COMMENT

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Pigs raised for food production are exempt from welfare laws governing how research animals are treated.

Part-revived pig brains raise ethical quandaries

Researchers need guidance on animal use and the many issues opened up by a leap in brain restoration, urge **Nita A. Farahany**, **Henry T. Greely** and **Charles M. Giattino**.

Scientists have restored and preserved some cellular activities and structures in the brains of pigs that had been decapitated for food production four hours before. The researchers saw circulation in major arteries and small blood vessels, metabolism and responsiveness to drugs at the cellular level and even spontaneous synaptic activity in neurons, among other things. The team formulated a unique solution and circulated it through the isolated brains using a network of pumps and filters called Brain*Ex* (see page 302). The solution was cell-free, did not coagulate and contained a haemoglobin-based oxygen carrier and a wide range of pharmacological agents.

The remarkable study, published in this week's *Nature*[1], offers the promise of an animal or even human whole-brain model in which many cellular functions are intact. At present, cells from animal and human brains can be sustained in culture for weeks, but only so much can be gleaned from isolated cells. Tissue slices can provide snapshots ▶

of local structural organization, yet they are woefully inadequate for questions about function and global connectivity, because much of the 3D structure is lost during tissue preparation[2].

The work also raises a host of ethical issues. There was no evidence of any global electrical activity — the kind of higher-order brain functioning associated with consciousness. Nor was there any sign of the capacity to perceive the environment and experience sensations. Even so, because of the possibilities it opens up, the Brain*Ex* study highlights potential limitations in the current regulations for animals used in research.

Most fundamentally, in our view, it throws into question long-standing assumptions about what makes an animal — or a human — alive.

SIGNS OF WHAT?

The pig brains used in the study, which was conducted by a team based largely at Yale School of Medicine in New Haven, Connecticut, produced a flat line on an electroencephalogram (EEG) of brain activity. Had any degree of sentience been recovered, let alone consciousness, one would expect to see low-amplitude waves in the alpha (8–12 Hz) and beta (13–30 Hz) range, at the very least[3,4]. In consultations with the Neuroethics Working Group of the US National Institutes of Health (NIH) BRAIN Initiative and in discussions with us, the researchers have stated that if they had detected such activity, they would have administered anaesthetic agents to prevent any experience similar to pain or distress, and would have reduced the brain temperature to swiftly quell the activity.

Researchers already study whole organs, and maintain cellular activity for a few seconds to minutes in slices of animal and human brains. Thus, on the face of it, in the absence of EEG activity, the Brain*Ex* study does not raise fundamentally different issues from those encountered in the use of animal or human brain tissue after death.

Yet, until now, neuroscientists and others have assumed two things. First, that neural activity and consciousness are irretrievably lost within seconds to minutes of interrupting blood flow in mammalian brains. Second, that, unless circulation is quickly restored, there is a largely irreversible progression towards cell death and the death of the organism[1].

The Brain*Ex* study used pig brains that had received no oxygen, glucose or other nutrients for four hours. As such, it opens up possibilities that were previously unthinkable.

Take the lack of EEG activity. This activity could have been lost irreversibly when the pigs were slaughtered. Another possibility, however, is that the lack of EEG activity was a function of the study design. The researchers used several chemical agents in their solution that inhibit neural activity, hypothesizing that the tissues would be more likely to show some recovery if cellular activity were reduced. Had these blockers been removed at some point, perhaps the team would have detected EEG activity.

Another possibility needing investigation is that something similar to shock treatment for the heart is required to reset the firing of neurons in the brain to a level that is detectable. Or maybe it takes longer than six hours (the length of the Brain*Ex* perfusion, following the four hours after death) for the cells to recover sufficiently for this kind of brain activity to emerge[5]. Physicians sometimes lower the core body temperatures of people who have had a heart attack, to induce a hypothermic coma. This can limit damage caused by swelling in the brain, for instance, and aid cellular recovery. In these cases, patients seem to need at least 24 hours of 'cooling treatment'.

Obviously, more data are needed, including the replication of the Brain*Ex* findings in other laboratories by other groups. But we're reminded of a line from the 1987 film *The Princess Bride*: "There's a big difference between mostly dead and all dead. Mostly dead is slightly alive." Even with all the unknowns, the discovery that mammalian brains can be made to seem 'slightly alive', hours after the animals had been killed, has implications that ethicists, regulators and society more broadly must now think through.

"The BrainEx study opens up possibilities that were previously unthinkable."

ANIMAL RESEARCH

To be clear, the Brain*Ex* study did not breach any ethical guidelines for research. The team sought guidance from Yale University's Institutional Animal Care and Use Committee (IACUC), which exists to ensure that the use of animals aligns with what is required by US law for federally funded research. The committee decided that oversight was unnecessary. The pigs, having been raised as livestock, were exempt from animal welfare laws and were killed before the study started. In the United States, the 1966 Animal Welfare Act is the only federal law that regulates how animals are treated in research, and applies to either living or dead animals. It explicitly excludes animals raised for food. Meanwhile, the policies and regulations of the US Public Health Service, which funds most US research involving animals — mainly through the NIH — do not specify any protections for animals after their death.

Had the research been conducted outside the United States, the response from ethics or regulatory bodies would almost certainly have been the same. The European Union's Directive on the Protection of Animals Used for Scientific Purposes largely aims to prevent (or minimize) any pain, suffering or distress experienced by live animals. It, too, specifically excludes animals raised for agriculture (see go.nature.com/2cpdgjr). In China, both the Ministry of Science and Technology and the provincial bureaus of science and technology ensure that researchers follow local regulations and that they abide by the National Standard on Laboratory Animal Welfare in China[6]. Here, too, the protections exclude animals raised for food, and the main focus is on eliminating or reducing live animals' potential pain and distress.

In our view, new guidelines are needed for studies involving the preservation or restoration of whole brains, because animals used for such research could end up in a grey area — not alive, but not completely dead. Five issues in particular need addressing.

First, how should researchers try to detect signs of consciousness or sentience? On its own, EEG activity would not reliably signal a conscious brain; such activity is nearly always detected in people who are under general anaesthesia[7]. EEG activity might provide an appropriate measure should it be detected along with responsiveness to transcranial magnetic stimulation (TMS) — a non-invasive way of stimulating brain activity, using a magnetic coil held near the head. Together with other measures, this would determine the brain's perturbational complexity index, a way of identifying the level of consciousness[8]. Furthermore, recent research in humans using functional magnetic resonance imaging indicates that certain patterns of neuronal activity may provide a correlate for consciousness[9].

Second, which species make appropriate models for this type of research on brain perfusion? And what kinds of research and results would be needed to justify the use of other models? (In our view, investigators should proceed cautiously with testing in other mammals, particularly in pigs, dogs or primates, at this time[10].)

Third, until more is known, is the use of neuronal activity blockers sufficient to safeguard against the emergence of capabilities associated with sentience, such as the capacity to feel pain? It might be necessary to apply Brain*Ex* or similar systems to mice or rats, both with and without neuronal activity blockers, to better understand the blockers' role.

Fourth, under which scenarios should anaesthetics be used in follow-on studies, to safeguard against the possibility of inducing any experience similar to pain or distress? And under what scenarios might it be permissible not to use them? (We think that the use of anaesthetics in follow-on studies should be mandatory at this time, given all of the unknowns.)

Finally, for how long should Brain*Ex* or

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A woman is silhouetted as she walks past an exhibition piece displaying a brain slice from a person who underwent surgery for epilepsy in the 1950s.

similar artificial circulatory systems be run? Such systems might be effective for only a certain period of time, or there could be a limit as to how much recovery can be achieved. This knowledge will inform analyses of risks and benefits.

HUMAN RESEARCH

Although it is a long way off, researchers might one day consider using a system similar to Brain*Ex* to treat humans for brain damage caused by a lack of oxygen. Until now, neuroscientists and physicians have assumed that the cell death caused by this is irreversible. Treatment generally involves working with a person's remaining healthy brain tissue to help rehabilitate mobility, motor and other skills.

Before developing whole human-brain models outside the body — and certainly before the use of brain perfusion in the clinic — investigators need to arm people with enough information for them to make informed decisions. Most fundamentally, patients or donors will need to understand what kinds of brain activity could result and what that activity could mean. They will also need to know the chances of recovery being only partial, and the implications that will have.

Another question is what information, if any, could plausibly be retrieved from the brain. Various groups are developing ways to decode the neural activity of living people, for instance to probe their memories or the images they have seen in their dreams[11,12]. Could such approaches one day be applied to brains after death?

Such possibilities (if they come to pass at all) are far in the future. Yet we need to think through at least some of them now. Hundreds of people worldwide have already paid to have their brains frozen and stored, in the hope that scientists will one day be able to revive them. It's easy to imagine misapplications of brain perfusion following the publication of the Brain*Ex* study alone.

GUIDELINES

It might not be easy for others to replicate the study, despite the Brain*Ex* team providing detailed information on the device, perfusate and methods. As a first step, the investigators, their home institutions and the NIH should facilitate the transfer of the technology and know-how to other researchers and institutions. Any follow-up and independent studies should be just as transparent as this one.

Crucially, future researchers will need guidance through the potential scientific, ethical and political questions opened up by this research.

Precedents exist. Internationally, research involving stem cells derived from human embryos has successfully been steered by the 2005 *Guidelines for Human Embryonic Stem Cell Research* released by the US Institute of Medicine and US National Research Council — the substance of which was almost entirely adopted by the International Society for Stem Cell Research. Ongoing efforts to set guidelines for human genome-editing research hold lessons, too. Key actors here are the US National Academy of Sciences, the US National Academy of Medicine, the UK Royal Society, the Hong Kong Academy of Sciences, the Chinese Academy of Sciences and the Nuffield Council on Bioethics.

In other contexts, such as in biomedical engineering (see, for example, go.nature.com/2t6kon5), artificial intelligence[13] and debates around the definition of death[14], international conferences are being held to help find common ground across countries and to develop frameworks that enable responsible scientific progress.

We think that the latest research on brain resuscitation demands the same kind of

international attention. A starting point could be the guiding principles issued last December by the Neuroethics Working Group of the NIH BRAIN Initiative, which held a 2018 workshop on research with human neural tissue[15].

Citizens must be part of the process. Engaging non-scientists in delineating the ethical boundaries of this research doesn't guarantee its public acceptance in the future; and nor should it, necessarily. But not engaging other stakeholders could help to precipitate its rejection.

In our view, discussion about the appropriate path for this research should not wait for follow-up studies. The Yale group was conscientious and consulted the local institutional IACUC, Yale bioethicists, NIH programme officers and even the NIH Neuroethics Working Group. The researchers did what they could, and probably more than many would have done, to ensure that they were acting appropriately in a void of ethical analysis on the issue.

Now is the time to fill that void. ■

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1. Vrselja, Z. *et al. Nature* **568**, 336–343 (2019).
2. Farahany, N. A. *et al. Nature* **556**, 429–432 (2018).
3. Koch, C., Massimini, M., Boly, M. & Tononi, G. *Nature Rev. Neurosci.* **17**, 307–321 (2016).
4. Mashour, G. A. & Hudetz, A. G. *Trends Neurosci.* **41**, 150–160 (2018).
5. Karnatovskaia, L. V., Wartenberg, K. E. & Freeman, W. D. *Neurohospitalist* **4**, 153–163 (2014).
6. Ogden, B. E., Pang, W., Agui, T. & Lee, B. H. *ILAR J.* **57**, 301–311 (2016).
7. Brown, E. N., Lydic, R. & Schiff, N. D. *N. Engl. J. Med.* **363**, 2638–2650 (2010).
8. Casali, A. G. *et al. Sci. Transl. Med.* **5**, 198ra105 (2013).
9. Demertzi, A. *et al. Sci. Adv.* **5**, eaat7603 (2019).
10. Nuffield Council on Bioethics. *The Ethics of Research Involving Animals* (Nuffield Council on Bioethics, 2005).
11. Huth, A. G. *et al. Front. Syst. Neurosci.* **10**, 81 (2016).
12. Horikawa, T., Tamaki, M., Miyawaki, Y. & Kamitani, Y. *Science* **340**, 639–642 (2013).
13. Azoulay, A. 'Towards an Ethics of Artificial Intelligence.' *UN Chronicle* (December 2018).
14. Shemie, S. D. *et al. Intensive Care Med.* **40**, 788–797 (2014).
15. Greely, H. T. *et al. J. Neurosci.* **38**, 10586–10588 (2018).

Pig brain study could fuel debates around death

The restoration of some functions in pig brains after death raises tensions over when to take human organs for transplant, warn **Stuart Youngner** and **Insoo Hyun**.

In this week's *Nature*, researchers describe restoring certain structural and functional properties to pigs' brains, even four hours after the animals had been killed[1]. They used an artificial perfusion system called Brain*Ex*.

Electrophysiological monitoring did not detect any kind of neural activity thought to signal consciousness, such as any evidence of signalling between brain regions (see 'Between life and death'). Nonetheless, the study challenges the long-held assumption that large mammalian brains are irreversibly damaged a few minutes after blood stops circulating. It also raises the possibility that researchers could get better at salvaging a person's brain even after the heart and lungs have stopped working.

Advances following on from the Brain*Ex* study could exacerbate tensions between efforts to save the lives of individuals and attempts to obtain organs to donate to others. (Such advances could also affect the use of human brains and brain tissue in research; see page 299.)

In our view, as the science of brain resuscitation progresses, some efforts to save or restore people's brains might seem increasingly reasonable — and some decisions to forego such attempts in favour of procuring organs for transplantation might seem less so.

The transplant community, neuroscientists, emergency medical personnel and other stakeholders must debate the issues[2]. Eventually, it might be useful for groups such as the US National Academy of Medicine to offer guidelines for physicians and hospitals. These would help to protect the interests of individuals for whom sufficient recovery is a possibility, as well as the interests of potential organ recipients.

DETERMINATION OF DEATH

For decades, bioethicists and transplantation-policy researchers have had to wrestle with the question of when to switch from trying to save someone's life to trying to save their organs for the benefit of another person.

Invariably, this comes down to a moral decision — namely about futility, which is a contentious and value-laden concept[3]. There are few data to support decisions. And clinicians disagree about when there is a chance of recovery. There is also little consensus on what level of recovery is 'good enough' from the perspective of patients and their families, as well as when these factors are weighed against limited medical resources.

In most countries, a person can be legally declared dead if they show irreversible loss of all brain function (brain death) or irreversible loss of all circulatory function (circulatory death).

In recent decades, most organs for transplant have been taken from those who have been declared brain dead, often after a catastrophic brain injury resulting from a stroke, trauma or prolonged lack of oxygen to the brain, caused for instance by drowning. (In these cases, the person's heart and lung functions are maintained in the intensive care unit.)

Increasingly, however, those who are declared dead after their hearts and lungs have stopped working are being deemed eligible for organ donation. This shift has largely been driven by an increased need for organs as transplantation surgeries have become more successful. According to the US non-profit organization the United Network for Organ Sharing, someone is added to the US transplant waiting list every ten minutes. In 2017, around 18 people in the United States died every day while waiting for a transplant.

"Someone is added to the US transplant waiting list every ten minutes."

If technologies similar to Brain*Ex* are improved and developed for use in humans, people who are declared brain dead (especially those with brain injuries resulting from a lack of oxygen) could become candidates for brain resuscitation rather than