Brain Death: Investigating the Electrophysiological Events at Death in Animal and Human Research

Regan A. Gallagher[1]*, Brooke A. Goldstein[1]*, Jonah A. Joffe[1]*, Kyle A. Kidd[1]*, and SooYoung H. VanDeMark[1]*
[1]Geisinger Commonwealth School of Medicine, Scranton, PA 18509
*Master of Biomedical Sciences Program
Correspondence: svandemark@som.geisinger.edu

Abstract

Brain death is an understudied and often misunderstood process. Identifying when death occurs in the clinical setting is not always the biological start point of when death actually occurs. Once circulation and perfusion of neuronal tissue ceases, a cascade of many processes ensues leading to eventual iso-electricity and true death. Identifying processes in the brain during active death are mostly studied in animal models, but recent studies have examined terminal patients removed from life-sustaining care. Measurement of brain electrical activity provides insight to the function of neuronal tissue, and the spreading and nonspreading of electrical depression has been studied in several patients. Among the various research articles examined in this paper, there is consensus that death is a transitional process, not a singular point in time, and that the brain is still active for a limited time after electrical silencing.

Introduction

Brain death has been an elusive area of medical science throughout the ages and is only beginning to be understood in the modern era. The development of new medical practices and scanning techniques have shed light on previous misconceptions in the study of brain function and dysfunction. As our collective scientific knowledge on pre- and post-neurological death improves, new definitions and clinical practices for patients' rights need to be determined.

The act of declaring brain death is a culturally unique phenomenon across the globe that is often determined by institutional and legal procedures. However, it varies greatly in its execution depending on a country's economic status as low-, middle-, or high-income, and the existence of a national organ transplant network (1).

Similarly, death has been redefined over time. Historically, the classification of death (and subsequently brain death) fell into one of three categories: decapitation or decay, non-response to painful stimuli, or the absence of respiration and pulse (2). Before life-sustaining technologies such as ventilators were developed, the practice of declaring death was much simpler than it is today. Due to our present ability to prolong life with medical machinery, it has become increasingly difficult to define when brain death has specifically occurred. The current diagnosis of brain death is multifaceted; focusing on the irreversible loss of brain function resulting in coma, absence of brainstem reflexes, and apnea (2, 3).

The clinical process for determining and certifying brain death in humans involves three measures: identifying a potential cause, ruling out confounding conditions, and performing a complete physical and neurological examination (2). Diagnosis begins with identifying a potential cause of brain death, such as severe head injury, hemorrhage, hypoxia, ischemia, infection, or other terminal conditions (2). A physician must also rule out any condition that may mimic or confound neurological death, such as shock, hypotension, hypothermia, and injection or ingestion of neurologic substances (e.g., drugs, toxins, or other neuro depressants). Guillain-Barré syndrome and other diseases that cause neuromuscular paralysis should also be investigated prior to a diagnosis of brain death (4).

After identifying possible causes and ruling out confounders, a physical examination is performed. Identification of outside stimuli; pupillary, gag, and oculovestibular reflexes; reaction of heart rate to atropine; and respiratory movements would contradict a diagnosis of brain death (2). An apnea test is then performed following the brainstem reflex exam. A patient must first be hemodynamically stable and ventilated to normal levels. Once stable, the patient is removed from ventilation to begin the apnea test. If no spontaneous respirations are present, an arterial blood gas is drawn after 8 to 10 minutes. If the returned PCO_2 value is >60 mmHg or 20 mmHg over the baseline value, it supports the diagnosis of brain death (3). Testing respiratory reflexes is generally the last test performed.

The precise clinical examination performed to determine brain death was found to vary globally, specifically when conducting the apnea test (1). Some institutional protocols recommend that the apnea test should only be carried out after two failed brain stem exams, as described above, separated by at least a 6-hour period (2). The American Academy of Neurology was unable to recommend a minimally acceptable period of time for observation prior to declaring brain death (3).

Determining neurologic death is a precarious event for patients, families, clinicians, and others including the organ transplant community. However, this determination is not guided by evidence-based methods but rather opinion-based ones (3) due to the lack of evidence and limitations of research on this topic. In this review, we examine the prevailing science on the dying brain immediately pre- and post-mortem within animal and human models in order to illuminate the complexity of defining and determining brain death even with the advancement of new technologies.

Methods

Findings from several peer-reviewed journal articles were examined to create a cohesive understanding of the classification and current understanding of the dying brain. Journal articles were obtained from a variety of reputable sources, utilizing database searches of Google Scholar, PubMed, Cochrane Reviews, and the National Center for

Biotechnology Information (NCBI). Search terms included: *brain death, dying brain, perfusion, neuronal death, hypoxia, ischemia, and clinical death*. After the initial round of articles were collected, additional source material was found within the references of said articles.

Discussion

Small animal models

Given the difficulty of examining the dying brain in humans due to the delicate nature of the topic and various ethical constraints, much of what is known about this topic comes from studying animal models.

In an experiment by Borjigin et al., 9 rats had electrodes placed directly onto the bilateral frontal, parietal, and occipital cortices. After a period of recovery, the rats were lethally injected to induce cardiac arrest. Electrical brain activity of the rats was recorded during wakefulness, sedation, and post-cardiac arrest using an electroencephalogram (EEG). [Note: This article refers to the use of an EEG, but based on its description, electrocorticography (ECoG) or intracranial electroencephalogram would be the more accurate term.] Electrical activity was seen for approximately half a minute after the last heartbeat in all 9 subjects. Electrical silence occurred after this period, as EEG recordings fell to under 10 µV. The collected data and analysis of the gamma, theta, and delta waves; the global coherence across the brain of activity; and the feedback-feedforward connectivity found, led the researchers to suggest that a high degree of information processing may occur during clinical death (5). They interpreted this activity as a possible scientific and physiological explanation for human near-death experiences. However, an alternative explanation for the electrical activity points to a cellular interpretation, positing that the cessation of blood flow into the brain causes an influx of calcium into cells, leading to cell damage that the EEG was recording as electrical brain activity (6).

Rodent brains have also been studied at the cellular level to explore the activity of injured neurons. Typically, neurons do not divide after birth in the mammalian brain. However, new evidence based on the presence of cell cycle associated proteins has shown that some neurons can reenter the cell cycle after brain injury (7–9), passing the G1/S-phase checkpoint and resuming DNA synthesis. The ability for damaged cells to pass this checkpoint and synthesize new DNA is dependent upon how long the apoptotic cells can survive (10, 11).

As brain tissue becomes hypoxic and dies, glutamate is released by neurons triggering the release of inflammatory cytokines and producing a small-scale positive feedback loop (12). Cytokine upregulation intensifies at the injured area. Hypoxic conditions activate anaerobic metabolism and cause a decline in synaptic signaling (13) as well as the reentry by neurons into the cell cycle (14).

Adult mice were anesthetized, and there was a permanent blockage of both carotid arteries. Following sedation, the mice were placed in a controlled atmospheric chamber that was infused with 7.5% oxygen and 92.5% nitrogen for 4 hours inside a hypoxic chamber (14). Blockage of only one carotid artery or an experience of hypoxia alone did not cause brain damage. Only with both insults, the hypoxic environment and the blockage of the carotid arteries, was enough to induce brain damage. Three days after the brain damage had ensued, the hippocampus was populated with pyknotic nuclei, which showed characteristic features of apoptosis, such as the condensing of chromatin and the preservation of the nuclear membrane. Bromodeoxyuridine/5-bromo-2'-deoxyuridine (BrdU) assays were used to identify proliferating cells on days 2, 6, 10, and 14, after the hypoxic-ischemic conditions were induced. BrdU-labeled cells were present in the damaged area of the hippocampus, and not present in any non-damaged region of the brain (14). The presence of the BrdU-labeled cells in the region of brain damage indicates that hypoxic-ischemic conditions may be able to induce apoptotic neurons to synthesize DNA and reenter the cell cycle (14).

However, it is important to note that there was a failure to detect phosphorylated histone, H3, after cerebral hypoxia-ischemia. H3 being used as a late G2/M phase marker. This suggests that neurons that incorporate BrdU were *unable* to complete the cell cycle division (14). Nonetheless, the discovery of neurons entering the G1/S phase of the cell cycle triggered by hypoxia-ischemia is novel. In the future, this finding could be used for potential treatment of neurodegenerative diseases — once, if ever, completion of the cell cycle does occur.

Injured neurons can undergo DNA synthesis in parts of the genome as long as these neurons do not die immediately after injury or disease. Under hypoxic and ischemic combination, neurons from adult rodents were induced to resume DNA synthesis. Reentry into the cell cycle could be an important, yet not fully understood, mechanism of treatment for Alzheimer's disease, stroke and other neurological conditions and diseases. While there may be concern surrounding damaged neurons reentering the cell cycle, when these neuronal cells do reenter the cell cycle, they typically resume apoptotic-associated DNA synthesis (14).

Large animal models

Moving from rodents to larger mammal models (dogs, pigs, and primates) has provided additional insights into the processes that occur when the brain is dying and dies.

In a study by Chen et al., 10 adult mongrel dogs underwent brain death using an inflated subdural balloon. Following induced brain death, all 10 subjects presented Cushing reflex, a hyperdynamic response, and diabetes insipidus (15). Cushing reflex describes an increase in both systolic and diastolic blood pressures (16). After brain death, blood pressure increased 321% and 296% from baseline values (15). A hyperdynamic response is characterized by an increase in cardiac output, contractility, and heart rate (17). This response lasted on average 13.3 minutes. Cardiac output increased the longest, returning to baseline after 90 minutes, while contractility returned to baseline values 45 minutes post-neurological death (15). Diabetes insipidus is a hormonal abnormality that causes an imbalance of fluids (18). Catecholamine levels (dopamine, epinephrine and norepinephrine) were increased in the 15 minutes immediately post-brain death and then tapered off with time. Additionally, adrenocorticotropic hormone, cortisol, and vasopressin

values were significantly decreased over the course of the experiment accounting for the fluid imbalance (15). Overall, the results in this study were consistent with previous findings of the process of brain death in animal models; and indicates animal brain death models are comparable to the human model of neurological death (15).

In a separate study, scientists worked with freshly slaughtered pigs in an attempt to reperfuse dead brain tissue. Their goal was to determine if any signs of activity could be measured post-mortem (19). The swine brain tissues (n=300) were connected to a special instrument the researchers created called BrainEx. BrainEx was a custom, "pulsatile-perfusion" machine; requiring new surgical techniques in order to connect the tissue and machine for reperfusion in large animals (19).

Data analysis found restoration of cellular activity and function at the 6- and 10-hour marks post-mortem (19). Six hours post-mortem, upon administering nimodipine to increase blood flow, blood vessels within the brain were able to dilate and constrict, allowing reperfusion. At that same time point, the amount of microglial and astroglial cells were at normal levels compared to control groups at 1 hour post-mortem. Hemoglobin levels at a normal concentration similar to that of live tissue were detected 10-hours post-mortem with perfusion. While sentience was in no way returned to the dead brain tissue, the results of the BrainEx experiment advances our knowledge on the functional and structural integrity of dead brain tissue in larger animals many hours after death (19).

In 1974, 25 rhesus monkeys underwent complete cerebral ischemia, a lack of blood flow to the brain, caused by intrathoracic clamping of the brachiocephalic and left subclavian arteries for a period of 60 minutes (20). After an hour, the clamps were released, and the brain was reperfused for 19 subjects; resulting in a recovered group and a non-recovered reperfusion group. A variety of physiological measures were taken over the course of 45 minutes up to 24 hours. For the monkeys who experienced reperfusion, the experiment found significant results including an *increase* in cerebral blood flow, mean arterial blood pressure, arterial PO_2, serum potassium, thrombin time, and a *decrease* in cortical pH, as compared to the controls' (before ischemia) values. For the monkeys that did not recover, they had a significantly *decreased* cerebral blood flow, arterial PO_2, cortical pH and *increased* serum potassium values compared to the reperfused monkeys. Interestingly, the non-recovered monkeys showed a significant increase in blood glucose levels as compared to the control group, but not to the recovered group.

Neurological activity was recorded using ECoG with electrodes placed 3 millimeters apart over the dura mater of the sensory cortex. Researchers activated the pyramidal tract by electrically stimulating the motor cortex. During ischemia, electrical silencing occurred quickly in seconds to a few minutes. After reperfusion, 11 of 19 subjects were able to regain pyramidal response and electrical activity; suggesting the return of neuronal function (20). Ultimately, the research by Hossmann et al. found that no major neurological damage resulted if complete blood flow was restored after the ischemic event, which was consistent with findings on cats and dogs.

In another experiment involving 8 rhesus monkeys, the whole head of one monkey was detached from the cervical spine and then attached to the spinal cord of a different decapitated monkey to investigate the continuation of cerebral functioning. The researchers posited that prior brain transplants were unsuccessful because the brain was unable to regenerate in the host body to receive and send sensory input. However, in this experiment, the key was keeping the cranial nerves and the vasculature as intact as possible, so the brain within the head could remain functional (21).

Before and after the procedure on both the receiving and giving monkey, levels of arterial blood gas, carotid pressure, pH, and body temperature, as well as EEG levels, were recorded. A drop in blood pressure was expected due to surgery, and the researchers gave a dose of norepinephrine to prevent hypotensive shock in the body. The specimens were also put on mechanical ventilators to keep them breathing. Before the head could be put onto the new body, researchers cauterized the carotid and jugular arteries to ensure no blood loss. These two blood pathways were previously found to sustain adequate blood supply to a monkey's brain upon dissection (21).

All 8 monkeys survived for periods ranging from 6 to 36 hours after cephalic transplantation. To test neurocognitive ability, food was placed into the subject's mouth and attempts to chew and swallow food were made. Similarly, the monkeys were able to track objects in front of their face with their eyes. These actions suggest awareness of their environment and intact neurological functioning of the cranial nerves post-surgical procedure (21).

The results from the EEG showed patterns characteristic of wakefulness such as small amplitude waves with occasional saw tooth peaks when arterial pressure remained above 40 mmHg (21). When mean arterial pressure decreased in the body, the EEG showed a corresponding decrease with reduced peaks and slower waves, but nevertheless electrical activity persisted (21).

No subjects survived past 36 hours due to blood loss at the surgical site. Post-mortem, all brain areas showed adequate perfusion and uptake (confirmed with Evans Blue vital dye). Under light microscopy, there was no cellular damage to the cerebrum, cerebellum and brainstem (21). As monkeys are our closest relatives, these results have many implications on human brain death, transplantation, and future studies.

Another primate experiment induced brain death via intracranial hypertension in 11 chacma baboons to analyze the mechanism of pulmonary edema after death. Eight baboons received a midline sternotomy for insertion of two electromagnetic instruments to measure flow. The other 3 baboons had Swan-Ganz catheters placed to monitor pulmonary artery pressure and pulmonary capillary wedge pressure (22). A multichannel recording system was used to simultaneously record all pressure values in order to calculate systemic vascular resistance (SVR) and pulmonary vascular resistance (PVR) (22). Baseline measurements were taken for both systemic and pulmonary hemodynamics. Subjects were sedated with ketamine, and normal saline was introduced into the subdural space to induce intracranial hypertension and subsequently brain death. Measurements were recorded

every 15 minutes for the first hour and then every 30 minutes for hours 2 through 7. Once finished, the lungs and heart were histologically examined (22).

Most notably, an increase in circulating catecholamines occurred in the first 5 minutes after induced brain death followed by a return to baseline levels after 15 minutes and continuous reduction. Specifically, in the first 5-minute time period, epinephrine levels increased 11 times the normal amount, while norepinephrine increased threefold, and dopamine levels doubled (22). This is indicative of increased sympathetic activity (the "fight or flight" response), which is believed to be the primary reason for peripheral vasoconstriction. A cascade of events followed vasoconstriction, eventually leading to the pulmonary edema associated with brain death. Under normal conditions, the left side of the heart contains 4% of the total blood volume while the lungs contain about 20% (23). After brain death, a rise in SVR by 537% led to decreased arterial blood flow, and thus, an increase in both pulmonary and left ventricular blood volumes to about 72% of the total blood volume (22). This, combined with the low PVR in the lungs, allowed blood to pool in the lungs without being pumped back out into systemic circulation. This study identified the mechanism of post-brain-death pulmonary edema, which could be important in decreasing risks associated with organ transplantations.

Using animal models to explore the dying brain has provided a repository of invaluable information regarding cellular viability, reperfusion capability, and external stimuli responses; however, the question remains: what does the human brain experience during death?

Human research

With such fraught legal and ethical consideration, it is not unexpected that much of our knowledge on brain death has been gained from animal models. However, while limited, it has been possible to study neurological death in humans.

Understanding the various processes which occur leading up to and during active brain death can widen our understanding of neuronal complexity. The brain is highly dependent on adequate oxygen perfusion and a constant supply of nutrients in order to remain functional. Loss of oxygen leads to a state of acute hypoxia, and eventually, death of neuronal tissue (12). Glutamate is released by neurons as hypoxic tissue dies. Additionally, inflammatory cytokines such as tumor necrosis factor alpha, nuclear factor kappa B (NFκB), and interleukin 1-beta (IL-1β) are released by microglia, astrocytes, as well as neurons. The release of cytokines is initiated by hypoxia-inducible factor 1-alpha (HIF-1α) binding to hormone response element (HRE) like binding sites; leading to the upregulation of cytokine release. These inflammatory agents exacerbate an already injurious situation. Oxygen sensors switch from aerobic to anaerobic metabolism due to the acute hypoxia and synaptic signaling decreases (14). Within less than 5 minutes, as much as 90% of adenosine triphosphate (ATP) in brain tissue is depleted (12).

The existence of a hypoxic condition activates the signaling cascade of NFκB. Reactive oxygen species can either activate or deactivate NFκB, and expression of NFκB increases during hypoxia. Ultimately, it is the HRE binding that directly upregulates NFκB. Once upregulated, NFκB acts on transcription pathways for inflammatory genes and HIF proteins (24). The upregulation of IL-1β cytokines leads to the activation of tyrosine kinases. Activated tyrosine kinases allow an excess flow of calcium ions and the hyperexcitability of neuronal tissue and ultimately tissue damage (25).

As systemic ischemia of neuronal tissue increases, the silencing of spontaneous neuronal electrical activity has been found to happen simultaneously in both humans and animal models. This simultaneous depression of electrical activity has been termed *nonspreading depression* (26). Global ischemia begins a process of hyperpolarization in oxygen-starved neurons. This process happens after only 20 to 40 seconds of ischemia in affected tissue and signals the electrical shutdown process across all hypoxic areas simultaneously. This nonspreading depression gets its namesake because of the simultaneous nature of electrical silencing (26).

Following 1 to 5 minutes after nonspreading depression, neurons have reached the end of their ATP reserve, starving sodium-potassium pumps of energy. The ATP starved pumps fail to restore resting membrane potential, and ultimately neurons lose their ability to maintain polarization and become iso-electric (26). As one neuron becomes iso-electric, other surrounding neurons follow suit in a domino pattern. The result is a self-propagating wave of neuron depolarization; originating either from a single point or from many. Like ripples in a pond, the depolarization spreads, giving rise to the term *spreading depolarization*. It is this spreading depolarization that begins the process of toxic changes, cytotoxic edema, and extracellular rise in glutamate (26).

As discussed above, one of the most important factors to consider when determining brain death is cerebral ischemia (27). Computer tomography perfusion (CTP) used in conjunction with radionuclide scans were found to be the most useful in determining brain death. Researchers compared CTP, computer tomography angiogram (CTA), radionuclide scans, cerebral angiograms, magnetic resonance imaging (MRI), and nonenhanced CT (NECT) for diagnostic accuracy when investigating suspected cases of brain death. These scanning techniques were compared against the accepted standard of a clinical brain death diagnosis across 74 patients. The study found all scanning techniques except NECT to have 100% specificity; meaning the tests correctly avoided flagging a non-brain-dead individual as brain dead (aka a false positive). Among the tests performed, CTP and radionuclide scan were found to have the highest sensitivity (96.7% and 92.9% respectively) and interrater reliability (κ=1 for both techniques); correctly identifying individuals who were clinically diagnosed as brain dead (aka true positives) (27).

Another study conducted a literature review of Medline and Embase from 1996 to 2009 and explored the capabilities of advanced imaging tests, including MRI, magnetic resonance angiogram, and CTA, in determining brain death. Insufficient evidence was found that these tests accurately assessed brain function cessation (3). CTP and radionuclide scanning techniques were not discussed within that paper.

Conclusion

As technological advances have been made, the concept of brain death has become more obtuse. Advances, such as new medical practices and scanning techniques have eliminated false conclusions that have been previously made. While medical advances have been able to prolong life, they have at times made it difficult to define brain death in some patients. As previously mentioned, the clinical process for determining neurological death in humans is to identify a potential cause of brain death and rule out confounding conditions, and then perform a complete physical and neurological examination (2). It is difficult to examine the dying brain in humans due to ethical considerations, so animal models have provided much of the information that establishes our knowledge of the dying brain.

Through gathering information from peer-reviewed journal articles, we were able to analyze and compare animal and human models of the dying brain. Electrical activity on an EEG showed rats having neurological activity for approximately 30 seconds after cardiac arrest. The activity and coherence in the brain was interpreted to potentially be a high degree of internal information processing occurring moments before complete neurological death (5). Although, there is controversy about how to interpret these findings, they provide one plausible scientific and physiologic explanation for human near-death experiences.

Neuronal death in Alzheimer's disease has shown that in some cases, damaged neurons are able to reenter the cell cycle. By understanding both the oxygen and blood perfusions of the dying human brain in conjunction with neuronal death found in Alzheimer's disease and brain injury, additional research and future treatments should be considered (13). Using the various techniques that were examined in this paper, accurate diagnosis of true positive individuals enables the further exploration of Alzheimer's disease and brain injury at the level of the dying neurons. The accurate diagnosis from CTP, CTA, radionuclide scans, cerebral angiograms, MRI, and NECT provide much needed information about an individual and the state of the human brain. It is extremely important that any diagnostic test be accurate in declaring brain dead patients from non-brain-dead patients (27). Additionally, the speed of this determination is vitally important so that organ retrieval and transplantation may occur.

The study performed on mongrel dogs took a deeper dive into the hemodynamics and hormonal changes that follow immediately after brain death (15). This study found a Cushing's reflex, followed by a hyperdynamic response and diabetes insipidus. The Cushing's reflex was shown by an extremely large increase in both systolic and diastolic pressures. A hyperdynamic response was shown by an increase in cardiac output, heart contractility, and heart rate. Diabetes insipidus was shown with hormonal abnormalities leading to an imbalance of fluids (15). These findings explain how the body, at large, reacts to brain death.

Post-mortem pig brains were tested for cellular viability and restoration (19). It was also found that the BrainEx perfused tissue showed normal pyramidal cells compared to the non-perfused samples which showed only swollen tissue. Additionally, the researchers found normal levels of astrocytes, microglia, and hemoglobin in both the 6- and 10-hour post-mortem tissue with the BrainEx samples compared to an in vivo brain. In these animals, when ischemic conditions were induced, electrical silencing occurred shortly afterwards. The animals were reperfused and nearly half of the subjects showed electrical activity, suggesting the return of neuronal function (19). The researchers were in fact successful in restoring both the molecular and cellular processes. While these were significant findings, they are not completely generalizable, as these findings are not associated with the same cognition of a living brain. As shown with both the small and large animals, ischemic conditions may enable nervous system cells to restore function.

A study on monkeys examined complete cephalic transplantation at the cervical vertebrae (21). All monkeys survived, and they showed awareness of their environment and intact neurological functioning of the cranial nerves post-surgical procedure. Another monkey study focused specifically on cerebral perfusion and found an unexpected relationship between complete ischemia and degree of recovery (20), which potentially harkens back to the rat study on neuronal regeneration in hypoxic-ischemic conditions.

Intracranial hypertension in chacma baboons showed interesting results such as an increase in circulating catecholamines that were shown shortly after brain death (22). This was followed by a return to baseline levels after about 15 minutes. Although these findings occurred, most notable was the study's identification of the mechanism of post-brain-death pulmonary edema (22). This finding may be important in decreasing risks that are linked to organ transplants.

In both animal and human models, systemic ischemia caused silencing of neuronal electrical activity. Once this occurs, the neurons will have depleted their ATP reserves, preventing the sodium-potassium pumps from functioning. As the sodium-potassium pumps break down, neurons are forced into a state of iso-electricity and maintain polarization. This creates a ripple effect, causing the neighboring neurons to also be forced into an iso-electric state, creating the spreading depolarization phenomenon (26).

Among the various research articles examined in this paper, there is consensus that death is a transitional process, not a singular point in time, and that the brain is still active for a limited time after electrical silencing. These conclusions are based on our examinations of the various research articles in this paper. With new technologies hijacking and bypassing a person's autonomous ability to breathe and circulate blood, the definition of neurological death will need to be continually reexamined. As a society, we are far from bringing an "offline" brain back online. Eventually, there may be a time in the future where we are able to restore life to someone who previously would have met the criteria of brain death.

Acknowledgments

The authors would like to thank Brian Piper, PhD, professor of Foundations of Neuroscience, and Iris Johnston in Library Services at Geisinger Commonwealth School of Medicine for their support in this endeavor.

Disclosures

None.

References

1. Wahlster S, Wijdicks EF, Patel PV, Greer DM, Hemphill JC, Carone M, et al. Brain death declaration: practices and perceptions worldwide. *Neurology*. 2015; 84(18):1870-9.
2. Goila AK, Pawar M. The diagnosis of brain death. *Indian J Crit Care*. 2009; 13(1):7.
3. Wijdicks EF, Varelas PN, Gronseth GS, Greer DM. Evidence-based guideline update: determining brain death in adults: report of the quality standards subcommittee of the American Academy of Neurology. *Neurology*. 2010; 74(23):1911-8.
4. Pritchard J. Guillain-Barre syndrome. *Clin Med (Lond)*. 2010; 10(4):399.
5. Borjigin J, Lee U, Liu T, Pal D, Huff S, Klarr D, et al. Surge of neurophysiological coherence and connectivity in the dying brain. *Pro Natl Acad Sci*. 2013; 110(35):14432-7.
6. Yong E. In dying brains, signs of heightened consciousness. *National Geographic*. 2013. Available from: https://www.nationalgeographic.com/science/phenomena/2013/08/12/in-dying-brains-signs-of-heightened-consciousness/ [Accessed 22 January 2020]
7. Copani A, Sortino MA, Nicoletti F, Bruno V, Ubertia D, Memo M. Activation of cell-cycle-associated proteins in neuronal death: a mandatory or dispensable path? *Trends Neurosci*. 2001; 24(1):25-31.
8. Liu DX, Greene LA. Neuronal apoptosis at the G1/S cell cycle checkpoint. *Cell Tissue Res*. 2001; 305(2):217-28.
9. Herrup K, Arendt T. Re-expression of cell cycle proteins induces neuronal cell death during Alzheimer's disease. *J Alzheimers Dis*. 2002; 4(3):243-7.
10. Yang Y, Geldmacher DS, Herrup K. DNA replication precedes neuronal cell death in Alzheimer's disease. *J Neurosci*. 2001; 21(8):2661-8.
11. Kuan CY, Whitmarsh AJ, Yang DD, Liao G, Schloemer AJ, Dong C, et al. A critical role of neural-specific JNK3 for ischemic apoptosis. *PNAS*. 2003; 100(25):15184-9.
12. Mukandala G, Tynan R, Lanigan S, O'Connor JJ. The effects of hypoxia and inflammation on synaptic signaling in the CNS. *Brain Sci*. 2016; 6(1):6.
13. Kuan CY, Schloemer AJ, Lu A, Burns KA, Weng WL, Williams MT, et al. Hypoxia-ischemia induces DNA synthesis without cell proliferation in dying neurons in adult rodent brain. *J Neurosci.* 2004; 24(47):10763-72.
14. Sharp FR, Bernaudin M. HIF1 and oxygen sensing in the brain. *Nat Rev Neurosci.* 2004; 5(6):437-48.
15. Chen EP, Bittner HB, Kendall SW, Van Trigt P. Hormonal and hemodynamic changes in a validated animal model of brain death. *Crit Care Med*. 1996; 24(8):1352-9.
16. Cushing H. Some experimental and clinical observations concerning states of increased intracranial tension. *Am J Med Sci*. 1902; 124(3):375.
17. Chiari P, Hadour G, Michel P, Piriou V, Rodriguez C, Budat C, et al. Biphasic response after brain death induction: prominent part of catecholamines release in this phenomenon. *J Heart Lung Transplant*. 2000; 19(7):675-82.
18. Fiser DH, Jimenez JF, Wrape VI, Woody RO. Diabetes insipidus in children with brain death. *Crit Care Med*. 1987; 15(6):551-3.
19. Vrselja Z, Daniele SG, Silbereis J, Talpo F, Morozov YM, Sousa AM, et al. Restoration of brain circulation and cellular functions hours post-mortem. *Nature*. 2019; 568(7752):336.
20. Hossmann KA, Zimmermann V. Resuscitation of the monkey brain after 1 h complete ischemia. I. Physiological and morphological observations. *Brain Res*. 1974; 81(1):59-74.
21. White RJ, Wolin LR, Massopust LC, Taslitz N, Verdura J. Cephalic exchange transplantation in the monkey. *Surgery*. 1971; 70(1):135-9.
22. Novitzky D, Wicomb WN, Rose AG, Cooper DK, Reichart B. Pathophysiology of pulmonary edema following experimental brain death in the chacma baboon. *Ann Thorac Surg*. 1987; 43(3):288-94.
23. Matzen S, Perko G, Groth S, Friedman DB, Secher NH. Blood volume distribution during head‐up tilt induced central hypovolaemia in man. *Clin Physiol*. 1991; 11(5):411-22
24. Eltzschig HK, Carmeliet P. Hypoxia and inflammation. *N Engl J Med*. 2011; 364(7):656-65.
25. Viviani B, Bartesaghi S, Gardoni F, Vezzani A, Behrens MM, Bartfai T, et al. Interleukin-1β enhances NMDA receptor-mediated intracellular calcium increase through activation of the Src family of kinases. *J Neurosci*. 2003; 23(25):8692-700.
26. Dreier JP, Major S, Foreman B, Winkler MK, Kang EJ, Milakara D, et al. Terminal spreading depolarization and electrical silence in death of human cerebral cortex. *Ann Neurol*. 2018; 83(2):295-310.
27. MacDonald D, Stewart-Perrin B, Shankar JJ. The role of neuroimaging in the determination of brain death. *J Neuroimaging*. 2018; 28(4):374-9.