venous lactate; **h** Hemoglobin. **i** Daily weight normalized to day −1 pretransplant. The gray bands are normal ranges. Data are mean ± s.d. Statistical tests were Wilcoxon (**b**, **d**, **e**, **f**, **g**, **i**) or *t*-test (**c**, **h**). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; NS not significant. Source data are provided as a Source Data file. Full statistical treatment in Supplementary materials.

function are discussed in the Supplementary Discussion. Altogether, we infer that (1) fresh control transplants in rats are analogous to living donor transplants in humans (best outcomes), (2) 24-h cold stored kidneys are similar to standard criteria deceased donor transplants, and (3) 60-h cold stored kidneys are at the limits of expanded criteria transplants. As such, we can speculate that nanowarmed kidneys will have long-term outcomes similar to those seen in standard deceased donor transplantation.

We note that Fahy and colleagues first demonstrated vitrification of rabbit kidneys in 1984[11] and reported a single successful rabbit kidney transplant[31], but have never reproduced that finding. Their studies were critical for proof-of-concept, but they were limited by their rewarming technique (convective warming on the organ surface), which was unable to reproducibly achieve the needed rates or homogeneity of heating required. Due to limitations in the convective warming rates, a higher concentration of CPA was required (9.3 M M22) with more significant toxicity than VMP. To overcome that toxicity, they reduced the loading temperature to −22 °C, which slowed CPA delivery due to increased viscosity and decreased diffusivity and membrane permeability. Inadequate tissue CPA concentration, combined with suboptimal rewarming, led to ice formation, particularly in the renal medulla, and failure in almost all transplants[21,31]. Additionally, their single surviving rabbit kidney transplant was vitrified for only 8 min prior to rewarming, whereas we demonstrated stable storage for up to 100 days. Further, their transplant recipient had a nadir creatinine of ~4 mg/dL and a terminal creatinine of ~6 mg/dL, which was much higher than what we observed (nadir 0.4 mg/dL and terminal 0.6 mg/dL, each in the normal range for rats). While their transplant did sustain the life of the recipient, it did so with significantly impaired renal function and the need for repeated blood

Table 1 | Serum chemistries 30 days post transplant

Serum	Control, $N = 5$[a]	V–N, $N = 5$[a]	P value[b]
Renal labs			
BUN (mg/dL)	19.20 (1.79)	18.40 (2.41)	0.7
Creatinine (mg/dL)	0.44 (0.19)	0.62 (0.24)	0.3
Na+ (mmol/L)	141.00 (3.16)	141.20 (1.64)	>0.9
K+ (mmol/L)	4.64 (0.32)	4.66 (0.50)	>0.9
Cl– (mmol/L)	102.00 (1.41)	102.00 (2.35)	0.8
HCO3– (mmol/L)	25.06 (1.99)	25.02 (1.08)	>0.9
Ca2+ (mg/dL)	9.58 (0.45)	9.82 (0.36)	0.2
Mg2+ (mg/dL)	1.98 (0.11)	1.76 (0.37)	0.2
Phos (mg/dL)	4.56 (0.82)	4.50 (0.66)	>0.9
Extrarenal labs			
Total protein (g/dL)	6.22 (0.63)	6.76 (0.74)	0.3
Albumin (g/dL)	3.66 (0.61)	4.04 (0.93)	0.6
Globulin (g/dL)	2.72 (0.43)	2.84 (0.23)	0.5
CK (U/L)	174 (12)	147 (17)	**0.032**
Total Bilirubin (mg/dL)	0.18 (0.08)	0.52 (0.42)	0.2
ALP (U/L)	150 (36)	149 (47)	0.8
GGT (U/L)	3.54 (0.67)	3.10 (0.34)	0.4
ALT (U/L)	39 (11)	47 (17)	0.8
AST (U/L)	63 (17)	53 (31)	0.7
Amylase (U/L)	569 (85)	807 (177)	**0.016**

Statistically significant P values are in bold.
[a]Mean (SD).
[b]Wilcoxon rank sum test; Wilcoxon rank sum exact test.

Table 2 | Urinalysis 30 days post transplant

Urine	Control, $N = 5$[a]	V–N, $N = 5$[a]	P value[b]
Specific gravity	1.028 (0.013)	1.022 (0.008)	0.5
pH	7.50 (0.61)	6.88 (0.85)	0.3
Protein (mg/dL)	7.78 (0.79)	8.28 (0.67)	0.6
Protein			
Neg	5 (100%)	4 (100%)	
Glucose			
Neg	5 (100%)	4 (100%)	
Urobilinogen			
Neg	5 (100%)	4 (100%)	
Ketones			>0.9
Neg	1 (25%)		
Trace	5 (100%)	3 (75%)	
Occult blood			
Neg	5 (100%)	4 (100%)	
RBC (per HPF)			
0–5	4 (100%)	3 (100%)	
WBC (per HPF)			
None	4 (100%)	3 (100%)	
Bacteria			
Not seen	4 (100%)	3 (100%)	
Casts			
Not seen	4 (100%)	3 (100%)	
Crystals			
Not seen	4 (100%)	3 (100%)	

[a]Mean (SD); n (%).
[b]Wilcoxon rank sum test; Wilcoxon rank sum exact test; Pearson's Chi-squared test.

transfusion. Further, we can predict that their convective rewarming approach will be insufficient for human-sized organs. In contrast, nanowarming is independent of size.

Other methods for warming cryopreserved organs have been attempted, but with limited success. Direct electromagnetic rewarming of cryopreserved materials, such as dielectric heating at radio and microwave frequencies, uses high-frequency electromagnetic fields to stimulate the oscillation of the polar molecules, thereby converting electromagnetic energy into heat[32]. These approaches have failed to produce adequately rapid and uniform rewarming due to variations in the material dielectric properties, attenuation of the higher frequency fields (e.g., caused by skin depth), and the shape of the sample[33]. For example, microwave rewarming leads to thermal hotspots from limited tissue penetration and standing wave formation[34,35]. Another group attempted nanowarming in rat hearts but did not test for viability or function after rewarming[20]. Another approach called "directional freezing" was tested for liver cryopreservation with partial success, but with only limited biologic outputs (including no survival transplants) and absent follow-on study[36].

Further, a range of other technologies are in use or are being studied to prolong preservation times for kidneys and other organs, including hypothermic machine perfusion[37], normothermic machine perfusion[25], subnormothermic machine perfusion[38], high subzero supercooling[39,40], and partial freezing[41]. While these extend preservation for broader organ sharing, they only increase preservation by hours to a few days and do not achieve prolonged banking for months and potentially years. These approaches, and ours, allow for national organ sharing, more time to perform cross-matching, and converting transplants to daytime events. However, nanowarming uniquely would improve donor/recipient matching by increasing the number of organs available for consideration at one time, whereas other approaches continue the practice of one at a time decision making for every potential organ offer. Further, nanowarming would allow better patient preparation prior to surgery, enable tolerance protocols that take days to weeks of conditioning or preparation, and increase organ utilization by banking organs even if an appropriate recipient has not yet been identified.

Success in prolonged organ preservation has tremendous potential to transform human organ transplantation but will also require significant changes in the current organ transplantation system. The current system is based on severe time constraints from organ acquisition to transplant, typically measured in hours. This has contributed to a U.S. system characterized by wide variations in organ availability by region and transplant center, inefficient matching of organs and recipients, significant inequities in organ availability, and excessive organ nonuse[42]. Successful banking and relief from time constraints will invite a transformation in the system to address these problems and benefit patients. Additionally, the ethical, legal, and social implications of this technology would be considerable. In particular, quality and safety issues will be forefront in developing a framework for governmental and regulatory oversight.

Our study does have several important limitations. First, it was performed in a rat model with limited sample size ($n = 4$–8 per group) rather than in human or human-sized (e.g., porcine) kidneys. Second, we only studied organ storage for up to 100 days. Third, we only followed transplant recipients to our 30-day endpoint and did not assess longer-term survival. Fourth, we performed only a limited number of allogenic transplants and have not characterized any changes to the host immune response. Fifth, we have not determined which mechanisms and modes of injury (e.g., apoptosis vs. necrosis, protein denaturation from high concentration CPA solutions) led to the observed initial transplant dysfunction in the nanowarmed kidneys. Sixth, we have not fully characterized how nanowarmed organs perform compared to other categories of transplants, such as 36–48 h of cold storage. And finally, while the organs eventually recovered fully, they experienced a period of 2–3 weeks of initial graft

dysfunction, which might have been treated with dialysis in some clinical settings.

Future directions to address those limitations and other questions include scaling up to human-sized kidneys, conversion to clinical-grade reagents and processes, thorough investigation of the modes and mechanisms of injury (e.g., apoptosis and mitochondrial content and function) to develop injury mitigation strategies, characterizing preservation limits and durability, and defining the host immune response.

Methods

Our study complies with all relevant ethical considerations. The Institutional IACUC committee from the University of Minnesota (protocol #2204-39970 A) approved all animal studies.

Animals

450–525 gm, 16–32-week-old, male Lewis (Strain #004) and Sprague Dawley rats (Strain #400) were purchased from Charles River Laboratories. Lewis rats were used as donors and recipients for syngeneic transplants and Sprague Dawley for allogeneic transplants and in vitro studies. Male sex was selected for their larger size and to avoid Y chromosome encoded-antigen immunoreactivity. Rats were housed in a conventional housing facility with a 12-h on/12-h off light cycle, 68–74 °C ambient temperature, 30–50% humidity, and free access to food (Envigo Lab Diet #2918) and water.

CPA solutions and nanoparticles

VMP (16.8 wt% ethylene glycol, 22.3% DMSO, 12.9% formamide, 1% X-1000, 1% Z-1000) and VS55 (16.8 wt% propylene glycol, 24.2% DMSO, 14.0% formamide, 0.24% HEPES) were prepared in carrier solutions previously used[18,21], except that the carrier for VMP experiments (LM5-XZ) was modified from the original LM5[43] by adding synthetic ice blockers 1% X-1000 (w/v) and 1% Z-1000 (w/v) (21st Century Medicine, Fontana, CA). For these experiments, we used silica-coated iron oxide nanoparticles (IONPs) that were synthesized from EMG308 iron oxide core nanoparticles (Ferrotec, Santa Clara, California) that were coated with a silica shell and surface modified with polyethylene glycol and a small hydrophobic ligand, trimethylsilane[22], suspended in CPA (VMP), and filtered before use. To avoid misinterpretation of experiments where IONPs may have aggregated after filtering, we set prospective exclusion criteria wherein the perfusion flow rate during unloading had to be >45% of the observed flow rate during loading.

Kidney cannulation and recovery

Rats were anesthetized with isoflurane, and a cruciate laparotomy was performed. The bowel and mesentery were retracted. The abdominal aorta, inferior vena cava, and left renal artery and vein were mobilized for cannulation. The kidney was dissected free of Gerota's fascia. A 20G bulb tip catheter (FTP-20-30, Instech Laboratories, Plymouth Meeting, PA) was used to cannulate the abdominal aorta below the renal arteries. A second cannula (Male Luer to Hose Barb Adapter, 45518-46, Cole Parmer, Vernon Hills, IL) was used to cannulate the inferior vena cava for venous drainage. A third cannula (PE-10-100 polyethylene tubing; 0.011″ ID × 0.025″ OD; SAI Infusion Technologies) was used to cannulate the ureter. The suprarenal aorta was cross clamped, and 15 mL of University of Wisconsin (UW) solution containing 500 IU of heparin was perfused at 0–4 °C. The suprarenal aorta and vena cava were ligated and divided above the left renal vessels. The kidney was explanted and stored in UW at 4 °C until use (30 min to 2 h).

Perfusion CPA loading

Kidneys were connected to a custom-built multithermic perfusion system for pressure- or flow-regulated perfusion[17,18]. When VS55 was used as the CPA, ramp loading was performed as previously reported[44]. When VMP was used as the CPA, we first used the loading protocol developed for that CPA[21], but changed to a modified protocol as follows. The kidney was first flushed for 20 min with a modified carrier solution (diluent in preservation solution in which CPA cocktails are prepared). In this case, the carrier was LM5-XZ, based on the original VMP carrier (LM5)[21], but containing synthetic ice-blockers X-1000 and Z-1000[43]. This solution helped improve vascular perfusion due to its oncotic properties. We then introduced VMP by ramping the concentration from 0 to 5 M by 50 mM/min, during which the flow rate decreased due to rising viscosity. At 5 M, we held the concentration steady for 10 min to allow for osmotic equilibration and then stepped up to full-strength VMP (8.4 M) to minimize exposure times at the highest CPA concentrations. Simultaneously, we increased the perfusion pressure from 40 to 60 mmHg to maintain adequate flow rates during the 25 min full-strength VMP perfusion. The perfusion loading of VMP took 155 min in total.

Loading of nanoparticles

Following the last step of VMP loading, 10 mg Fe/mL IONPs in VMP was perfused via syringe pump at 4 °C. Perfusion duration was 4–5 min at a constant flow rate of 0.5 mL/min, and the loading pressure was monitored[18]. After perfusion, the kidney was disconnected and placed in a 2 × 3-inch polyethylene bag (McMaster-Carr, Elmhurst, IL) prefilled with ~20 mL of VMP with 4 mg Fe/mL IONPs at 4 °C. A fiberoptic temperature probe (Qualitrol, Fairport, NY) was placed in the solution adjacent to the kidney to record the temperature, and a data logger (Qualitrol T/Guard, Fairport, NY) was used to record the thermal history at 1 s intervals.

Mass transport model

CPA and water transport inside the kidney were simulated by Krogh cylinder model[45]. In the Krogh model, the kidney is represented by many parallel identical cylindrical/hexagonal prism units, each consisting of a central capillary surrounded by tissue. The mass transfer in the whole kidney is analyzed by considering the processes in such a unit to be typical and representative of the processes in the whole organ. The transport from the capillary vasculature into the extravascular space is assumed to occur across a membrane (composite basement and cell membrane) that can be described by coupled solute/solvent flow using irreversible thermodynamics as described by the Kedem-Katchalsky (KK) equations. Three important parameters used in the KK equations are L_p (hydraulic conductivity, [m^3/(N s)]), ω (CPA permeability, [mol/(N s)]), and σ (reflection coefficient). The values of those parameters were $L_p = 1.5 \times 10^{-14}$ m^3/(N s), $\omega = 7.0 \times 10^{-13}$ mol/(N s) and $\sigma = 0.1$, which were determined in preliminary experiments. The combined Krogh cylinder and KK modeling were applied to the previous VMP loading protocol[21] and the modified protocol developed in this study.

Vitrification of kidneys

Vitrification was achieved in a controlled rate freezer (Kryo 560-16, Planer Ltd, Middlesex, UK). The controlled rate freezer was pre-programmed to start at a chamber temperature ($T_{chamber}$) = 0 °C and cool down to $T_{chamber}$ = −122 °C at a ramp rate of −40 °C/min. A 25 min annealing step was introduced when the chamber reached −122 °C (just above the glass transition temperature (T_g)) to allow the organ to thermally equilibrate and reduce the introduction of thermal stress before the glass transition. After the annealing step, a slower ramp rate of −5 °C/min was used to cool from −122 °C down to −150 °C, reducing the build-up of thermal stress as the organ entered and cooled in the glassy phase. At $T_{chamber}$ = −150 °C, a 10-min temperature-hold step was used to equilibrate the organ to the storage temperature (−150 °C) before rapid transfer to a −150 °C freezer (PHC Corporation of North America, Wood Dale, IL) for storage until rewarming.

Nanowarming of kidneys

Nanowarming was conducted using a 15 kW radiofrequency (RF) coil (AMF Life Systems, Auburn Hills, Michigan) at 94% power (provides an RF field at 63 kA/m and 180 kHz with field variation ≤ ±5% across the ~80 mL coil bore)[14]. The bag containing the vitrified kidney was transferred from the −150 °C storage freezer to a Styrofoam container chamber containing liquid nitrogen and vapor (which maintained the temperature near −150 °C for > 30 min) to move the kidney to the RF device. The sample was then transferred from the Styrofoam container to the center of the coil, and the alternating magnetic field was switched ON to initiate nanowarming. A data logger was used to record the thermal heating history. The field was switched OFF when the temperature reached −25 °C (above the melting temperature of VMP). The bag containing the kidney was removed from the coil, placed on ice, and CPA unloading began within 3 min.

Perfusion unloading of kidneys

The kidney was reconnected to the perfusion system and unloading started by perfusing 4.2 M VMP with 300 mM mannitol for 15 min. Mannitol was added to reduce cell swelling during exposure to the relatively hypotonic unloading solutions. Then the concentration of CPA was ramped down from 4.2 M VMP with 300 mM mannitol to 0 M (LM5-XZ) over 120 min. The VMP ramping rate was −35 mM/min, and the mannitol ramping rate was −2.5 mM/min. The kidney was then flushed with LM5-XZ for 30 min. The perfusion unloading took 165 min in total, and pressure was kept at 40 mmHg and temperature at 0-4 °C for the entire process. After unloading, the kidney was flushed with cold UW hypothermic preservation solution, disconnected from the perfusion circuit, and placed back in UW solution on ice.

Micro-CT imaging and histology

Micro-CT was performed at a resolution of 0.061 mm. Briefly, the kidneys were scanned on a micro-CT imaging system (NIKON XT H 225, Nikon Metrology, MI) with an accelerating voltage of 65 kV and current of 95 μA[46]. The vitrified kidney in a cryobag was held in LN2 vapor (−150 °C) in a Styrofoam container during imaging. Separate tubes of water and air at room temperature were attached to the top of the container to serve as calibration references for determining radiodensity in Hounsfield units (HU). The images were reconstructed to reduce beam hardening artifacts and improve image quality (3D CT pro, Nikon Metrology, MI). The images were converted to unsigned 16-bit float images, post-processed (VGstudio Max 3.2, Volume Graphics, NC), and exported as DICOM images for a final analysis using MATLAB (MathWorks).

Histology with hematoxylin and eosin (H&E) or Periodic acid–Schiff (PAS) was also performed as routine[18]. The kidney slices were digitized for histopathological analysis, and histologic interpretation was performed in a blinded fashion by a clinical pathologist.

Differential scanning calorimetry

A differential scanning calorimeter (DSC, Model Q1000, TA Instruments, New Castle, DE) was used to record thermograms (heat flow vs. temperature) of crystallization events in samples during cooling and rewarming. All calculations for DSC measurements are performed in the thermal analysis software (Universal Analysis 2000, TA Instruments). A parameter (i.e., ice fraction (%)) was primarily calculated at a given cooling and/or warming rate, as described previously[47]. CCR and CWR were calculated from the measured heat flow using ice fractions following previously published methods[48,49]. T_m (peak) and T_g (inflection point) of heating were calculated using the heating cycle of DSC heat flow curves thermogram[50].

Normothermic machine perfusion

Normothermic machine perfusion of kidneys was performed as we have reported for livers[19] with brief modifications. The arterial perfusate was a modified Krebs–Henseleit Bicarbonate Buffer (KHB, 120 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4,1.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 0.1 mM EDTA) that was supplemented with 5.54 mM glucose, 5 g/L BSA, 1 g/L amino acids, and 0.5 g/L creatinine. Pressure was measured with a sensor at the arterial perfusion catheter (PREPS-N-000, PendoTECH, Princeton, NJ), and the flow rate was adjusted to maintain 90–110 mmHg. Oxygenation was achieved with carboxygen (95% O_2 and 5% CO_2), and the temperature was maintained at 37 °C with an inline heat exchanger. Samples from the vein and ureter were taken at 10-min intervals and analyzed using an ABL90 FLEX Plus point of care system (Radiometer, Brea, CA) and enzymatic creatinine assay (Rat Creatinine Kit #80340, Crystal Chem, IL, USA). Oxygen consumption rate was calculated as described[51], creatinine clearance as described[52], and glucose consumption rate was calculated as (arterial glucose concentration − venous glucose concentration) × arterial flow/kidney weight.

Kidney transplantation

Inbred male Lewis rats (450–525 g) were used as donors and recipients for syngeneic transplants, and outbred Sprague Dawley rats (450–525 g) were used as donors and recipients in a limited number of outbred rat transplants. Baseline body mass and laboratories were obtained on pre-operative day −1. Recipient rats were anesthetized with isoflurane, and a laparotomy was performed. The bowel and mesentery were mobilized and retracted. The abdominal aorta, inferior vena cava, and left renal artery and vein were dissected free of adherent tissue. The left native kidney was mobilized free of Gerota's fascia and skeletonized down to the hilar vessels. The left native ureter was transected close to the hilum for maximum length. The left renal artery was ligated and divided. A microvascular clamp was placed on the renal vein, and the vein was divided to explant the native kidney.

Two arterial microvascular clamps were placed proximal and distal to the anastomotic site on the recipient's infrarenal aorta. An arteriotomy was performed and immediately flushed with heparinized saline. The donor kidney was flushed with cold normal saline, wrapped in cold gauze, lowered into the field, and periodically dripped with cold saline to maintain hypothermia until reperfusion. An end-to-side (donor end aorta to recipient side aorta) anastomosis was performed using running 10–0 Prolene suture. The venous anastomosis was then performed either using the cuff technique[53] in an end-to-end fashion with the native left renal vein or by an end-to-side sutured anastomosis between the donor renal vein and the inferior vena cava. Once the arterial and venous anastomoses were complete, the venous and arterial clamps were released to reperfuse the kidney. Warm normal saline was poured onto the reperfused kidney. After visualization of urine in the catheter, the right native renal artery, vein, and ureter were ligated and divided, and the native right kidney was explanted. An end-to-end anastomosis of the donor and recipient ureter was performed over a PE-10 stent. The abdomen was closed in layers using 4-0 PDS in the abdominal wall and 5–0 PDS in the skin. The rat was incubated on a heating pad until recovery from anesthesia while providing supplemental oxygen.

Venous blood gas was measured daily from day −1 to day +20 (ABL90 FLEX Plus). Serum creatinine was measured daily from day −1 to day +30 (Rat Creatinine Kit), as was body weight. The rats were euthanized on postoperative day 30. The kidneys were recovered for histology, and serum and urine were collected for analysis by a clinical Vet Med laboratory. The estimated glomerular filtration rate (eGFR) was calculated as described[28].

Statistical analysis

Statistical analysis was performed in R version 4.2.1 (R Foundation for Statistical Computing, Vienna, Austria). The number of biological replicates is indicated in each figure legend and the accompanying

statistical treatment summary in the Supplementary materials. All measurements represent distinct biological replicates taken from individual kidneys or rats, except for time series data, where kidneys/rats were resampled at each time point. For comparison of continuous variables, normality was established using the Shapiro–Wilk test or qq plots and distribution histograms. Homogeneity of variance was assessed using Levene's test. For normal (or near-normal) group comparisons, ANOVA testing with pairwise post hoc *t*-test for single comparisons, Tukey HSD test for multiple comparisons with equal variance, or the Games–Howell test if unequal variance were used to determine statistical differences. Non-normal variables were tested using the non-parametric Kruskal–Wallis test for overall significance and the pairwise Wilcox (Mann–Whitney *U*) test for individual group comparison. Categorical variable comparison was performed via Pearson's Chi-squared test. *P* values were adjusted for multiple comparisons. Complete statistical treatment for each figure is presented in the supplementary files (Supplementary Data 1). Statistical testing was two-sided, and a P value of <0.05 was considered significant.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

All data generated or analyzed during this study are included in this published article (and its supplementary information files). Source data are provided with this paper.

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Author contributions

Z.H. performed the CPA loading and unloading, vitrification and nanowarming, micro-CT experiments, and mass transport modeling. He also prepared figures and edited the paper. J.S.R. performed the surgical procedures, normothermic machine perfusion, kidney transplants, and histology. He also prepared figures and edited the paper. B.N. assisted with the development of the surgical model and edited the paper. J.P.A. refined nanoparticle synthesis, production, CPA stability, and delivery and edited the paper. L.G. performed some experiments and edited the paper. M.L.E. managed the project, performed some experiments, and edited the paper. S.M.W. and T.L.P. edited the paper and added content. E.B.F. and J.C.B. conceived and supervised the project, analyzed the data, prepared figures, and wrote and edited the paper.

Competing interests

The authors declare the following intellectual property related to this work: "Cryopreservative compositions and methods" Pending U.S. Patent Applications 14/775,998 and 17/579,369 (M.L.E and J.C.B.). All other authors declare no competing interest.

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THE CONVERSATION

from THECONVERSATION.COM

Illuminating the brain one neuron and synapse at a time –

5 essential reads about how researchers are using new tools to map its structure and function

The U.S. BRAIN Initiative seeks to elucidate the connection between brain structure and function. Science Photo Library - PASIEKA/Brand X Pictures via Getty Images

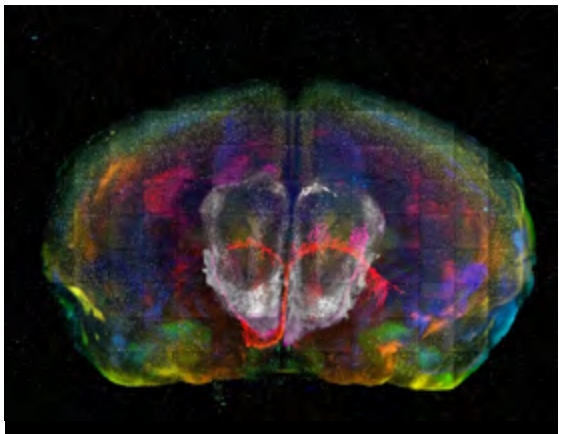
Scientists know both a lot and very little about the brain. With billions of neurons and trillions of connections among them, and the experimental limitations of examining the seat of consciousness and bodily function, studying the human brain is a technical, theoretical and ethical challenge. And one of the biggest challenges is perhaps one of the most fundamental – seeing what it looks like in action.

The U.S. Brain Research Through Advancing Innovative Neurotechnologies (BRAIN) Initiative is a collaboration among the National Institutes of Health, Defense Advanced Research Projects Agency, National Science Foundation, Food and Drug Administration and Intelligence Advanced Research Projects Activity and others. Since its inception in 2013, its goal has been to develop and use new technologies to examine how each neuron and neural circuit comes together to "record, process, utilize, store, and retrieve vast quantities of information, all at the speed of thought."

Just as genomic sequencing enabled the creation of a comprehensive map of the human genome, tools that elucidate the connection between brain structure and function could help researchers answer long-standing questions about how the brain works, both in sickness and in health.

These five stories from our archives cover research that has been funded by or advances the goals of the BRAIN Initiative, detailing a slice of what's next in neuroscience.

1. Mapping the brain



Zooming in on this high-resolution image of a mouse brain reveals rectangular lines where individual image tiles were stitched together, each colored dot representing a specific cell type. Yongsoo Kim, CC BY-NC-ND

Attempts to map the structure of the brain date back to antiquity, when philosophers and scholars had only the unaided eye to map anatomy to function. New visualization techniques in the 20th century led to the discovery that, just like all the other organs of the body, the brain is composed of individual cells – neurons.

Now, further advances in microscopy that make use of artificial intelligence and genomics have allowed scientists not just to see each individual neuron in the entire brain, but also to identify the connections among them and begin to ascertain their function.

Neuroscientist Yongsoo Kim of Penn State likened this method to a photo mosaic, piecing together areas of the brain that haven't been charted before. "It's like building a Google map of the brain," wrote Kim. "By combining millions of individual street photos, you can zoom in to see each street corner and zoom out to see an entire city." Creating these high-resolution maps, he wrote, could help scientists develop new theories on how the brain works and lead to better treatments for brain disorders like dementia.

Read More: [Mapping how the 100 billion cells in the brain all fit together is the brave new world of neuroscience]

Harvard researchers modeled how folding reduces instability caused by differential growth rates in the brain.

2. Brain folds and wrinkles

Another fundamental question researchers have been puzzling over is how the brain develops the bumps and grooves that riddle its surface. Until roughly the second trimester of fetal development, the human brain is completely smooth.

Scientists have proposed a number of theories on the mechanics of brain folding. One of them, differential tangential growth, posits that folds form because of a mismatch in growth rates between the outer and inner layers of the brain. To ease the forces compressing the outer layer and restore structural stability, the layers buckle and fold.

Biomechanical engineer Mir Jalil Razavi and computer scientist Weiying Dai of Binghamton University created models to clarify this theory. They identified other factors that may also be at play, like the number of axons – the part of the neuron that transmits electrical signals – in a particular area. "Our brain models provide a potential explanation for why brains may form abnormally during development, highlighting the important role that the brain's structure plays in its proper functioning," they wrote.

Read More: [Brain wrinkles and folds matter – researchers are studying the mechanics of how they form]

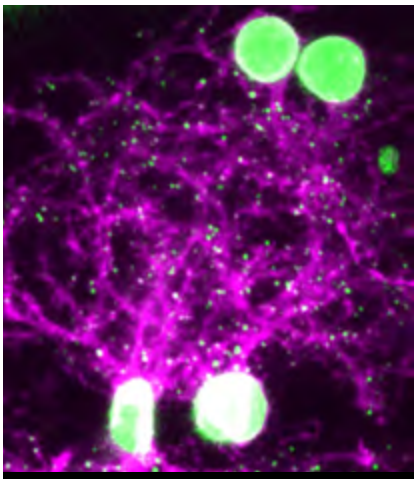
3. Where memories are stored

Just like the RAM in a computer, memories take up physical space in the brain. Researchers have hypothesized that memories may be stored by rearranging the connections, or synapses, among neurons. While this theory has largely been confirmed by observing changes in the electrical signals neurons produce after memory formation, what triggers these changes has been unclear.

Biomedical engineer Don Arnold of the University of Southern California and his colleagues took a mapping approach. They compared 3D maps of zebrafish synapses before and after memory formation – namely, learning to associate a light with an unpleasant stimulus. They found that one brain region gained synapses while another's were destroyed, indicating that associative memories may be a result of the formation and loss of connections among neurons.

These findings imply that it might one day be possible to treat conditions like PTSD by physically erasing the associative memory linking a harmless trigger with a traumatic experience. More research is needed, and there are obvious ethical considerations to address. "Nevertheless," Arnold wrote, "it's tempting to imagine a distant future in which synaptic surgery could remove bad memories."

Read More: **Where are memories stored in the brain? New research suggests they may be in the connections between your brain cells**



Neurons in a live fish brain, with synapses colored green. Zhuowei Du and Don B. Arnold, CC BY-NC-ND

4. Seizures hijack memory pathways

Seizures are sudden surges of electrical activity in the brain. People who experience temporal lobe seizures are sometimes unable to remember what happened immediately prior. This may be due to disruptions to the circuitry in the hippocampus, the part of the temporal lobe key to memory consolidation.

Neurology researchers Anastasia Brodovskaya and Jaideep Kapur of the University of Virginia hypothesized that seizures can cause memory loss by using the same pathways the brain uses to process memories. They mapped the neurons of mice learning to navigate a maze and during induced seizures, finding that both cases activated the same brain circuits.

"Because they use the same brain pathways, seizures can disrupt the memory consolidation process by taking over the circuit," they wrote. "This meant that seizures can hijack the memory pathways and cause amnesia."

Read More: **Seizures can cause memory loss, and brain-mapping research suggests one reason why**

5. What the nose knows

What the eye can't see, the nose can for many organisms. From dogs to mosquitoes, many animals behave in ways that allow them to detect and pursue an odor long before its source comes into view.

Scientists John Crimaldi, Brian Smith, Elizabeth Hong and Nathan Urban of the Odor2Action research network use technology to study olfaction, or sense of smell. They trace how the shape of an odor plume informs how it will be detected, how those odor molecules are translated into electrical signals in the brain, and how these electrical signals are reformatted into useful information that influence behavior.

This video from the Wachowiak Lab at the University of Utah shows the activity of the olfactory bulb in a mouse brain. Each odor the mouse is exposed to makes different combinations of neurons light up.

A better understanding of the olfactory system, they wrote, can lead to the development of electronic noses that make searching for chemical weapons and disaster victims safer for people and animals. They also believe that examining the olfactory system can help advance study of the brain. "Its relative simplicity is what allows scientists like us to study it from end to end and learn how the brain works as a whole," they wrote.

While a grand unified theory of the brain still remains elusive, new tools and techniques are helping researchers excavate its hidden depths. As Crimaldi and his team put it, "An exciting future in scientific and medical development, we believe, is right under our noses."

Read More: **From odor to action – how smells are processed in the brain and influence behavior**

MEDICAL NEWS TODAY

from MEDICALNEWSTODAY.COM

Scientists use genetic rewiring to increase lifespan of cells

Have scientists found new clues to improve longevity?

- **Human lifespans have increased throughout the 20th and 21st centuries, but those increases are slowing down, so scientists continue to hunt for ways to improve longevity.**
- **Healthful diets, hygiene, and medical care have all contributed to the increases in lifespan, and now researchers are looking to genetics.**
- **In a new proof-of-concept study, researchers almost doubled the lifespan of yeast cells by genetically rewiring the circuit that controls aging.**
- **Their findings may pave the way to increasing longevity in more complex organisms and, possibly, even in people.**

We all strive to live long and healthy lives, but can you lengthen your life? The National Institutes of Health (NIH) tell us that the best way to increase lifespan is to eat well, get quality sleep, exercise regularly, get regular medical checkups, and avoid bad habits such as smoking and drinking excessive alcohol.

Scientists working to combat the aging process have extended the lifespans of worms, mice, and even monkeys. But could they do the same for people?

Now, a team from the University of California, San Diego, has managed to extend the lifespan of a simple organism by around 80% by manipulating the genetic circuit that controls aging.

The proof-of-concept study carried out in yeast is published in Science.

Synthetic biology behind cell aging

The UC San Diego research team has been studying cell aging for several years, discovering that cells follow a cascade of molecular changes throughout their life until they

eventually degenerate and die. However, they found that not all cells age in the same way, and this was the focus of their new research.

They first used computer simulations of cell aging to test their ideas before moving on to modifying the aging circuits in the single-celled yeast Saccharomyces cerevisiae.

They discovered that the cells followed one of two aging routes. Around half of the cells underwent a gradual decline in the stability of their DNA (nucleolar aging); for the rest, the aging path was characterized by a decline in their mitochondriaTrusted Source – the organelles that provide energy for the cell (mitochondrial aging).

Manipulating gene expression to increase lifespan

To control the aging of the cells, they manipulated the expression of two conserved transcriptional regulatorsTrusted Source — molecules that determine which genes are active in the cell. Silent information regulator 2 (Sir2) drives nucleolar decline (leading to DNA instability), and heme activator protein 4 (Hap4) is associated with the mitochondrial activity.

When one of these regulators is expressed and therefore active, it stops the other from being expressed, so the researchers engineered a synthetic gene oscillator to rewire this mechanism. By generating sustained oscillations between the two types of cellular degeneration in individual cells, they prevented the cells from following either of the two aging routes. The lifespan of these cells increased.

Prof. Nan Hao, senior author of the study and co-director of the UC San Diego's Synthetic Biology Institute, told Medical News Today:

"Our work is a proof-of-concept, showing that, like mechanical engineers can fix and enhance our cars so that they can last longer, we can also use the same engineering approach to modify and enhance our cells to live longer. The highlight is our approach to achieve that: using computers to simulate the natural aging system and guide the design and rational engineering of the system to extend lifespan."

Lifespan almost doubled after genetic rewiring

By creating the gene oscillator, the scientists made the yeast cells continually switch between the two aging pathways, preventing them from committing to their pre-destined path of decline and death, slowing the cells' degeneration.

Those yeast cells that were synthetically rewired and aged under the direction of the synthetic oscillator had an 82% increase in lifespan compared with control cells.

And the genetic manipulation did not appear to adversely affect them, according to Prof. Hao, who told MNT: "The yeast cells survive nicely with a fast growth rate."

Potential application to improve longevity

> *"This is the first time this computationally-guided engineering-based approach [has been] used in aging research. I can't see why we cannot apply the same strategy to human cells."*
>
> *– Prof. Nan Hao*

All cells contain gene regulatory circuitsTrusted Source that are responsible for many physiological functions, including aging, so in theory, a similar approach could work in human cells.

The aim may not only be to extend the life of more complex organisms but to extend the life of some cells within organisms to prevent degenerative diseases.

However, Prof. Hao cautioned that they do not know whether increasing longevity might affect the cells in other ways:

"That's a deep biological question. Our current hypothesis is that the longevity of the cell is not a trait selected through evolution. Cells have to first be able to survive in the rapidly changing, unpredictable stressful environment."

"There is a possibility that our engineered long-lived cells will be less resistant to certain types of stresses in the environment. So basically, extending longevity might sacrifice some normal functions, but that's just a hypothesis," he added.

Implications on increasing healthy life years for people?

Prof. Hao suggested that there may be potential for this approach in people:

"Both of the two regulators have counterparts in humans, so I do believe that the same strategy could be applied to human cells. In fact, that's our next step in the future."

And Prof. Howard Salis, Principal Investigator at the Salis Lab, Penn State University, who was not involved in the study, agreed:

"If the collective objective of these interventions is to maintain healthier cell states, then the risk and morbidity of age-associated diseases will be reduced."

However, it is very early days, and although this study shows that it is possible to switch off aging mechanisms in a single-celled organism, there are many questions to be answered before the technology might be applied to people.

ScienceNews
INDEPENDENT JOURNALISM SINCE 1921

from SCIENCENEWS.ORG

An AI can decode speech from brain activity with surprising accuracy

The research is still a ways away from helping people who can't communicate through speech

An artificial intelligence can decode words and sentences from brain activity with surprising — but still limited — accuracy. Using only a few seconds of brain activity data, the AI guesses what a person has heard. It lists the correct answer in its top 10 possibilities up to 73 percent of the time, researchers found in a preliminary study.

The AI's "performance was above what many people thought was possible at this stage," says Giovanni Di Liberto, a computer scientist at Trinity College Dublin who was not involved in the research.

Developed at the parent company of Facebook, Meta, the AI could eventually be used to help thousands of people around the world unable to communicate through speech, typing or gestures, researchers report August 25 at arXiv.org. That includes many patients in minimally conscious, locked-in or "vegetative states" — what's now generally known as unresponsive wakefulness syndrome (SN: 2/8/19).

Most existing technologies to help such patients communicate require risky brain surgeries to implant electrodes. This new approach "could provide a viable path to help patients

with communication deficits ... without the use of invasive methods," says neuroscientist Jean-Rémi King, a Meta AI researcher currently at the École Normale Supérieure in Paris.

King and his colleagues trained a computational tool to detect words and sentences on 56,000 hours of speech recordings from 53 languages. The tool, also known as a language model, learned how to recognize specific features of language both at a fine-grained level — think letters or syllables — and at a broader level, such as a word or sentence.

The team applied an AI with this language model to databases from four institutions that included brain activity from 169 volunteers. In these databases, participants listened to various stories and sentences from, for example, Ernest Hemingway's The Old Man and the Sea and Lewis Carroll's Alice's Adventures in Wonderland while the people's brains were scanned using either magnetoencephalography or electroencephalography. Those techniques measure the magnetic or electrical component of brain signals.

Then with the help of a computational method that helps account for physical differences among actual brains, the team tried to decode what participants had heard using just three seconds of brain activity data from each person. The team instructed the AI to align the speech sounds from the story recordings to patterns of brain activity that the AI computed as corresponding to what people were hearing. It then made predictions about what the person might have been hearing during that short time, given more than 1,000 possibilities.

Using magnetoencephalography, or MEG, the correct answer was in the AI's top 10 guesses up to 73 percent of the time, the researchers found. With electroencephalography, that value dropped to no more than 30 percent. "[That MEG] performance is very good," Di Liberto says, but he's less optimistic about its practical use. "What can we do with it? Nothing. Absolutely nothing."

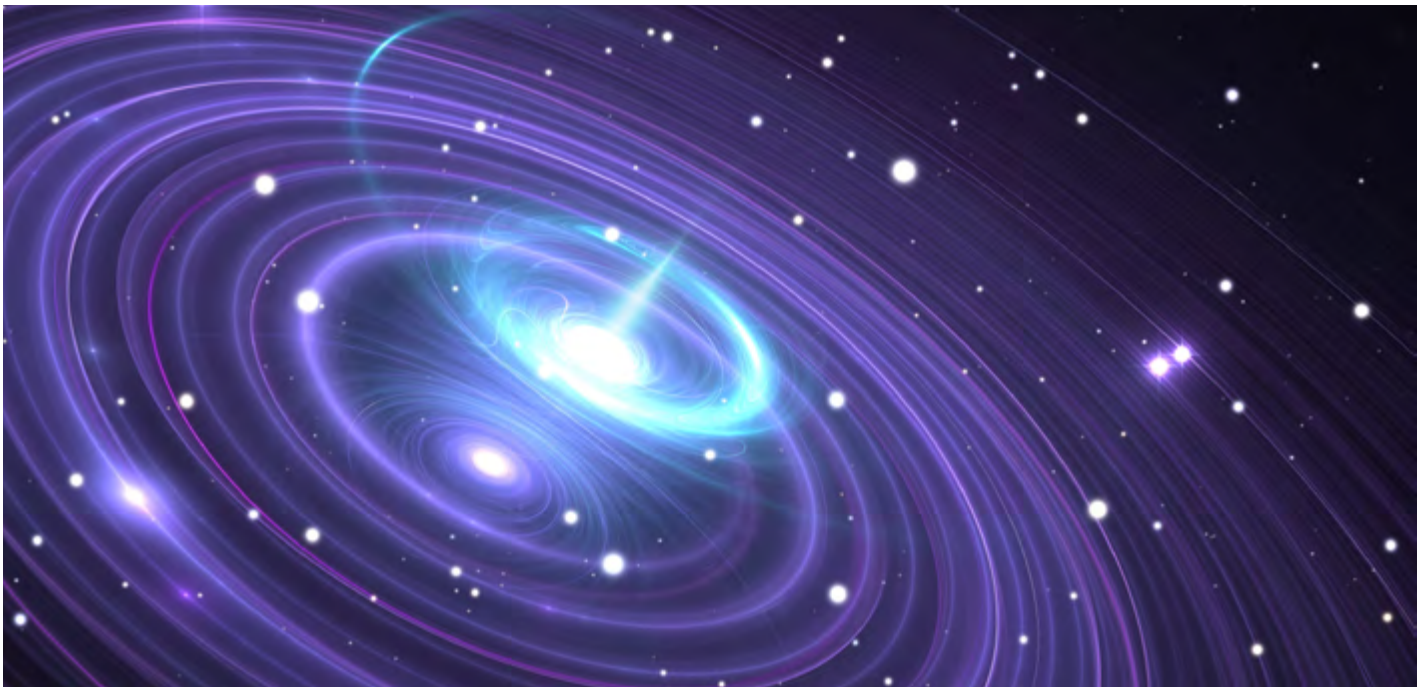
The reason, he says, is that MEG requires a bulky and expensive machine. Bringing this technology to clinics will require scientific innovations that make the machines cheaper and easier to use.

It's also important to understand what "decoding" really means in this study, says Jonathan Brennan, a linguist at the University of Michigan in Ann Arbor. The word is often used to describe the process of deciphering information directly from a source — in this case, speech from brain activity. But the AI could do this only because it was provided a finite list of possible correct answers to make its guesses.

"With language, that's not going to cut it if we want to scale to practical use, because language is infinite," Brennan says.

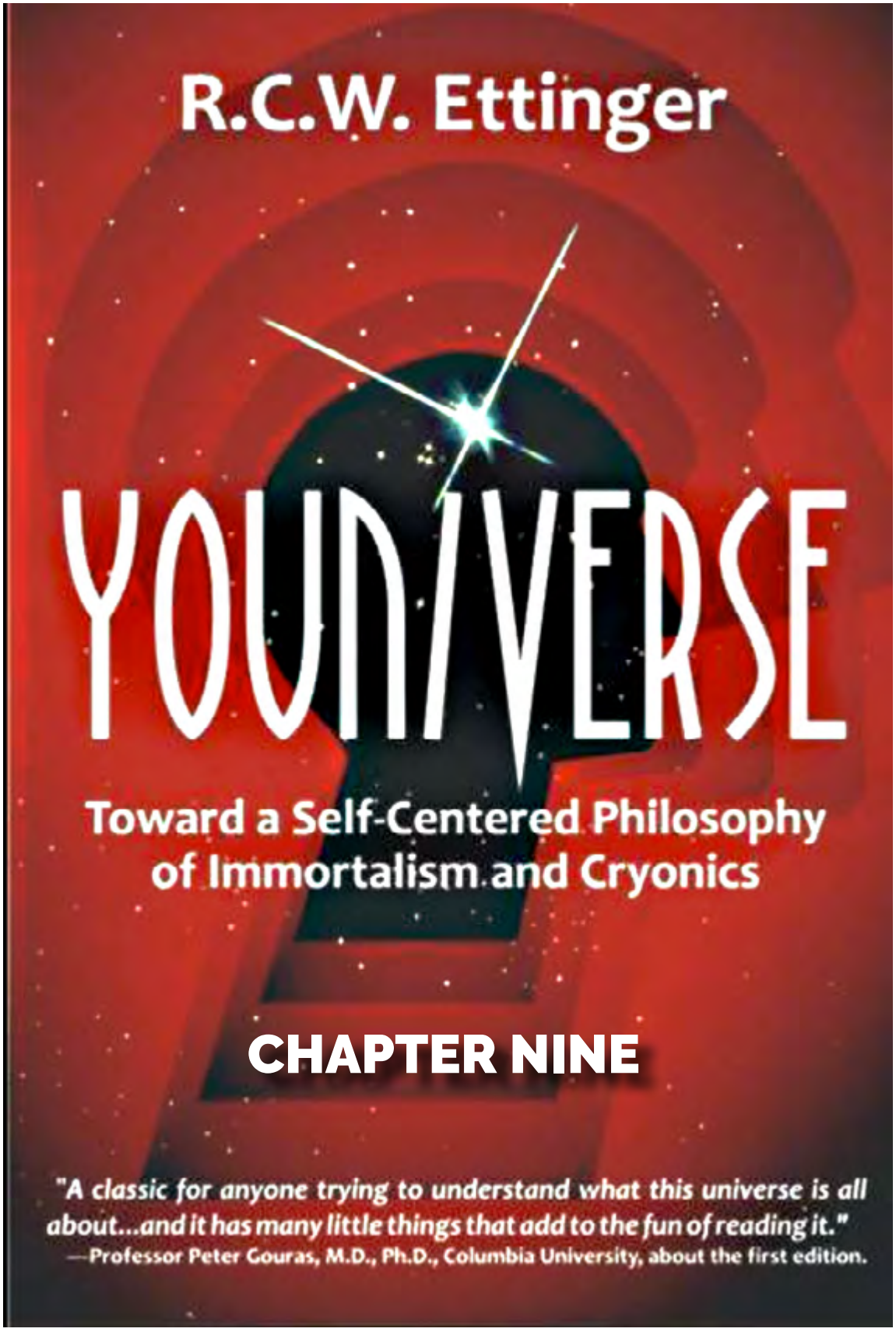
What's more, Di Liberto says, the AI decoded information of participants passively listening to audio, which is not directly relevant to nonverbal patients. For it to become a meaningful communication tool, scientists will need to learn how to decrypt from brain activity what these patients intend on saying, including expressions of hunger, discomfort or a simple "yes" or "no."

The new study is "decoding of speech perception, not production," King agrees. Though speech production is the ultimate goal, for now, "we're quite a long way away."



CI Reading Room

Serializing essential works on cryonics



Chapter 9

Identity & Survival 1 - Meat

Introduction: If your body were frozen after clinical death and revived-cured of your fatal illness, traumas repaired, perhaps rejuvenated and improved—would it be "you" that walks eagerly into the sunlight? Or if you live into a Star Dreck era and patronize a beam-me-up machine for commuting, would the person walking out be the same one who walked in? If the beam-me-up machine were to input one of you and output two, would they both be you? If the "many worlds" interpretation of quantum theory is correct (championed by eminences including David Deutsch), are countless versions of you living and dying right now, enjoying or suffering everything possible? Could "you" live as a computer simulation or emulation?

We have probable answers in the two extreme cases:

a) You are cryopreserved immediately after clinical death by advanced methods, with relatively little damage, and later repaired and revived. This is almost certainly survival, the revived patient being the "same" (in the appropriate sense) as the person who died.

b) You die and are buried or cremated, go to gas and dust, and millions of years later a super-computer "restores" you as a simulation or emulation. This is almost certainly not survival and the emulation, in my opinion, could not reasonably be said to share your identity-if indeed it would be a person at all.

For the intermediate cases, the answers are even less clear, but let's look into the matter.

In 1962, my discussion of criteria of identity and survival (in the first version of The Prospect of Immortality) was the most rigorous and thorough then available, as far as I know. Since then, the field has burgeoned, but remains limited mainly to thought experiments of uncertain value. Our ignorance is still highlighted by the facts that:

1. There is no agreement, or anything approaching it, on the correct criterion or set of criteria of identity or survival. We don't know who or what we are, or what it means to survive.

2. There is no agreement even on the correct or most useful way to phrase the questions.

3. There is not even any agreement on how to choose criteria (criteria for criteria), or what would constitute a proof of a theory of survival.

But I do have a new suggestion or two, and the situation is not hopeless.

Preview: **We will go into considerable detail on the various prior suggestions and viewpoints, for two simple reasons: (1) The issue is vitally important. (2) The questions are very hard to understand, with some of the smartest people in the world calling each other idiots, squabbling for years or decades on end. If you think you understand the problems quickly, you probably don't have a clue. This initial suc- cinct summary isn't going to explain much, but I think it helps to have road signs.**

My tentative view is that the essence of "you" is in your "qualia" as I define them, subjective experiences. The physical basis or anatomy/ physiology of what you "feel" or directly experience has not been ascertained, but I suspect it may be something in the order of a standing wave in the brain, with modulations. I call this the "self circuit."

The key point is that I also postulate that every system and every event has extension or spread in space and time. We bind space and time. We do not live in a film-frame universe; there is no such thing as a point of space or an instant of time. There are also no "homunculi" in the brain, no "central observer" to whom things appear or happen.

Instead, the experience and the experiencer are the same. A quale is not a representation of anything-it is a thing-in- itself, irreducible. In other words, you don't have feelings-rather, you are the sum of your feelings.

To be sure, there is important additional baggage, notably your memories and personality, which are partly separate from your subjective state or qualia. Many people feel they would not exist, or would not want to exist, if deprived of too much of their memories and personalities. The question, however, is not what you would or might choose, but what you rationally ought to choose if suitably informed and given the chance, and my position is that-in almost all circumstances-you ought to choose to continue to exist, to survive.

And, finally, we come to the explicit criterion of survival. You do not survive if your brain is destroyed, or too badly damaged, and then duplicated or emulated as it was when whole. But you survive in the ordinary course of events, from moment to moment and day to day, even though you change, because you overlap your predecessors and successors or continuers. You-future and you-past share space and time and matter with you-present, and this tends to validate your concern for the future and your connection with the past. In other words, the criterion of survival is just a sufficient degree of physical continuity (even though countless scholars have offered thought experiments purporting to disprove this). Now let's start the detailed discussion by reviewing my 1962 summary, almost verbatim, after another brief reminder of why we need to invest this effort.

The most important of all scientific questions-and possibly the profoundest is the nature of the self or of personal identity.

Unless you know what you are, you can scarcely know what you ought to want, which means you cannot know whether your values are valid or how to live. And unless you know what constitutes survival, you can hardly plan for it.

Laugh or cry, the fact remains that only a few lonely souls-even among "philosophers"!-take this problem seriously or personally. I hope here to clarify the problem and also to persuade a few readers that some of the most rarefied strata of philosophy are also among the most personally important.

We begin with a nearly verbatim reproduction of my 1962 discussion in the preliminary version of The Prospect of Immortality. This was seriously off the mark, but nevertheless is a good introduction. After that we will look at some im- proved ideas, as well as new puzzles.

The Problem of Identity, Criteria of Survival: In considering the chances of reviving, curing,

rejuvenating and improving a frozen man, we have to envisage the possibility of some very extensive repairs and alterations. This leads to a number of very perplexing puzzles. As an extreme case, imagine an elderly cancer victim who is not frozen until several hours after death, and then only by crude methods. Almost all the cells of his body have suffered severe damage and are thoroughly dead by present criteria, although some would grow in culture and we assume a small percentage of them have degenerated relatively little. But after enough centuries pass, medical art at last is ready to deal with him, and for the sake of emphasis, let us assume a grotesque mixture of techniques is used.

When our resuscitee emerges from the hospital, he may be a crazy quilt of patchwork. His intemal organs-heart, lungs, liver, kidneys, stomach, and all the rest-may be grafts, implanted after being grown in the laboratory from someone else's donor cells. His arms and legs may be bloodless artifacts of fabric, metal and plastic, directed by his own will and complete with sense of touch but extended and flexed by tiny motors. His brain cells may be mostly new, regenerated from the few which could be saved, and some of his memories and personality traits may have had to be imprinted on or into the new cells by microtechniques of chemistry and physics, after being ascertained from the written records.

Striding eagerly into the new world, he feels like a new man. Is he?

Who is this resuscitee? For that matter, who am I and who are you?

Although most resuscitees will not represent such extreme cases-we hope most of us will be frozen by nondamaging methods-nevertheless we cannot sidestep the issue. We are now face to face with one of the principal unsolved problems of philosophy and/or biology, which now becomes one of prime importance in an exceedingly practical way, namely that concerning the nature of "self".

What characterizes an individual? What is the soul, or essence, or ego? This seemingly abstruse question will shortly be seen to have ramifications in almost every area of practical affairs; it will be the subject of countless newspaper editorials and Congressional investigations, and will reach the Supreme Court of the United States.

We can bring the problem into better focus by putting it in the form of two questions. First, how can we distinguish one man from another? Second, how can we distinguish life from death?

Later I shall enlarge on some tentative partial answers. First we can illuminate the question, and perceive some of its difficulties and subtleties, by considering a series of experiments. Some of these experiments are imaginary, but perhaps not impossible in principle, while others have actually been performed.

Experiment 1. We allow a man to grow older.

Legally, he retains his identity; and also subjectively, and also in the minds of his acquaintances (usually). Yet, most of the material of his body is replaced and changed; his memories change, and some are lost; his outlook and personality change. It is even possible that an old acquaintance, seeing him again after many years, might refuse to believe he is the same person.

On first considering this experiment, we are apt to feel slightly disturbed, but to retain a

vague conviction that "basically" the man is unchanged. We may feel that the physical and psychological continuity has some bearing on the question.

Experiment 2. We watch a sudden, drastic change in a man's personality and physique, brought about by physical damage, or disease, or emotional shock, or some combination of these. Such has often occurred. Afterwards, there may be little resemblance to the previous man, mentally or physically. There may be "total" amnesia, although he may recover capability of speech. Of course he retains, e.g., the same fingerprints, and the same genes. But it would be absurd to say the main part of a man is his skin; and identical twins have the same genes, yet are separate individuals.

Although the physical material of his body is the same stuff, he seems-and feels like a different person. Now we are more seriously disturbed, because the main continuity is merely physical; there is a fairly sharp discontinuity in personal- ity. One might say with some plausibility that a man was destroyed, and another man was created, inheriting the tissues of his predecessor's body.

Experiment 3. We observe an extreme case of "split personality".

It is commonly believed that sometimes two (or even more) disparate personalities seem to occupy the same body, sometimes one exercising control and sometimes the other. Partly separate sets of memories may be involved. The two

"persons" in the same body may dislike each other; they may be able to communicate only by writing notes when domi- nant, for the other to read when his turn comes.

We may be inclined to dismiss this phenomenon by talking about psychosis or pathology. This tendency is reinforced by the fact that apparently one of the personalities is usually eventually submerged, or the two are integrated, leaving us with the impression that "really" there was only one person all along. Nevertheless, the personalities may for a time seem completely distinct by behavioral tests, and subjectively the difference is obviously real. This may leave us with a disturbing impression that possibly the essence of individuality lies after all in the personality, in the pattern of the brain's activity, and in its memory.

Experiment 4. Applying biochemical or microsurgical techniques to a newly fertilized human ovum, we force it to divide and separate, thereby producing identical twins where the undisturbed cell would have developed as a single indi- vidual. (Similar experiments have been performed with animals.)

An ordinary individual should probably be said to originate at the "moment" of conception. At any rate, there does not seem to be any other suitable time-certainly not the time of birth, because a Caesarean operation would have produced a living individual as well; and choice of any other stage of development of the foetus would be quite arbitrary.

Our brief, coarse, physical interference has resulted in two lives, two individuals, where before there was one. In a sense, we have created one life. Or perhaps we have destroyed one life, and created two, since neither individual is quite the same as the original one would have been.

Although it does not by any means constitute proof, the fact that a mere, crude, mechanical

or chemical manipulation can "create a soul" suggests that such portentous terms as "soul" and "individuality" may represent nothing more than clumsy attempts to abstract from, or even inject into, a system certain "qualities" which have only a limited relation to physical reality.

Experiment 5. By super-surgical techniques (which may not be far in the future) we lift the brains from the skulls of two men, and interchange them.

This experiment might seem trivial to some. Most of us, after thinking it over, will agree it is the brain which is important, and not the arms, nor the legs, nor even the face. If Joe puts on a mask resembling Jim, he is still Joe; and even if the "mask" is of living flesh and extends to the whole body, our conclusion will probably be the same. The assemblage of Joe's brain in Jim's body will probably be identified as Joe. But at least two factors make this experiment non-trivial.

First, if the experiment were actually performed and not merely discussed, the emotional impact on the parties concerned would be powerful. The wives would be severely shaken, as would the subjects. Furthermore, Joe-in-Jim's-body would rapidly change, since personality depends heavily on environment, and the body is an important part of the brain's environment.

Also, we may be willing to admit that Joe's arms, legs, face, and intestines are not essential attributes of Joe-but what about his testicles? If Joe-in-Jim's-body lies with one of their wives, he can only beget Jim's child, since he is using Jim's gonads. The psychiatric and legal problems involved here are formidable indeed.

Some people might be tempted to give up on Joe and Jim altogether, and start afresh with Harry and Henry. In one sense, this is an impractical evasion, since the memories, family rights and property rights cannot be dismissed. From an- other view, it may be a sensible admission that characterization of an individual is to some extent arbitrary.

Once again, the suggestion is that physical systems (i.e., real systems) must in the end be described by physical parame- ters (operationally) and that attempts to pin profound or abstract labels on them, or to categorize them in subjective terms, cannot be completely successful.

Experiment 6. By super-surgical techniques (not yet available) we divide a man's brain in two, separating the left and right halves, and transplant one half into another skull (whose owner has been evicted).

Similar, but less drastic, experiments have been performed. Working with split-brain monkeys, Dr. C. B. Trevarthen has reported that the surgically separated brain halves may learn side by side at the normal rate, as if they were quite indepen- dent. This is most intriguing, even though the brains were not split all the way down to the brain stem, and even though monkeys are not men.

There is also other evidence in the literature which we can summarize, with certain simplifications and exaggerations, as follows. Either half of a brain can take over an individual's functions independently. Normally, one half dominates, and loss of the other half is not too serious. But even if the dominant half is removed, or killed, the other half will take over, learning the needed skills.

There is presently no conclusive evidence that so drastic an experiment as ours would neces-

sarily succeed; but in princi- ple, as far as I know, it might, and we are not at the moment concerned with technical difficulties.

If it did succeed, we would have created a new individual. If the left half was dominant, we might label the original individual Lr; the same skull containing the left half alone after surgery we might call L, and the right half alone, in a dif- ferent skull after the operation, is R.

L thinks of himself as being the same as Lr. R may also think of himself as Lr, recuperated after a sickness, but to the outside world, he may seem to be a new and different, although similar, person. In any case, R is now an individual in his own right, and regards his life to be as precious as anyone else's. He will cling to life with the usual tenacity, and if he sees death approaching, will probably not be consoled by the knowledge that L lives on.

Even more interesting is the attitude of L, the formerly dominant half, now alone in the skull. Suppose that, before the operation, we had told Lr that the dominant half of his brain was diseased, and would have to be removed, but that the other half would take over, albeit with some personality changes and possibly some loss of memory. He would be worried and disturbed, certainly-but he would probably not regard this as a death sentence. In other words, Lr would be consoled well enough by the assurance that R would live on. Yet after the splitting, and transplanting opera- tion, L would regard his own destruction as death, and it would not satisfy him that R lived on, in another body. This experiment seems to suggest again that, psychologically if not logically, the physical continuity is an important consideration.

Experiment 7. A man is resuscitated after a short period of clinical death, with some loss of memory and some change in personality.

This experiment has actually been performed many times. (97) Death was real by the usual clinical tests (no respiration, no heartbeat) but of course most of the cells remained alive, and most people would say that he had not "really" died, and that he was certainly the same person afterward. This experiment is important only as background for the following ones.

Experiment 8. A man dies, and lies unattended for a couple of days, passing through biological death and cellular death. But now a marvel occurs; a space ship arrives from a planet of the star Arcturus, carrying a supersurgeon of an elder race, who applies his arts and cures the man of death and decay, as well as his lesser ailments. (It is not, of course, suggested that any such elder race exists; the experiment is purely hypothetical, but as far as we know today, it is not im- possible in principle.)

The implications are apt to shake us. If decay is to be regarded as just another disease, with a possibility of cure, then when may the body be considered truly dead? If "truly" dead be taken to mean "permanently" dead, then we may never know when we are in the presence of death, since the criterion is not what has already happened to the man, but what is going to happen to him in the (endless?) future.

Experiment 9. A man dies, and decays, and his components are scattered. But after a long time, a super-being somehow collects his atoms and reassembles them, and the man is re-created.

Once more, the difficulty or even impossibil-

ity of the experiment is not important. We also disregard the question of the possibility of identifying individual elementary particles. Is it the "same" man, in spite of the sharp physical discontinuity in time? If memory, personality, and physical substance are all the same, perhaps most of us would think so, even though we are disturbed by the black gulf of death intervening. But if we so admit, we must open the door even wider.

Experiment 10. We repeat the previous experiment, but with a less faithful reproduction, involving perhaps only some of the original atoms and only a moderately good copy. Is it still the same man?

Again, perhaps, we wonder if there is really any such thing as an individual in any clear-cut and fundamental sense.

Experiment 11. We repeat experiment 10, making a moderately good reconstruction of a man, but this time without trying to use salvaged material.

Now, according to the generally accepted interpretation of quantum theory, there is in principle as well as in practice no way to "tag" individual particles, e.g., the atoms or molecules of a man's brain; equivalent particles are completely indistinguishable, and in general it does not even make sense to ask whether the atoms of the reconstructed body are the "same" atoms that were in the original body. Those unfamiliar with the theory, who find this notion hard to stomach, may consult any of the standard texts.

If we accept this view, then a test of individuality becomes still more difficult, because the criteria of identity of material substance and continuity of material substance become difficult or impossible to apply.

Experiment 12. We discover how to grow or to construct functional replicas of the parts of the brain-possibly biological in nature, possibly mechanical, but at any rate distinguishable from natural units by special tests, although not distinguishable in function. The units might be cells, or they might be larger or smaller components. Now we operate on our subject from time to time, in each operation substituting some artificial brain parts for the natural ones. The subject notices no change in himself, yet when the experiment is finally over, we have in effect a "robot"! Does the "robot" have the same identity as the original man?

Experiment 13. We perform the same experiment as 12, but more quickly.

In a single, long operation, we keep replacing natural brain components with artificial ones (and the rest of the body likewise) until all the original bodily material is in the garbage disposal, and a "robot" lies on the operating table, an artifi- cial man whose memories and personality closely duplicate those of the original.

Perhaps some would feel the "robot" was indeed the man, basing the identity in the continuity, on the fact that there was never a sharp dividing line in time where one could say man ended and robot began. Others, well steeped in democracy and willing to apply political principles to biology, might think the robot was not the man, and ceased to be the man when half the material was artificial.

The subject himself, before the operation, would probably regard it as a death sentence. And yet this seems odd, since there is so little real difference between experiments 13 and 12; 13 merely speeds things up. Perhaps sufficient persuasion could convince the subject

that the operation did not represent death; he might even be made to prefer a single operation to the nuisance of a series of operations.

Experiment 14. We assume, as in the previous two experiments, that we can make synthetic body and brain components. We also assume that somehow we can make sufficiently accurate nondestructive analyses of individuals. We proceed to analyze a subject, and then build a replica or twin of him, complete with memories.

Does the identity of our subject now belong equally to the robot" twin? It might seem absurd to say so-but compare the previous experiment. There is scarcely any difference, especially since in 13 the subject was under anesthesia during the operation; 13 was virtually equivalent to destroying the subject, then building a robot twin. The only real difference be- tween 13 and 14 is that, in 14, both the original and the duplicate survive.

Experiments 15, 16 and 17. We repeat experiments 12, 13, and 14 respectively, but instead of using artificial parts we use ordinary biological material, perhaps obtained by culturing the subject's own cells and conditioning the resultant units appropriately. Does this make any difference?

In logic, one would think perhaps not, but blood is thicker than water. Some people might make a different decision on 15 and 16 than on 12 and 13.

Experiment 18. We assume the truth of an assertion sometimes heard, viz., that in certain types of surgery, a patient under certain types of anesthesia suffers pain, although he does not awaken and afterwards does not remember the pain. The experiment consists in performing such an operation.

Most of us do not fear such operations, because we remember no pain in previous experiences, and because authoritative persons assure us we need not worry. Even a warning that the pain under anesthesia is real is unlikely to disturb us much, if we are not of very nervous temperament. Still less do we fear ordinary deep anesthesia, in which there seems to be no pain on any level, even though for the conscious mind, this gulf is like that of death. Yet a child, or a person of morbid imagina- tion might be intensely frightened by these prospects.

Thus again we note a possible discrepancy between the logical and the psychological.

Experiment 19. A Moslem warrior is persuaded to give his life joyfully in a "holy war", convinced that the moment his throat is cut he will awaken in Paradise to be entertained by houris.

We draw the obvious but useful conclusion that, from the standpoint of present serenity, it is merely the prospect of immortality that is important.

Experiment 20. We pull out all the stops, and assume we can make a synthetic chemical-electronic-mechanical brain which can, among other things, duplicate all the functions of a particular human brain, and possesses the same per- sonality and memory as the human brain. We also assume that there is complete but controlled inter-connection between the human brain and the machine brain: that is, we can, at will, remove any segments or functions of the human brain from the joint circuit and replace them by machine-components, or vice versa.

In a schematic sense, then, we envisage each of the two brains, the biological one and the

mechanical one, as an electronic circuit spread out on a huge "bread board" with complete accessibility. From the two sets of components, by plugging in suitable leads, we can patch together a single functioning unit, the bypassed elements simply lying dormant.

To make the picture simpler and more dramatic, let us also assume the connections require only something like radio communication, and not a physically cumbersome coupling.

We might begin the experiment with the man fully conscious and independent, and the machine brain disconnected and fully dormant. But now we gradually begin disconnecting nerve cells or larger units in the man's brain, simultaneously switching on the corresponding units of the machine. The subject notices no change-yet when the process is completed, we "really" have a machine brain controlling a "zombie" human body!

The machine also has its own sensory organs and effectors. If we now cut off the man's sensory nerves and motor leads and simultaneously activate those of the machine, the first subjective change will occur, namely, an eerie transportation of the senses from one body to another, from the man's to the machine's.

This might be enjoyable: perhaps the machine's sense organs are more versatile than the man's, with vision in the infra- red and other improvements, and the common personality might feel wonderful and even prefer to "live" in the machine. At this stage, remember, the man is entirely dormant, brain and body, and the outside observer may be inclined to think he is looking at an unconscious man and a conscious machine, the machine suffering from the curious delusion that it is a man controlling a machine.

Next, we reactivate the components of the man's brain, either gradually or suddenly, simultaneously cutting off those of the machine, but leaving the machine's sensories plugged in and the sensories of the human body disconnected. The subject notices no change, but we now have a human brain using mechanical senses, by remote control. (We disregard such details as the ability of the human optical center to cope with infra-red vision, and the duplication of the new memories.) Finally, we switch the human effectors and sensors back in, leaving the man once more in his natural state and the machine quiescent.

If we perform this sort of exchange many times, the subject may become accustomed to it, and may even prefer to "inhabit" the machine. He may even view with equanimity the prospect of remaining permanently "in" the machine and having his original body destroyed. This may not prove anything, but it suggests once more that individuality is an illusion.

Discussion and Conclusion: In discussing these hypothetical experiments, we have touched on various possible criteria of individuality-identity of material substance, continuity of material substance, identity of personality and mem- ory, continuity of personality and memory-and seen that none of these is wholly satisfactory. At any rate, none of them, nor any combination, is both necessary and sufficient to prove identity.

The simplest conclusion is that there is really no such thing as individuality in any profound sense. The difficulty arises from our efforts first to abstract generalities from the physical world, and then to regard the abstractions, rather than the world, as the basic reality. A rough analogy will help drive home the point:

The classification "man" is useful, but not sharply definable. Is a freak a man? Is an aborted fetus a man? Is a pre- Neanderthal or other "missing link" a man? Is a corpse a man if some of the cells are still alive? And so on. A label is bandy, but objects may be tagged arbitrarily. In the physical world, there is no definite collection of objects which can be called men, but only shifting assemblages of atoms organized in various ways, some of which we may choose to lump together for convenience.

Let us then cut the Gordian knot by recognizing that identity, like morality, is man-made and relative, rather than natural and absolute. Identity, like beauty, is partly in the eye of the beholder. It is only partly existent, and partly invented. Instead of having identity, we have degrees of identity, measured by some criteria suitable to the purpose.

The result is wonderful: we have lost our souls, but gained heaven, in a certain sense. Perhaps few of us, even if intellec- tually convinced that identity is an illusion and death therefore unimportant, may be able to translate this into emotional acceptance, or will want to. But we can now persuade ourselves that death need never be regarded as absolutely final- since it is always possible, at some distance in space, time, and matter, for reasonably close duplication or resuscitation to occur that is, for physical reincarnation, with memory or without. This possibility can dull the edge of desperation for those unable to obtain first class freezer accommodations for themselves or their families.

Additional Thought Experiments: Because it is so difficult to wrap one's mind around these large and slippery questions, it may not be amiss, despite the time and space consumed, to add some variants of thought experiments before proceeding further.

Implanted History: Here's a thought experiment a bit different from those previously mentioned, involving im- plantation of false memories. It might be considered an extension of the old question of the effects if a cryonics resuscitee has memory gaps filled with best guesses.

We assume that somehow, with advanced technology, it becomes possible to modify a person's brain-without his knowledge, admittedly requiring some kind of elaborate conspiracy, including changes in official records-in such a way that, when he awakens after the procedure (after a night's sleep, as it seems to him), his subjective past is drastically changed, his memories those of a very different life. Everything still seems coherent-the new "memories" are consistent with each other and with external records, as far as the subject can tell. In any event, he has no suspicions and will not in- vestigate the question.

So, the "new" person is clearly inauthentic in some ways. Yet to a certain extent, the problem is no different-except in degree from ordinary life. We all have gaps in our memories, and we routinely, unconsciously, fill them in with guesses, and even sometimes with wishful thinking. Many of our memories, the specialists tell us, are really memories of memories of memories, degraded and spin-doctored.

And of course there is no guarantee that this "thought experiment"-or something similar-didn't actually happen to you. Maybe "you" are a simulation in a computer (although I consider this possibility extremely remote.) Most of us encounter apparent anomalies in life-little things seem unaccountably different than we remembered them, or different than they should be. We can't figure it out, so we shrug it off.

Is there a lesson in there somewhere? Perhaps the lesson is that the past doesn't, or shouldn't, matter very much. The altered you would still have feelings and potential, and would still be rationally compelled to do the best he can with what he has. After all, once more, you are what you are just through the luck of the draw. If past circumstances had been differ- ent, you would have been different-maybe for the better, maybe worse. All you can do is carry on.

In the end, you only have two general choices-self-destruction with probable oblivion, or charting of (hopefully optimal) pathways into the future.

Cheerful charting!

Leaving Your Future Behind: Another little variation to underscore the slipperiness of some of these ideas-this time the concept of a "future" version of "you" in the past.

If we can play with notions of either purposeful or accidental creation of duplicates of a person, at any stage of his development or implied (likely or possible) development (including a sufficient part of his environment), then imagine creation-long ago and far away-of you and your environment as you would become some time in your future (say late 21st Century).

Then "you" (far in the past and vastly distant) remember(ed) events still in the future of you-here-and-now. Does that mean you will have survived even if you die tomorrow? If that far-past person died after a while (in the slightly less distant past), does that mean you died before you were born?

Corbin's Frog, Greta Garbo, & Superman: Although it doesn't really break new ground, a thought experiment of Lee Corbin may be worth repeating.

Lee asks us to imagine starting out with a normal man, then slowly changing his atoms (or perhaps somewhat larger parts) until finally he has been turned into a different person-maybe an historical person such as Greta Garbo. Assuming the intermediate people could live and function (a stretch), who is who and when?

Or suppose the original man is gradually turned into a frog-perhaps an exact replica of a particular frog. (We disregard such questions as whether a part-man part-frog would be viable.) At the end of the experiment, Lee says, the human is no more; he does not exist-he is dead.

Most of us-at first-might find this not too disturbing. If you are gradually changed into a frog, certainly that final frog is not the original you. The continuity may be worrisome; it may bother us a bit that there was no particular point at which one could say the person ended or the frog began. But this may be seen as no different from other "paradoxes" of continu- ity. (Giving me one more penny cannot make me rich; therefore no matter how many pennies you give me, one at a time, I will never be rich. Etc.) We could simply say that anywhere near the beginning the subject was still a person, anywhere near the end of the line he was a frog, and in the middle he was part this and part that-the "quantitative solution". But wait. Imagine now that the human is gradually changed, not into a frog, but into a superman-such a superman, indeed, as some of us hope eventually to become through advanced technology, through growth and development and retrofitting. Somewhere down the line, that superman may resemble the original man scarcely more than does a frog or even an amoeba. At some point, that superman might even decide that his memories from his human era are no longer necessary, useful, nor

interesting; he jettisons them, or warehouses them in an external store. At this point, he is essentially both a different individual and a different species. Does it not follow, then, that the original human is non-existent? Is he not just as dead as if he had been made into a frog?

We recall that the only apparent rational life strategy of any person is to attempt to maximize personal satisfaction (Feel- Good) over future time. But this assumes the future person is you. If you will (or may) change into a not-you, have you not lost your compass?

One "solution" that tempts some people is to say that, yes, that future superman is me in a sense-i.e., he is the being I hope to become, my spiritual descendant or successor, even if not actual flesh of my flesh. But at this point, the individual with all his personal concerns has vanished!-because, if it isn't actually you, one future superman is as good as another, and we are back to concern for an abstract posterity. We are then essentially very near the position of Hans Moravec, who is willing to see himself as just a step on the "evolutionary" ladder leading to superior silicon intelligences. I am not ready to swallow that (which doesn't prove anything either).

At present, no clear-cut answer presents itself to me-nor, credibly, to anyone else, as far as I know. But on a pragmatic, common-sense basis, about all we can do for now is try to avoid extreme or sudden change, try to maintain the maximum physical integrity and psychological continuity, and leave this problem, or its more extreme variations, for the future. (But again a reminder of my conjecture on binding of time and space by the self circuit-overlapping successors, which may just possibly solve the problem.)

Retrospective: All of the 1962 analysis was useful, but fell far short, in light of decades of subsequent thought. In particular: I neglected the crucial importance of feeling and the "self circuit". I mentioned, but did not really investigate, the critical issues of objective and subjective time; and I did not emphasize the dubious nature of intuitive tests or develop any rigorous basis for investigation of identity. (It isn't a very good excuse that nobody else apparently did either.) Now I offer brief indications of some of my more recent thoughts, on these topics and others.

Feeling & the Self Circuit: I claim it should be nearly self-evident (after sufficiently long and hard thought!) that the basis of a person is in that subset or aspect of the brain that forms the seat of feeling (hence consciousness, which I define tentatively, with some oversimplification, as the integration of feeling and cognition.) I call this subset or aspect of the brain the "self circuit". Giving it a name doesn't accomplish much, but it does provide a focus. The self circuit is, after all, the most important phenomenon in the universe.

Needless to say, memory and personality are important, and many would reject "survival" if these could not be largely retained. Nevertheless, most of us feel we are the "same" people we were as infants, even though memory and personality have changed drastically. Most of us would also choose "survival" even at the expense of total amnesia. Of course, if the in- fant could think about it, he might not agree that he would survive if his developed brain retained no early memories. (Yes, the real question is what we should accept or choose, not what we "would" accept or choose; but we have to work into it gradually.)

In biological terms, it seems almost obvious

that the seat of feeling, the self circuit, is the core of life, or of life's potential. Just as the arms and legs and stomach etc. are merely appendages or supports of the brain, so also most of the brain itself is just an appendage of the self circuit. (And this is regardless of whether the self circuit is localized or distributed.)

The self (circuit) is surely the expression of some kind of semi-homeostatic feedback circuit in the electrochemistry of our brains. It probably resides (in the main) in a primitive region of the brain, since some form of consciousness doubtless began to arise in animals well below us on the evolutionary scale. And, as noted, it may well be unitary, in the sense that it cannot be shaved off in sections without destruction.

In Alfred Bester's novel, The Demolished Man, a criminal is punished and rehabilitated by having his personality and memories stripped away down to infancy, to begin again with a clean slate. Some would regard this as a death sentence- but the self circuit remains; feeling is continuous.

Of course, the questions of material/continuity still apply to the self circuit. But until we actually understand the physical nature of the self circuit, we have to be hesitant. For example, we do not know whether it can be assembled/disassembled piece-meal. If it has a unitary quality, if it is an "emergent" phenomenon with some irreducible minimum complexity, then there are many implications.

Feeling & Consciousness: I postulate that the self circuit (roughly synonymous with your qualia) is the seat of feeling and hence the core person in some sense. Feeling is the sine qua non of consciousness. Automata (without feeling) can in some sense think, and possibly even match or surpass every intellectual achievement of organic brains; but without feeling there can be no consciousness or awareness.

At the same time, feeling by itself does not constitute or permit awareness of anything beyond sensation. Larger awareness also requires computation, some kind and degree of ordering of sensations, of correlation and categorizing and manipulation of mental events; among other things, this demands some kind of memory. That is why I call consciousness the integration of feeling and computing, or feeling and cognition.

And a note on self-awareness or self-consciousness: I do not regard this as a particularly significant question. Obviously we have to distinguish between consciousness itself (or potential consciousness) and the content of consciousness. The ability to have the concept of "I" is just a matter of intellectual sophistication, a step up the computation ladder. Lower animals, or very young children, who may not have this concept, or may not have it clearly, nevertheless have an obviously strong sense of personhood, of being the center of the subjective universe. They "know" that they are themselves, even though they do not know that they know it, cannot articulate it.

The Skunk's Insight: As the skunk philosopher, Smelvin told his good friend PhueAnn: "Stinko, ergo sum." But Descartes had a more logically solid version: "Cogito, ergo sum." A bit better would have been, "Sentio, ergo sum." ("I feel, therefore I am.") After all, "think" might be something a computer could do.

This has been mistakenly attacked by other philosophers including Leibniz and down to the present. Leibniz complained that thinking is just "representation" and leaves open the

necessity of a cascade of representations of representations. This has also been called the "homunculus" problem-the apparent necessity to postulate, at every level, another little observer inside the last mentioned observer.

The confusion arises from the fact that the brain has many functions, and cognition indeed involves a lot of representa- tion. Much of our thinking is by analogy and metaphor. Signals inside the brain correspond to conditions both within and outside the brain. It then becomes easy to slip into the error of presuming that all significant brain activity is just informa- tion processing.

An immediate feeling-a quale-is not a representation. Qualia are not codifications; they do not stand for other things, even though they are consequences of other things. They are things in themselves, the last ditch, the bottom line, the essence of being. "Thinking" is possible without qualia, and computers can think, albeit so far very crudely. But feeling requires qualia-feelings are qualia-and until we better understand brain anatomy and physiology, we can only guess at the biophysics of qualia. (My crude guess, once more, is that a quale is some kind of standing wave, or modulation thereof, with spatial and temporal extent.)

Reminder-there is no professionally agreed definition of quale. One should not be too cavalier about introducing new terminology or deviating from accepted usage, but here there is no well-accepted usage. So for my purposes, a quale is de- fined (somewhat vaguely) as a feeling or subjective experience.

Descartes' insight ("I think, therefore I am.") was close to rock solid. We can be mistaken about the implications of our immediate feelings, or their relation to the outside world, but we cannot be mistaken about having those feelings. Our im- mediate feelings and only those- are what we know for sure.

Subjectivity, Evolution, & Epiphenomenalism: Some strong AI people or "upmorphists" or "pattern people" have questioned the evolutionary basis of subjective experience. If zombies can exist, for instance-unfeeling automatons that nevertheless talk and act like people, and I claim they could exist-then, since their observable behavior is no different than that of real people, how could feeling arise and be preferentially selected by evolution?

This is a very strange question in some respects. After all, we know first-hand that consciousness exists. We also know that automata can be very efficient at least in some respects, and human-level (or higher) capabilities in advanced future computers are to be expected, barring the collapse of civilization. We also know that many ordinary organic systems show a degree of intelligence (the human intestine, for example, with neurons doing complex jobs, as well as lower animals) and yet give us no reason to suspect subjectivity. In other words, we are confident both that feeling exists and that "intelligent" activity can exist without it. So what are the upmorphists agitated about—or is the question merely one of scientific curiosity?

The motivation for the question appears mainly to be an effort to show that feeling or its lack cannot be demonstrated empirically, and that a zombie can never be proven to be such. I have more to say on this in the next chapter, but for the moment, let's just disregard the motivation and answer the question.

Consciousness, or the feeling at its root, can perhaps improve the efficiency of an organism by responding to categories of stimuli instead

of individual stimuli.

The human gut deals with the food passing through it in complicated ways that include responses to differences in chemical composition, viscosity and several other factors. Similarly, the person could-and in some cases doubtless does- respond differently to different types of potential food in the vicinity, or to different types of hazard. But people, and lower organisms, can respond more quickly to a simple, general clue such as "smell good" or "smell bad". You can turn on your "approach" or "attack" or "avoid" or "flee" reaction more quickly if you don't have to sort through a whole lot of individual mental files for your clues-you have already categorized a whole lot of individual cases and only need the generic label as your cue.

Sure, a computer could do that too-but the computer, with its greater internal speed, could also mimic human responses without any subjective experience. And if the computer did respond to categories, that would not necessarily require subjectivity. I am only saying that subjectivity might be sufficient for improved efficiency, not necessary.

Now a word about epiphenomena. The term has no precise, agreed definition, but roughly an epiphenomenon is something that is incidental to some other phenomenon but unimportant and perhaps lacking any reciprocal effect or any notable consequences.

Some claim that consciousness is an epiphenomenon, and we could do without it. Taken at face value, that's just silly, since without consciousness we would have nothing-we would not be alive at all as we know life.

Aside from the fact that consciousness is everything to us, can subjective experience be relegated to the role of sidelight, like the whistle of a boiling teakettle? The whistle is real, but from some points of view unimportant, at least after it has served its function of announcement. It has no significant effect on the boiling of the kettle.

Epiphenomenalists also note that, as revealed by experiments, people often make certain types of decisions before being aware of those decisions, the time difference being on the order of a few hundred milliseconds. (Search "Libet" on the web.) In other words, even though you are aware of your decision, the decision was actually made unconsciously, before your awareness of it. This suggests that your consciousness was only a spectator, not a player. But several things need to be said. First, there are certainly many occasions when you consciously weigh and ponder at some length, and only then come to an ultimate decision, which you proceed to implement. Consciousness is a major player here. It is the culmination of a causal chain, and the beginning of another causal chain.

Second, we must remember the role of habit. Soldiers, workmen and others routinely train themselves to perform certain operations automatically and promptly, with little or no conscious thought. The routine becomes almost a reflex. It is a little like walking, which is done in a way that is partly conscious and partly automatic. The fact that habit can take control, and relegate consciousness to the background, does not eviscerate consciousness, but only limits its role.

Third, in those instances where habit assumes the main burden, the formation of that habit was owing to conscious decisions. Aiming and firing a rifle becomes semi-automatic only after a decision to build the habit and many subsequent decisions to keep it up and reinforce the

habit.

Fourth, there are doubtless different levels of awareness. Walking is probably more automated than firing a rifle. Some emergency actions are very quick and with little awareness, but not zero. It isn't black and white. In particular, think of "flash" functions such as joy-stick game-playing. Your actions or reactions take much less than the half-second period noted by Libet and others as the lag between action and later consciousness, yet no one would claim that you were not consciously making those decisions. You were making them all right-just not dwelling on them or announcing them. Bottom line: Consciousness is a complex phenomenon, or set of phenomena, but it frequently has a major role in decision making. Beyond that, once more, it is the central feature of life as we know it.

When Does Life Begin? For Catholics, at conception. For Jews, after graduation from medical school or law school. For Protestants, somewhere in between.

Ah, well. A reasonable case can be made that human life begins when the fetus develops a brain. When the self circuit is formed is something we do not yet know. Neither do we know whether something deserving of the name "self circuit" exists in very low life forms-or even in intermediate life forms such as insects-or in the individual cells of our bodies. The answers to these questions have all kinds of implications.

My preliminary guess is that there is a sharp cut-off, and that it occurs fairly high on the evolutionary ladder, excluding insects. Systems without feeling can certainly evolve, can display capabilities of goal-seeking, can eat and excrete and grow and reproduce; we could call such "pseudo-life". But if the self circuit is unitary and emergent, then it either exists or does not-even though it might be developed from more primitive systems guiding the behavior of pseudo-life. In other words, there is no necessary contradiction between saying, on the one hand, that feeling evolved from previously existing biological systems of discrimination and control, and on the other hand that feeling is an all-or-none phenomenon. Speculation on these matters, of course, can have only limited usefulness; in the end, we need the actual experimental and theoretical results. Fortunately, these topics are attracting increasing interest.

But we remind ourselves again that even a thorough understanding of the biology will not necessarily answer all questions. For one thing, the self circuit is still a physical system, and all the old questions of identity criteria apply to the self circuit too.

Potential Existence: One of these questions bears further investigation-the difference (if any) between existence and potential existence.

Questions of identity, and of the meaning and value of existence, seem closely related to the problem of being vs. potential being. (Is potentially to be, to be? That is the question.)

Thought experiments on identity often involve gradual or abrupt changes in personality or/and physical composition, interruptions of active life, or even temporary destruction. These are followed by new or renewed or continued life, with possible changes in personality, memory, or/and physical substance, including the possibility of multiple doppelgangers. There is no present agreement on the implications of these thought experiments, but they all involve questions of the meaning and value of potential existence.

We note first that virtually all existence could perhaps be termed merely potential. The subjective present may be only a fleeting moment, probably not longer than about 1/20 second in objective time and of uncertain subjective duration. In almost all aspects of life, and certainly in our goals and strategies, we necessarily focus on the future (even though sometimes on the near future). But since the future is not (subjectively) here yet, and has only a certain probability of ever arriving in the form we expect, one might say that virtually all of our plans and values are tied to uncertain potentialities. Our future selves do not yet exist and there is a chance they may never exist.

(As I also mention elsewhere, "might-be" or "might-have-been" can play a part in physical reality, according to some interpretations of quantum mechanics. "Counterfactuals"- conditions that never existed or events that never happened- can have a physical influence on what does exist or happen.)

If we think about it at all-few do!-we probably decide that our potential (at least near future) existence has reality and value because the transition is expected to be smooth and its realization highly probable. But it is likely to make us uneasy to admit that we are recognizing degrees of potential existence: we are used to thinking of "to be or not to be" as all-or-nothing.

Now consider a frozen person. He has suffered both material damage and interruption of function. If active feeling-a functioning "self circuit" and the existence of qualia-is the criterion of being, then the frozen person may not exist, or may have only potential existence.

How about someone under deep anesthesia, and perhaps also deep hypothermia, as in certain kinds of surgery? He may have no EEG at all and almost certainly has no feeling on any level; hence he might also be said not to exist, except potentially.

But if we are willing to settle for mere potential existence, this seems to lead to some very queer conclusions. For one thing, we always had potential existence-even at the time of the Big Bang (if there was a Big Bang). Did you exist before your grandmother was born? And of course it opens the door to acceptance of doppelgangers as self.

In terms of practical psychology, there may not be much of a problem, at least for a while. If the beam-me-up-Scotty routine ever became prevalent, people would "remember" the transitions and become comfortable with them-which says more about psychology than about logic. If a future "you" (or a reasonable facsimile) seems almost sure to materialize, then most people would doubtless settle for that.

It may be worthwhile to emphasize once more the gap between logic/realism on the one hand, and psychology and conditioning on the other. People accept grotesque and absurd assurances, if these are delivered often enough by sufficiently authoritative people, or/and if acceptance relieves the burden of thought.

For example, some Moslems apparently believe promises that Paradise is waiting, complete with zaftig and ever-virgin houris (72 of them for each hero, we read), if only they will kill enough Americans or Israelis or the wrong kind of Moslems. Many communists (although in greatly diminished numbers now) believe it makes sense to dedicate/sacrifice your life in grinding labor for the benefit of the state and posterity-which, translated, means the commendation of authorities and your own skew-

trained conscience. Even nominally irreligious "humanists" generally find "higher" values in dedication to humanity or posterity.

Feeling comfortable is no guarantee you are doing the right thing: you may be deluded. What you want is not always what you ought to want. Figuring out what we ought to want is the primary problem of philosophy.

My view is that one ought to want whatever he estimates will maximize his future feel-good (assuming he expects a future lifetime preponderance of satisfaction over dissatisfaction, hence chooses to live). As previously noted, this is often an extremely difficult calculation, not only in data gathering and mathematical manipulation, but especially in comparing the many varieties of feel-good and in identifying the future self. We are dealing with complex feedbacks, shifting probabilities and, above all, uncertain premises.

Much remains unclear, always including the nature of time as well as the "self circuit". However much or little emphasis we put on continuity, problems and seeming contradictions remain. But the only reasonable working posture I can see is to seek as much continuity as possible both in information and physical substance. That way we keep our options open. Again (see above and below) the key may be the space and time overlap of successive versions of the self.

Reprise of the Duplicate Problem: Let us once more visit the question of survival in duplicate(s), or the validity of the beam-me-up routine, starting with a brief summary.

Patternists claim that the "person" is defined by his material configuration, and if several configurations differ significantly only in location, all of them are the "same" person, in different "instantiations", and if one is destroyed, he nevertheless should be considered to have survived, so long as at least one duplicate remains.

Again, this is just an assertion or definition or expression of personal preference. It is not a logical conclusion from agreed premises. It requires, but does not justify, a radical change in viewpoint.

In December of 2004, Dr. R.M. Perry on Cryonet wrote: "I am in basic agreement with the above-yes, it *is* an expression of personal preference, what I choose to consider important. It does require a radical change of viewpoint, and in and of itself it does not justify that change (is scientifically unprovable). Part of the justification, however, is in the good consequences I see that follow (the non-finality of death in particular), coupled with the property, as I maintain, that this alternative view of a person, strange though it will seem to many, is at least defensible on logical grounds and can be said to fit the observable facts of experience."

I now continue the elaboration of the problem:

We restrict ourselves here to the cases that are simplest, and most favorable for the patternists, atom-for-atom duplication with no time lapse. Clearly other cases, such as "isomorphic" duplication in silicon or duplication with hiatus in time, will be less plausible as constituting survival. Also, the atom-by-atom requirement bypasses the question of which parts or aspects are most important; we duplicate everything.

On the pro side: It is at least vaguely plausible to many, and "obvious" to some, that "you" are characterized, at most, by your detailed (atom-by-atom) description at a particular time. If a duplicate, or a successor or continuer is indistinguishable, then that person must be you. If there is more than one, they must all be you, at

least initially.

Putting it a slightly different way, if "two" systems behave exactly alike, they must be considered the "same". And if a duplicate of me is created somewhere else, with initially the same properties I have except location, then survival of that duplicate can reasonably be considered my survival.

A continuity requirement, some think, cuts no ice. At least, most of us would not be worried by a temporary interruption of metabolism—which occurs partially anyway in sleep or in anesthesia. Indeed, classical continuity may not even be allowed by the quantum laws of physics.

It is irrelevant that each of several duplicates might regard his copy as uniquely himself, or might not be consoled by the thought that others would live on after his death. The question is not what a copy or original would feel or might feel, but what one ought to feel, and this is what we are trying to determine.

On the contra side: First, we know that many people, probably most, would not be consoled by the thought that a duplicate (or more than one) would survive after the original's destruction. This doesn't prove anything, but if at all possible, we want a solution that fits our intuition.

Second, consider two houses built on neighboring lots from the same blueprint, differing slightly in street address but nothing else of consequence. We certainly do not regard them as the "same"-only similar. It surely would seem very odd to have laxer requirements for "identity" of people than for buildings.

Third, in broader terms, it is never the case that "two" systems are indistinguishable. If the question even arises, that already proves they are distinguishable. They must differ at least in location. Beyond that, a difference of location necessarily implies other differences as well.

Consider even tiny bits of dead matter. Two hydrogen atoms are identical in most respects, but if they differ in location, and perhaps in momentum, then, even without regard to gravitational aspects, they cannot substitute one for the other. Fourth, the physical continuity criterion-especially for people-cannot be dismissed out of hand. Although many thought experiments seem to suggest that physical continuity is not a necessary criterion of identity or survival, the worry persists that somehow it may be. For whatever it is worth, the intuition of most people probably demands physical continuity, no hiatus in space or time.

Fifth, acceptance of one duplicate as self seems to require acceptance of any number. Psychologically, if not logically, this seems like nonsense. If a whole mob of "you" are created, would all those people have interchangeable identities in some sense-at least temporarily-and would the original "you" survive unless all were wiped out?

Sixth, what if we shift attention from "survival" to enjoyment/suffering? If a duplicate "is" you, don't you have the same stake in "his" quality of life as in "your own"? The ramifications of this are dismaying, to say the least.

Seventh, compare creation (somehow) of a duplicate unbeknownst to you with one created after you give your consent. If the secret or even accidental creation of a duplicate could "save" your life, and destruction of a duplicate could threaten your life (by reducing the redundancy safety factor), then the whole picture becomes unpleasantly confused. Although the world

was clearly not created in a way designed to save us confusion, still we resist bizarre and "unnatural" viewpoints as long as we can.

Eighth, the patternists do not claim that, if duplicates are created, all the duplicates continue to be the same person, or that survival of one duplicate means survival of all. In a relatively simple case, say where several duplicates are created at the same time of a particular original, then all will thereafter diverge. If they continue to live, then life of any or all the duplicates, as well as of the original, would (they say) represent survival of the original as he was at the time of duplication. At any later time, the original or any duplicate (as he is at that later time) would not survive through survival of other once- duplicates. But these contortions-even applied to the simplest cases, let alone cases of temporal displacement-seem strained at best, barely plausible, and emphatically not established even as a good theory.

Ninth, some assert that any interruption of configuration is tantamount to extinction. If you are damaged to the extent that you cannot function-maybe if your brain is damaged so it can no longer give rise to or sustain qualia-then you no longer exist. Not only that, but you can never exist again, because any "restoration" would "really" be just creation of a new person or duplicate, not the original. This argument seems questionable to me, and not very useful in any case in terms of decision making, but it is not easily dismissed.

Upshot: Final conclusions must wait until we have more information, including the anatomical/physiological basis of feeling (the "self circuit") and the deeper nature of spacetime. But my tentative conclusion, again, is that we have extension in space and time, overlapping our predecessors and continuers, which tends to validate our connection with the future and past, and hence the importance of physical continuity.

Appealing again to (admittedly fallible) intuition, most of us probably feel that we cannot psychologically "exist" except as time binders. To feel or to be is dynamic. To feel is to feel that something is happening. It hardly seems anything can be happening unless our attention spans some segment of at least the recent past and perhaps the near future. We cannot "exist" in an arbitrarily thin slice of time. And, as noted, the minimum duration of a subjective "moment" seems to be about 1/20 of a second.

Now the question arises: can we expand or extend the objective duration of the subjective moment? Apparently we could do it by physical means-somehow slow down the processes in our nervous systems-but that scarcely seems helpful. A more important question is, could we extend the subjective duration of the moment?

At first, this seems a contradiction in terms. But if our sense of existence requires a sense of something happening, this seems to imply a feeling of change.

Now, must a feeling of change require actual change the passage of appreciable time? Or can a "feeling" be captured just in a physiological state at a particular moment, i.e., a particular configuration of the brain's atoms in a razor-thin slice of time?

If the latter, then "snap-shot" existence might be possible. If snap-shot existence is possible, then frozen people (for example) might "exist" and-just conceivably-other recently dead people, if the appropriate elements of brain configuration are still preserved.

Stay Tuned: Some of these questions will certainly be illuminated rather soon, at least in part, by laboratory investigations. We will learn more about how long it takes to have a feeling, and whether a single feeling can be extended in time. We will zero in on the self-circuit, and get a better handle on the physical/physiological nature of feeling.

Physics theorists, and perhaps even experimentalists, may develop and refine some of the new/radical ideas about the physical nature of time. We know, for example, that respected theorists seem to take seriously the possibility of changing the past, and of time loops. If these possibilities should be real, all bets are off.

Accidental You: We start out shaped by a set of genes-which we did not choose. Our bodies and minds grow and change, partly in response to environmental influences-also initially imposed and not chosen. If the environment is suboptimal, the best potentialities of our genes cannot be realized. As an extreme example, someone raised by wolves will be an idiot; as a slightly less extreme example, someone abused or neglected as a child will be psychologically warped. Suppose you had an unfortunate background-child abuse or neglect and all that, on top of genetically limited intelligence. (And don't most of us have both of those, in some degree?) You made a lot of bad choices. What is the "real" you? Is it the guilt-wracked wreck with the wretched memories and inferior capabilities? Is it the better and happier person you might have been, or could become after technological intervention? Even if you decide the "real" you is the historical one, is that the one you want revived and perpetuated?

Some would say yes: I want the truth. I want to change and improve-but not retroactively, not unconsciously, not without my knowledge and consent, and not suddenly. I don't want to keep on licking my wounds, but I want to remember them, or be able to remember them-otherwise there is no validity.

Again, this has a plausible ring, but little if anything more than that. Personally, I would just as soon be rid of my bad memories and traits and habits instantly, and "know" the history only as an archive that I can look up if I wish.

Questions for the Laboratory: Can philosophers help the experimentalists? To get the right answers, it helps to ask the right questions, and conceivably, we can contribute a little here.

First, we are not likely to find localized brain regions identifiable with the self by any of the currently known experiments. For example, we know that stimulation of certain points in the brain produces sensations-but so does stimulation of points on the skin and elsewhere. Those known points in the brain are more likely waystations or switching points than parts of the central self.

Second, once we have located and identified the self circuit or aspects of it, we have to learn what constitutes pleasure/ satisfaction and the opposite. Is it a single condition, state, or sequence of events? Or is there more than one kind? Can more than one kind coexist? If more than one kind can coexist, with different strategies favoring each, then life becomes even more complicated.

Reprise: A Tentative View of Survival-The Overlap: "You" consist essentially of your brain, or some parts and functions of it.

You cannot exist at a geometrical point in space, if there is such a thing. You necessarily enclose a non-zero volume-you have spatial

extension or you "bind space." In particular, your "self circuit" has spatial extension.

Since presumably nothing can happen in zero time, and it takes time for signals to move around in the brain, or for a self circuit to perform an oscillation, any subjective experience you have probably requires non-zero time. You can't feel anything at an "instant" of time, if there is such a thing. Thus an indivisible you has temporal extension or duration. You "bind time."

Your qualia or experiences are not "in" you or attached to you, but are you-or the most important part of you, the "alive" part-and have spatial and temporal extension.

This means that you overlap your predecessors, as well as your successors or continuers, in space and time. You are at least in part the same physical person as your recent and near-future selves, as well as the same psychological person. Thus "your" interests are at least to a considerable extent the same as those of your near-future self. It is therefore logical, as well as an inevitable result of evolution and an almost inescapable intuition, that we "identify" with our future selves, and more strongly with our nearer future selves.

Next Issue:

Chapter Ten: Identity & Survival 2 — Silicon

10 Worst Mistakes in Cryonics

Don't ruin your chance for a succesful suspension

1) Not signing up ahead of time

Becoming a member, having contracts in place, and having paperwork in order should not be a last minute decision. Waiting until the last minute or after death results in an unnecessary delay of care or worse- No suspension at all! Don't wait. Sign up here and be prepared. https://www.cryonics.org/membership/

2) Not providing proof of funding

Some people believe that they can worry about funding later or if they have funding, they have put off providing proof of funding to CI. This should be done annually. Failing to provide this can result in a delay of care while the funding clears, which can take weeks. Send your proof of funding to CI now to be prepared.

3) Not telling anyone your plans

Being reclusive or not telling family or friends your wishes is not recommended. You should not be afraid to tell those around you what your wishes are, especially your next of kin. Wearing a bracelet, necklace or having identification or other items in view can speak to your wishes. This is all you have when you can't speak for yourself. Disasters have resulted in the past from not sharing. Talk with your family, close friends and your estate attorney, so you can be prepared.

4) Not planning

Many think cryonics is a turnkey service where you can just sign up and let fate take over. No matter how much you pay for cryonics, you are the only one who can make sure that you will have the best chance by planning. CI has provided a lot of information on our website and in our standby manuals. Those who plan succeed those who don't fail.

For more information visit: https://cryonics.org/category/members/standby/

5) Not notifying CI of Emergencies

There is no way that your cryonics provider can help you if they do not know of your emergency. Your family, friends, standby group or next of kin must immediately contact CI when you are having health issues or worse. It is also important for CI to know if you have up and coming surgeries or procedures, including terminal illness. Patients with a diagnosed terminal illness could enter hospice care, which might help your cryonics situation vastly. Any delay in notifying us directly could result in a poor suspension. Those helping you must have simple and clear instructions.

6) Committing suicide

Anyone who commits suicide who is not terminally ill or breaks a local law in doing so is potentially putting both themselves and our organization at great risk. CI will not risk itself for people who engage in behavior that goes against our mission to preserve life. Such activity will likely lead to an autopsy and long delays, rendering the suspension process substandard or impossible to carry out.

Do not consider cryonics as a way out of your problems. You are likely to not get suspended under those circumstances. If you do not have a terminal illness and are considering suicide, you should seek mental health advice and treatment as soon as possible. https://www.mentalhelp.net/articles/depression-hotline/

7) Engaging in Risky or illegal activities

Risky behaviors or associations that lead to the patient dying around suspicious circumstances will also likely lead to mandated autopsies that will also stand in the way of your cryonics wishes. It is best to use common sense and not put yourself in harm's way. Not only could your life be ended, so too could your chances of cryonics suspension or future reanimation. Use common sense and stay safe.

10 Worst Mistakes in Cryonics

8) Providing financial or legal incentives that encourage your **not** being suspended.

Leaving all of your insurance or cryonics money to family if you are not suspended is certainly an option at CI, but ironically it does provide financial incentive for hostile family members to block your suspension. As often is the case, people will make sure you are not suspended to get a hold of your money.

One suggestion is to leave family and next of kin some separate money from cryonics funding while suggesting that Cryonics funding go to cryonics as a donation no matter if you are buried or suspended. In addition, family or next of kin can be further compelled to cooperate if they will actually lose the money that is allocated to them for not cooperating. It is also suggested that your family be made fully aware of your wishes and stipulations, so they know what the results of their actions will be. You want to make sure you put incentives and disincentives in the correct place, so that your wishes are honored. It is suggested that your will and cryonics documentation reflect this and get reviewed by an attorney. See https://cryonics.org/members/protect-yourself-from-legal-threats/

9) Not removing a hostile next of kin from rights to your remains and finances

In many states and areas you can legally remove a hostile family member or next of kin from your estate. You can reassign someone who is sympathetic to cryonics and who has the legal authority to disposition of your remains, as well as your assets. In some states and locations there are disposition of remains reassignment documents, as well as powers of attorney, both in regards to financial as well as medical decisions. The executor of your will or anyone involved with making decisions should be sympathetic to your cryonics wishes. It is your responsibility to make your wishes very clear and to remove any doubt or potential legal resistance from family or next of kin.

We suggest seeking legal advice to help you in this regard. Some members have even made a video statement of their wishes and given it to both their cryonics organization as well as their attorneys. Not being careful could mean that you don't get suspended, despite your wishes. Many are surprised to learn that they lose their rights upon legal death. See an attorney and prepare.

10) Dying under less then favorable conditions

This seems harder to control then the other situations, but there are some things you can do to make your situation more favorable. You can diet, exercise and follow the latest official medical advice to stay healthy longer. The longer you are alive, the better the technology will probably be for suspending you and the closer we will be to a future that may be able to reverse your condition.

You can also avoid travel to remote or hostile places where such travel is risky. Some overseas travel can result in long delays both logistically and bureaucratically. In general, dying near your cryonics provider or cryonics standby group helps your chances. Living a healthy lifestyle and staying sociable, while surrounding yourself with people who will act on your behalf is paramount. Building solid, positive relationships with good people is probably one of the most important things you can do to have your wishes honored. Take care of yourself and maintain social connectivity.

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Bulletin Board

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Got something to say?

The CI Newsletter is looking for submissions from our readers!

If you've got a great idea for a story, please forward it to:

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CRYONICS QUESTIONS?

Need some help with your membership?

Want to understand your suspension options?

Need to fill out important cryonics paperwork?

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1-(586) 791-5961

FREE Memberships?!!

Did you know the Cryonics Institute offers FREE LIFETIME Memberships for minor children of paid Lifetime Members? Any minor children (under the age of 18) of fully-paid Lifetime Members are eligible for a permanent Lifetime Membership of their own. If you'd like to give your children the priceless gift of a second chance of life with you in the future, please contact us at 1 (586) 791-5961 and ask about Lifetime Membership Benefits.

Show the world you support cryonics with CI gear from our **Cafe Press store**.


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