AMARANTH FOUNDATION

A research roadmap: replacing brain cells to combat brain aging

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Executive summary

The brain progressively fails with age. Brain aging exacts a massive human toll through a variety of pernicious effects: it relentlessly strips away memory, identity, and cognition and is linked to a wide range of deadly neurodegenerative diseases such as Alzheimer's disease. Combating brain aging is one of the greatest challenges facing society and is thus critical if we are to massively extend human lifespan. Even if the function of other aging organs can be rejuvenated, these efforts will be in vain if we cannot preserve the function of the brain, which constitutes the cradle of human thought, memory, and identity.

Creating new brain cells to replace aged, dysfunctional ones is one "moonshot" strategy to combat brain aging. One hurdle is that the brain is a complex multicellular community comprising different cell-types (e.g., neurons, astrocytes, oligodendrocytes, microglia, vascular cells, mesenchyme, and others) enmeshed in networks of extracellular matrix and other components. Creating new brain cells to replace aged, dysfunctional ones may be a viable strategy to combat brain aging. Either new external cells can be transplanted, or alternatively, new cells can be created endogenously. **Safely replacing aging brain cells with brand new ones could be transformative and will require intense focus and funding to realize.**

Moonshot idea #1: Transplantation of exogenous brain cells or tissues. Cells (e.g., neural progenitors, neurons, or glia), organoids, or actual brain tissue can be transplanted into the brain. While transplanting cells into the brain may sound quixotic, there have been multiple successes thus far, in both human patients and animal models. We need to amplify this exciting progress in brain cell transplantation.

Neuron transplantation: In clinical trials, transplantation of human fetal brain tissues into patients with either Parkinson's disease or Huntington's disease led to encouraging improvements, although variability between patients was observed[1–5]. Alternatively, large numbers of human neurons can be generated from human pluripotent stem cells (hPSCs, including embryonic or induced pluripotent stem cells) in a Petri dish. Many transplantation studies of hPSC-derived neurons or brain organoids in rodent brains have shown successful neuronal maturation and synaptic integration[6–13]. Notable findings include high survivability and extension of long-range axonal projections[14–16]. In some cases, there is also a marked improvement in animal neurological function[17,18].

Glia transplantation: Glia - which include microglia (the immune cells of the brain) - constitute about 50% of all brain cells[19]. Glia malfunction with age and thus targeting or replacing glia is critical to combat brain aging. Excitingly, drugs can be used to deplete pre-existing microglia within the brain, followed by infusion of new immune cells into the blood that enter the brain and create microglia-like cells[20–25]. This opens the possibility of replacing dysfunctional microglia with brand new ones, although further work is needed.

Challenges: Generating authentic, functional brain cells or brain tissues; minimizing the risk of surgical implantation; and overcoming immune rejection (in the event of immune-mismatched cell delivery).

Moonshot idea #2: Endogenous neurogenesis via stem-cell activation. New brain cells can be created endogenously from pre-existing neural stem cells found in specific brain regions (e.g., the subventricular zone and subgranular zone). New brain cells can also be potentially created *in vivo* by transdifferentiating brain cells of one type (e.g., glia) into another (e.g., neurons) by transcription factor overexpression. Endogenous cell production is advantageous because it will create immune-matched cells, thus overcoming immune rejection.

Challenges: Activating dormant neural stem cells in the brain to induce proliferation and migration to injury or disease sites; identity and function of transdifferantiated cells.

Moonshot idea #3: Learning from songbirds and turtles how to create new adult brain cells. While the birth of new brain cells slows to a trickle in adult humans and mice, other animals (e.g., songbirds and turtles) continuously create brain cells throughout life. Continued creation of new brain cells in songbirds is integral to their capacity to continuously learn new, sophisticated songs throughout life. Can we study songbirds and turtles to understand how to rejuvenate new brain cell production in adult humans? Biology teems with examples where tools from one species can ported to another (e.g., CRISPR).

Challenges: Applying cutting-edge transgenic techniques to songbirds and turtles to decode adult neurogenesis.

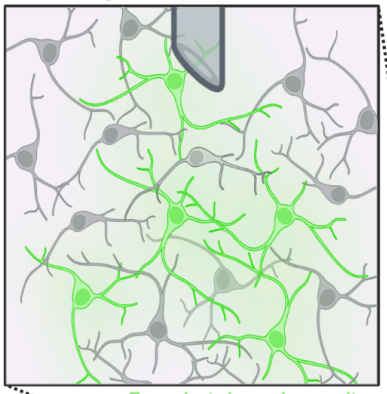
Moonshot idea #4: Brain extracellular matrix: understanding and engineering. The field largely focuses on brain cells, while the extracellular matrix (ECM) that supports brain structure and function has been largely overlooked. It is technically challenging to edit ECM *in vivo*, limiting studies of whether it drives brain aging. Developing new tools and/or therapies to replace or restructure the ECM will rapidly advance this field.

Challenges: Developing new enzymes/inducers/tools to safely edit brain ECM; test roles of specific ECM proteins in aging.

Purpose of the Workshop

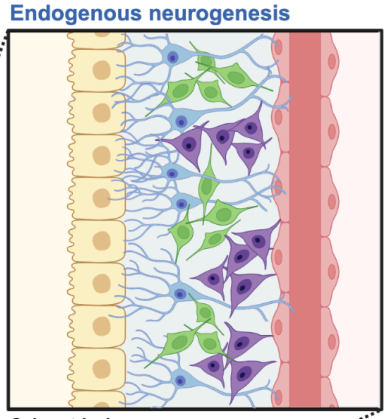
The Amaranth Foundation, part of the James Fickel family office, is a philanthropic organization that funds moonshot science, globally, with a focus on longevity research, and brain aging in particular. In February 2023, the Amaranth Foundation organized a workshop event in San Francisco consisting of the brightest scientific minds in the neuroscience field. The aim was to determine where the future of neuroscience research should be directed in extending the healthy human lifespan, combating neurodegenerative disease, and fostering new collaborations between the participants and their peers.

Moonshot idea #1:
Cell transplantation

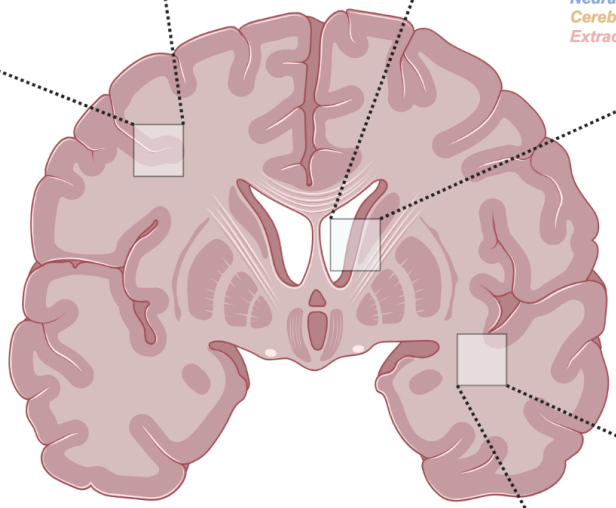


Transplanted neural progenitors
Mature neurons

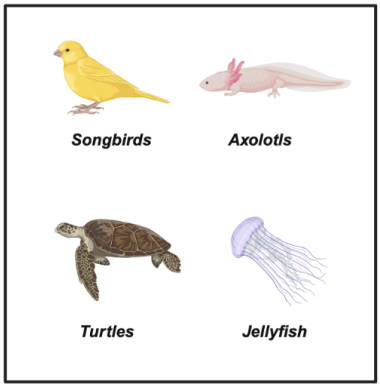
Moonshot idea #2:
Endogenous neurogenesis



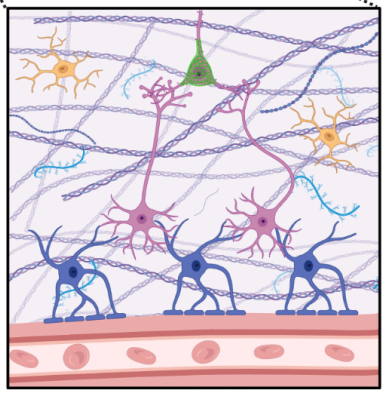
Subventricular zone
Neural progenitor cells
Cerebral spinal fluid
Extracellular matrix



Moonshot idea #3:
Exotic animal models

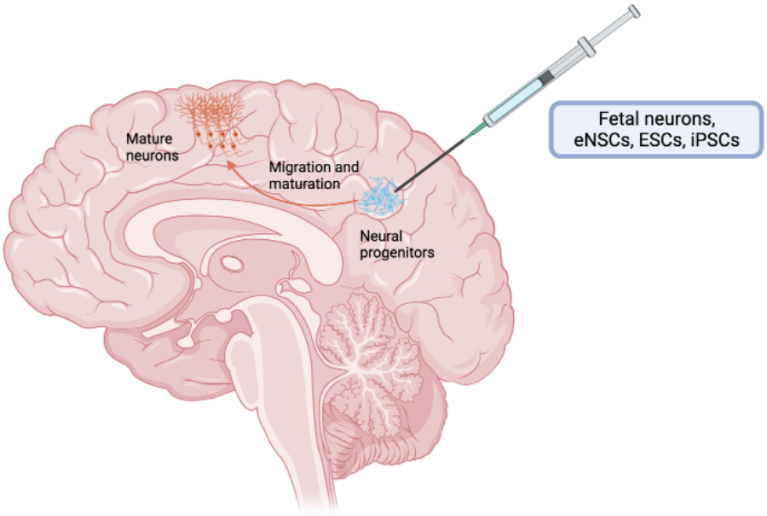


Moonshot idea #4:
Extracellular matrix



Astrocytes *Collagen/elastin* *Perineuronal net*
Neurons *Proteoglycans*
Microglia *Hyaluronan*

1. **Cell transplantation**



Overview:

- It is critical to develop therapeutics that are “proactive” in targeting the underlying causes of disease.
- Exogenous transplantation of neuronal and/or glial progenitors is a “proactive” solution for brain aging.
- Embryonic or induced pluripotent stem cells offer a reliable and scalable source for production of neuronal and glial cell types.
- Cell replacement therapy is hindered by our ability to generate desired cell types. This is due to the time it takes to differentiate into mature cell states, the efficiency of cell differentiation, and the need to optimize for high-throughput expansion and prevent genomic instability.

Degradation (such as in neurodegenerative disease) or damage (such as in stroke) of the brain leads to irreversible neuronal loss, and often results in debilitating symptoms such as cognitive and/or motor function deficits. Current therapeutic strategies are “reactive” to the manifestation of symptoms—namely, they are prescribed to manage existing symptoms and to limit further degradation of neurons. It is therefore critical to instead develop therapeutic strategies that are “proactive” in targeting the underlying causes of disease.

While mammalian brains (compared to other vertebrates) fail to regenerate, there is a small capacity for the mammalian brain to compensate for cell loss. In neuronal loss, network restructuring and synaptic plasticity can reduce the overall symptomatic burden. In Parkinson’s disease, for example, cognitive and functional impairments often occur when almost 80% of the nigrostriatal dopaminergic neurons are lost[26]. Whereas in Alzheimer’s disease, an increase in signal intensity for neuronal circuits recruited for memory compared to healthy controls has

been observed[27], highlighting the adaptability of the human brain during disease and even for those at an older age.

Compensatory mechanisms within the human brain are evident. The brain responds either by recruiting more neurons to 'fire' on any given task, or by recruiting other less affected brain regions to serve as de-facto motor, sensory or cognitive centers[28–30]. It is apparent that during damage to the human brain, some mechanisms of plasticity activate to compensate for cell loss. However, this plasticity is limited, and in cases where extensive degradation of injury-induced neuronal loss occurs - or in cases of heightened inflammation - irreversible damage can occur. It is necessary to develop more "proactive" strategies aimed at preventing or actively treating age-associated degradation.

Exogenous transplantation as a neuronal or glial replacement strategy is one such "proactive" solution and can be performed in any affected brain region. However, the disease pathology and/or cell type or source can affect the clinical outcome. For example, Huntington's disease is a good candidate for targeted cell transplantation using striatal medium spiny neurons. This is due to knowledge of the precise brain region to target and cell type to replace. By contrast, in cases such as stroke or other traumatic brain injuries, neuronal replacement is far more complex, due to the various cell types that die in the affected region[31]. Still, it is worth noting that transplanted neurons can extend axons and integrate into the existing neural network even after a stroke[32].

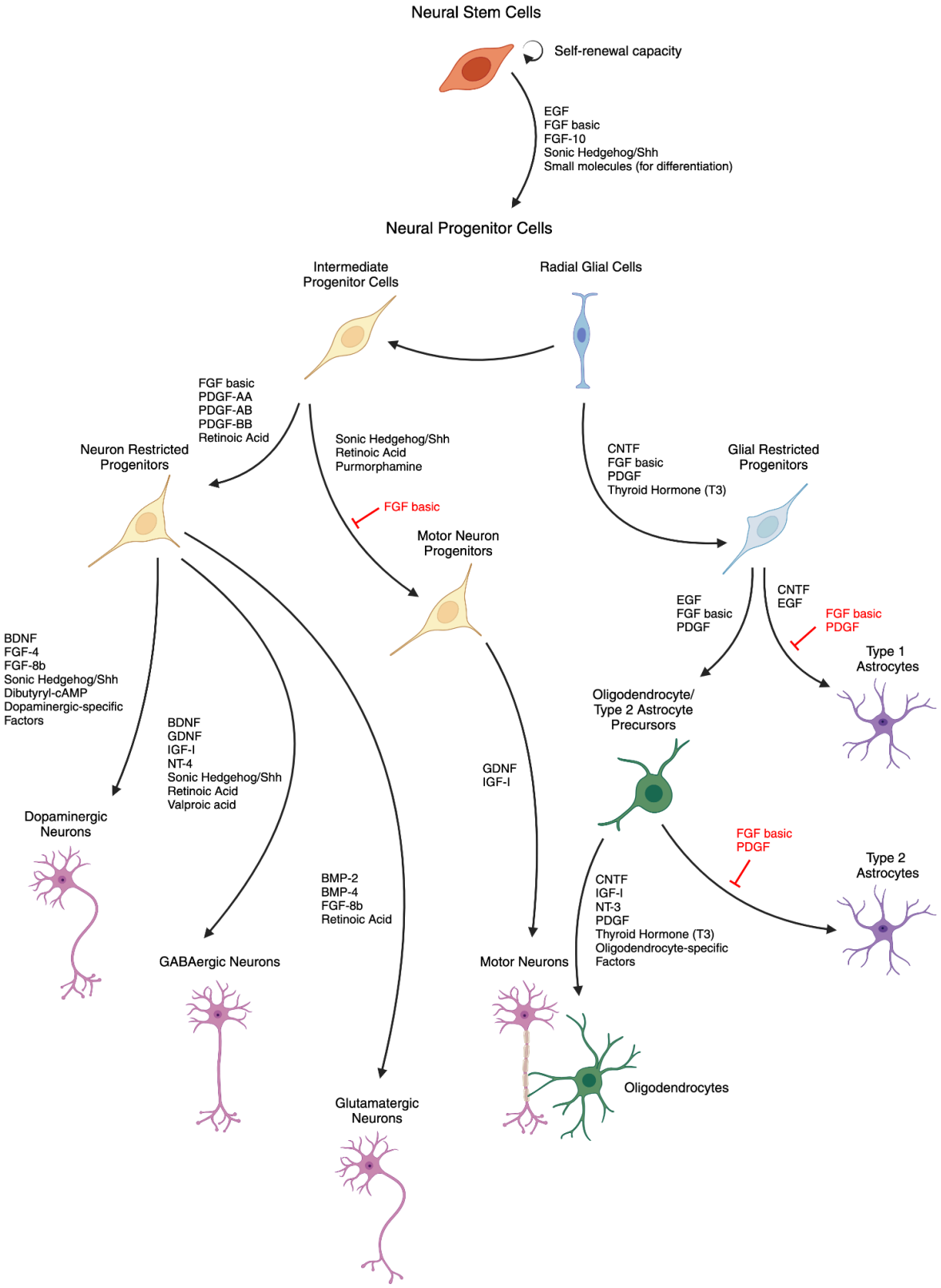
The choice of source cells and production of desired cell types is a vital component in the successful outcome of transplantation. Key criteria, such as availability, expandability, and differentiation into your desired cell type must be taken into consideration (see section 2). Groundbreaking work using cells obtained from fetal tissue directed to differentiate into dopaminergic neuronal subtypes were transplanted into animal models for Parkinson's disease, thus giving birth to the field of cell transplantation. Subsequent data demonstrated good cell survival and integration into the neural network resulting in improved motor function[33,34]. In subsequent decades, clinical trials involving fetal-derived tissue for cell replacement in patients with either Parkinson's disease or Huntington's disease showed encouraging improvements, although variability between patients was observed[1–5].

Embryonic or induced pluripotent stem cells offer an alternative, scalable source for production of neuronal or glia cell types. Numerous transplantation studies in rodent brains have shown successful neuronal maturation and synaptic integration, highlighting the power of this method[6–13]. Notable findings include high survivability and extended, long-range axonal projections[14–16]. In some cases, there is also a marked improvement in behavior[17,18].

Cells derived from pluripotent stem cell sources hold great promise as for cell-replacement therapy. This is because they are able to generate desired cell types upon transplantation[35]. However, a number of limitations persist. First, the time taken for stem cells to differentiate into mature neurons is currently prolonged. Second, the efficient conversion of stem cells into cells of a defined state is important in order to prevent the development of tumors or unwanted

tissues upon transplantation[36]. Third, high-throughput expansion of cells in culture pre-transplantation must be optimized in order to prevent the manifestation of genomic instability within cells, a hallmark of tumor formation[37,38]. Despite these limitations, it is evident that cell replacement therapies have greatly impacted the field in combating brain aging and traumatic brain injury as marked by successful rodent and clinical trial studies. It is also evident, however, that a greater effort must now be made to optimize the conditions of the cells transplanted to increase survivability, the safety profile, and the time taken to produce the cells in the first place.

2. Principles of stem cell differentiation



Overview:

- The number of cells that are able to differentiate and integrate into existing bran neural circuitry is relatively small, with a large proportion of cells dying post-transplantation.
- Our understanding of the molecular mechanisms that underpin cell differentiation is poorly understood.
- Production of a detailed cell differentiation roadmap to produce pure cell populations is a solution and would offer a limitless supply of cells for downstream applications.

Replacement of aged cells in the brain with younger, healthier cells is thought to restore brain function[39]. Recent research in this area has demonstrated that transplanted embryonic neurons in mice are capable of integrating successfully within the brain and is involved in complex activities, such as vision and motion[35,39,40]. However, the number of cells that differentiate and integrate into the neural circuitry is relatively small, with most cells dying soon after transplantation[41–44]. Our understanding of how cells differentiate, and the molecular mechanisms that underpin this remain poorly understood[45]. While there are many challenges to making cell replacement therapy in the brain a reality, the biggest among them is our ability to produce pure populations of human cells for transplantation from embryonic and induced pluripotent stem cells. The prospect of producing many different kinds of brain-specific cell types from stem cells offers production of a limitless supply of “young” cells for cell replacement. This approach also opens up the opportunity to produce genetically modified cells using CRISPR[46,47] and then to transplant cells that have carry “rejuvenating” genetic modifications that can prolong healthy brain function, or potentially reverse aging.

Pioneering work in recent years has accomplished the production of over 25 different human cell types ranging from blood to the brain [48–51]. To achieve this, a detailed cell lineage roadmap outlining the sequence of intermediate cell progenitor states that results in the production of desired, high purity cell types is needed. The efficiency of this system is down to two key aspects. First, the simultaneous activation of desired cell types alongside the blocking of unwanted cell types, and second, the timing of applied signaling factors to induce a change in cell type, typically within 24 hours. This approach highlights the complexity of controlling cell states and is in stark contrast to the established norms of differentiation via continuous activation or inhibition for many weeks.

Generating any desired cell type from stem cells offers a limitless supply for various downstream applications, such as disease modeling, transplantation/engraftment, and other basic research concepts. Using a detailed roadmap approach to specific cell type generation has been successfully reproduced by several academic groups[52–58], owing to this platform’s reproducibility. However, the challenge still remains to generate the thousands of the different brain cells that exist from stem cells[59], opening up the opportunity to provide a limitless supply of cells from transplantation. The steps to achieve such a goal will involve building a brain-specific roadmap; identifying the progenitor cells that give rise to brain cell type; and elucidating the extracellular signals that can induce target cell type. By repeating this process, a detailed map can be generated for each cell type lineage.

2.1. **Neurons**

Overview:

- The current standard to differentiate stem cells into brain progenitors, the “dual SMAD inhibiting” method, is capable of producing forebrain and midbrain cells, but not hindbrain cells.
- Ex-vivo differentiation into the estimated 110 neuronal subtypes that exist in the brain is not possible and remains an unresolved challenge in the field.
- Furthermore, the timeline to produce neurons commonly takes weeks to months. The need to develop strategies to speed up this process remains an open challenge.

The current standard to differentiate stem cells into brain progenitors involves inhibiting TGFβ and BMP, termed the “dual SMAD inhibition” method[60]. While this method is capable of producing cells specific to the forebrain and midbrain[13,16,17,61–66], it remains difficult to produce hindbrain cells, suggesting that these cells are derived from another progenitor type. This explanation goes against the prevailing model that a single neural progenitor can produce any brain cell type, and further highlights the complexity of brain development.

This is further complicated due to the multiple subtypes of cells that exist in the brain. For example, in the cerebral cortex, where our most high-level cognitive functions exist, there are two major cell types: cortical glutamatergic (excitatory) neurons and GABAergic (inhibitory) interneurons[67–69]. Of the cortical excitatory neurons, multiple subtypes exist across six distinct regions within the cortex[70–73], with a further 56 finely-grained excitatory subtypes so far discovered[70]. Looking at the cerebral cortex in its entirety, it is estimated that over 110 neuronal subtypes exist[70], underscoring the sheer challenge of being able to produce all of these cell types.

In order to generate cells for transplantation, the infrastructure to reliably expand and generate desired brain cell types in a time- and resource-efficient manner remains an unresolved challenge. Currently, differentiation of stem cells into neurons is a lengthy process taking weeks to months[74]. Work to rapidly and efficiently generate specific types of desired brain cells is pivotal for future cell replacement therapy. Further research into extracellular signaling factors[75], extracellular matrix[76], and/or small molecules[77] would be promising first steps to resolve this challenge.

2.2. **Glia – microglia, astrocytes, and oligodendrocytes**

Overview:

- Our understanding of differentiating stem cells into glia is poor. This approach not only takes several months but produces 'impure' glial populations. Maturation of glial progenitors into cells of a defined state with a high degree of purity is an unresolved challenge in the field.
- Microglia cell replacement offers an alternative method for neurodegenerative treatment. However, a major obstacle to microglia transplantation is due to resident microglia being highly regulated – making transplantation difficult.
- Pharmacological depletion of resident microglia using colony-stimulating factor 1 (CSF1R), followed by the transplantation of CSF1R resistant microglia offers a potential therapeutic strategy.

While great strides have been made in the field of cellular differentiation and reprogramming for neural progenitors into brain specific cell types, there is an unmet challenge in generating glia – microglia, astrocytes, and oligodendrocytes. Glia comprises about 50% of all brain cells[19], and there are multiple types of glia. Microglia are long-lived resident immune cells of the brain, acting as the first line of defense against invading pathogens. Activation of microglia, therefore, is an important protective mechanism in maintaining a healthy brain state[78]. Astrocytes are key regulators of brain function, promoting neurogenesis and synaptogenesis (the formation of synapses between neurons), and controlling blood brain barrier permeability[79]. Oligodendrocytes wrap myelin (a multilayered sheath of membrane) that insulate around the axon of neurons to enable fast electrical communication between cells[80].

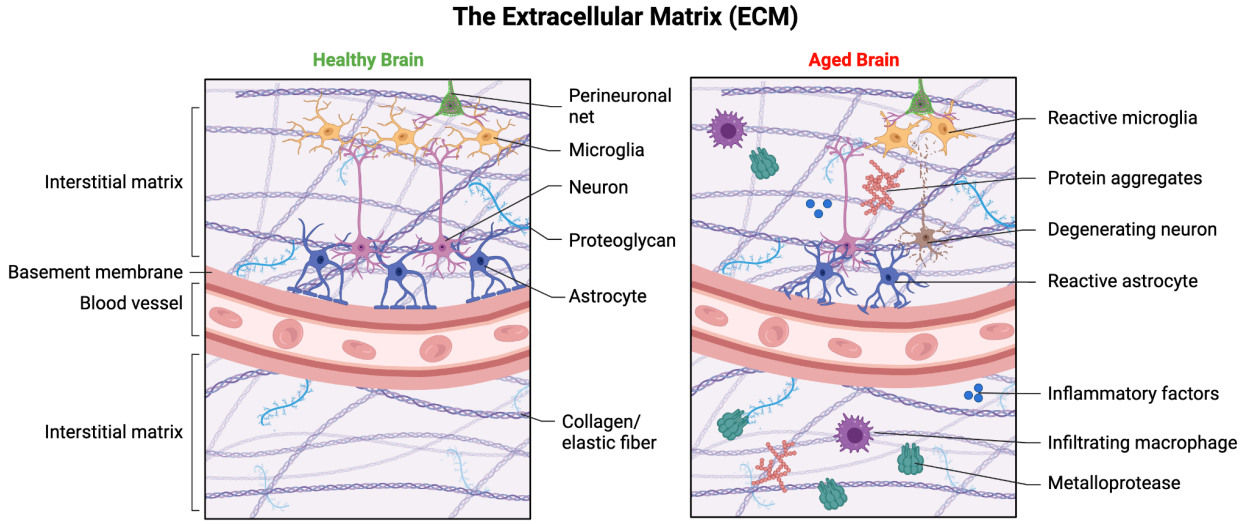
In age-associated neurodegeneration (such as Alzheimer's or Parkinson's disease), dysfunction of the glia is one of the major drivers of disease manifestation[81,82]. Generation of these pure, 'non-neuronal' cell types would enable future cell-replacement therapies capable of combating brain aging. However, generation of glia in this way is notoriously challenging, with current efforts taking months to create 'impure' cell populations[74]. Furthermore, the maturation of glia precursors into cells of a defined state remains undetermined with regards to which signaling factors to use, resulting in "unguided" differentiation at the final stages. Our understanding of the molecular mechanisms in glial biology remains poorly understood and an open challenge in the field. Applying a similar roadmap strategy, as highlighted above, would be a significant step in the right direction.

In more recent years, significant attention has been devoted to microglia. Microglia play a key role in maintaining neural circuitry and blood brain barrier development[83,84]. In some neurodegenerative diseases, such as Alzheimer's, a high proportion of genes that confer genetic risk are derived from microglia[85–88], highlighting the key role microglia play in maintaining normal brain homeostasis. Current therapeutic approaches targeting microglia are primarily pharmacological[89–91] – though some studies have explored microglia transplantation as a replacement of existing microglia in the brain[20–25]. A major obstacle to microglial cell replacement is that resident microglia are highly regulated, and engraftment of new, exogenous

microglia is very challenging[92]. One solution is to deplete the resident microglia via pharmacological depletion with colony-stimulating factor 1 receptor inhibitors (CSF1Ri) alongside bone marrow transplantation of peripheral monocytes to infiltrate the brain[21,25]. However, this approach is highly risky due to the irradiation or chemotherapy required to enable engraftment of peripheral myeloid cells. Even with successful long-term engraftment, the cells are still functionally and transcriptionally distinct from microglia[20,93,94]. In addition, the engraftment efficiency is very low[95]. It is evident that a simpler, more targeted approach to cell replacement is needed.

Methods to deliver stem cell-derived microglia that are functionally and transcriptionally more akin to the resident microglia[96,97] and can be delivered without the need for harmful conditioning must be developed. Recent research has sought to address this issue. Inhibitor resistant CSF1R microglia derived from immortalized hematopoetic progenitors have successfully been transplanted into mice. Clearance of resident microglia in the brain with CSF1Ri alongside replacement and expansion of CSFR1 inhibitor resistant microglia have been shown to exhibit similar gene expression profiles and activation response to inflammation[98]. However, future work in the field is needed to assess the potential aberrant effects of replacing resident microglia with engineered, drug-resistant microglia and to assess downstream effects such as longevity and long-term function. This approach opens up the opportunity to increase the efficiency of microglia replacement whilst reducing the overall risk to health compared to other methods. Further cell engineering capabilities would enable future transplantations with microglia that significantly improve human health, in addition to the rejuvenating effects of replacement. Directed research towards cell replacement of other glial cells – astrocytes and oligodendrocytes – should also be made for targeted therapies towards more glial-specific diseases.

3. Extracellular matrix



Overview:

- Damage to the extracellular matrix (ECM) is also a major contributing factor to neurodegeneration. Our understanding of the intrinsic "monitoring" systems that promote ECM repair are poorly understood and need to be further explored.
- Current therapeutic strategies to treat ECM degradation involve drugs that alleviate ECM degradation but are unable to reverse the damage already occurred.

Another aspect to aging involves damage accumulated outside of cells, in the extracellular matrix (ECM). Damage to the ECM is a particular issue, because unlike cells, the extracellular matrix is more challenging. It is reliant on the resident cell population and may incorporate molecules produced by different cell types. In general, the components of the ECM are deposited in the space by the cells and support cell function and viability[99]. However, in some cases, over the life of an individual, these deposits do not get turned over and/or the ECM accumulates damage (due to products of proteolytic ECM remodeling, advanced glycation end products, and pro-inflammatory or neuroplasticity-inhibiting epitopes)[100]. While there are receptors to ECM proteolytic products (such as brevican)[101] and short vs. long hyaluronic acids[102], there is a desperate need to better understand the intrinsic "monitoring" system within cells that is able to recognize and/or repair damage made to the ECM. One major contributor to neurodegeneration, for example, is damage accumulated to extracellular carbohydrates that are of particular importance in ECM collagen structure[103].

There is always a pool of ECM molecules that are not incorporated into the ECM and could potentially be recruited. It is hard to exclude that ECM structures exchange their content as some ECM molecules can be released, diffuse, and then become trapped to another ECM structure. Neighboring cells can sense the state of the ECM and produce more molecules or proteases that reshape the ECM structure[104]. Damage to the ECM is a major contributing factor

to neurodegeneration, modulating oxidative stress susceptibility and cell excitability[105,106]. Current therapeutic strategies to treat ECM degradation involve drugs that alleviate ECM degradation or inhibit synthesis of ECM components but are unable to reverse the damage already occurred[107].

In Alzheimer's disease, for example, carbohydrate-protein aggregates accumulate over time resulting in a stiffening of the ECM[108,109]. Small-molecule solutions to prevent or alleviate the degradation of the ECM are not "specific" enough, being unable to identify and reverse the complex damage that occurs as we age[107]. Furthermore, cell replacement strategies, such as what we have highlighted above, would not effectively rejuvenate the ECM, due to the already accumulated damage. Moreover, ECM aggregations enriched in cell migration- and neurite growth-inhibitory epitopes may substantially limit integration of transplanted cells into the tissue and their regenerative capacity[110].

ECM molecules are crucial to support cell viability and growth, and may be integral to the success of cell transplantation. However, while every other part of the human body can be surgically replaced, the brain cannot. An alternative solution to "rejuvenate" the ECM would be to replace damaged brain tissue with healthy tissue, replete with its own tissue. As our understanding of differentiating and producing cells of a particular type grows (see section 2), we could, perhaps, eventually grow tissues and organs that are tailored to each patient with regards to size and immunological compatibility. While the initial focus will be on treating injury and disease, it is theoretically possible to replace tissue in otherwise healthy individuals to reverse aging.

3.1. **Other challenges**

Overview:

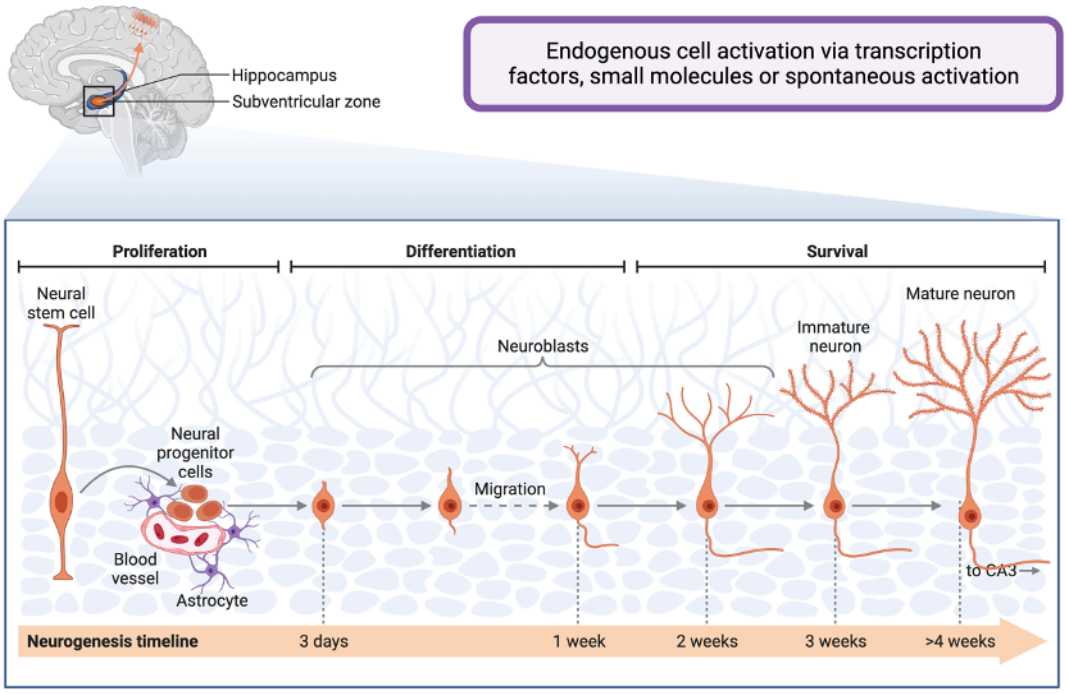
- Progressive replacement of damaged brain tissue with younger, healthy tissue that is size- and immunologically-matched could reverse the impact of disease, and potentially reverse brain age in healthy individuals.

The brain, unlike other organs, cannot simply be replaced in its entirety in “one shot” due to the important functions each region plays in maintaining cognitive function and life. There are two established principles in neurobiology that support progressive brain tissue replacement. First, it has been demonstrated that brain region functions can change over time. For example, in a case of benign gliomas slowly destroying the language centers of the brain, relocation of language centers in older adults were relocated to other brain centers[111]. This is in contrast to language loss due to sudden forms of damage, such as in stroke or traumatic brain injury[112]. As long as deterioration/destruction of brain tissue is progressive over time, other brain regions can act as de-facto brain centers for those being damaged, whether it be language, personality, sight, or motor function. Second, it is not necessary to understand the intricate development and function of brain tissue to treat disease or aging. Our brains develop following germinal cells in fetal development. Research using these cells grafted onto adult rodent brains exhibit an innate differentiation program that generates functional neocortical tissue[113-117]. However, despite the promising results highlighted above, grafting studies thus far have failed to produce functional tissue in adult human brains and remains an open challenge in the field.

The capacity for neurons (and glia) to regenerate is a significant factor in treating neurodegenerative disease and aging. In humans this capacity is greatly limited to neurodevelopment. However, in some mammals, birds, and reptiles, the ability for their brains to generate new neurons extends well into adulthood[118-120]. A key component to successful neuronal regeneration is due to radial glia, specialized brain stem cells give rise to neurons and glia. During embryonic development, radial glia cells extend long projections from the ventricles at the center of the brain to the outer surface. These serve as physical “highways” that enable newborn neurons to migrate from the sites of their birth to their final destinations within the brain[121,122].

However, in adult brains these “highways” disappear and affect how neurons can migrate. In songbirds, for example, newborn neurons were able to move through adult brains via “directed diffusion” and not via radial glia[123]. In an adult mouse brain, injected embryonic neurons into the visual cortex were able to integrate and cause a significant rejuvenation effect to vision[34]. However, work to understand the migration and integration process of neurons is poorly understood for brain regeneration. Possible solutions include controlled cell migration using implantable devices with chemical ligands or magnetic fields, or transplantation of cells sensitive to ultrasound to activate specific patterns of neuromodulation? These remain open challenges in the field.

4. Endogenous cell activation



Overview:

- In the adult mammalian brain, there are two neural stem cell (NSC) regions, the subventricular zone (SVZ) and subgranual zone (SGZ) capable of producing neurons and glial cells.
- These calls can become “activated”, proliferate, and migrate to different brain regions as they slowly differentiate.
- The capacity for NSC activation, differentiation and migration in the aged brain is greatly diminished, resulting in cognitive decline.
- Identifying the genes that can impact neural stem cell (NSC) activation is vital in understanding and preventing the effects of brain aging.
- Current work in the field has identified only a handful of genes, due to limited experimental designs in the past.
- Innovative work developing a high-throughput, genome-wide screen in old mammalian cells and rodents has identified over 300 genes which, if downregulated, become capable of activating old NSCs.
- Further work to expand this screen in more relevant models (such as non-human primates, or in human brain tissue) is required, and remains an unresolved challenge in the field.

In the adult mammalian brain, there are several neural stem cell (NSC) regions that have the capacity to differentiate and produce neurons or glial cells capable of repairing tissue[124–208]. The subventricular zone (SVZ) of the lateral ventricles in the cerebellum and subgranual zone (SGZ) in the hippocampus are the two active regions in the adult brain[125,126,127,130–133]. Both have the capacity to generate thousands of neurons or glial cells[134,135]. Within the SVZ, much like the SGZ, a population of quiescent NSCs becomes 'activated' and proliferates, generating neural progenitors that commit to a particular cell lineage. These 'committed' cells migrate out of the niche towards the olfactory bulb and slowly differentiate into neurons. However, in the aging brain, NSC activation is severely impacted, resulting in decreased regeneration and cognitive decline[124,125,130,136–139]. This is associated with changes in the structure and composition of the ECM in the SVZ neurogenic niche. Differentiation of glial progenitor cells can produce astrocytes or oligodendrocytes. Production of these cells is important for maintaining normal brain homeostasis and motor function.

Identifying the genes that can impact NSC activation is an important step in preventing the effects of aging in the brain. While seminal work has already been conducted to elucidate "old" NSC activation, only a few signaling pathways and transcriptional regulators have been discovered[140–149]. This is due to the limited capacity of the studies conducted, only focusing on a few genes at a time. A high-throughput, scalable approach is required, capable of screening hundreds or thousands of genes at a time over key aging timepoints.

As aging occurs at a cellular and organismal level, a screen able to capture a complete dataset on both levels will be highly informative. In 2022, innovative research has developed a CRISPR-Cas9 genome-wide screen for old mammalian cells and organisms capable of identifying novel gene targets whose manipulation potentially could restore brain function. Initial findings have discovered over 300 genes which, when downregulated ("switched off"), activate old NSCs in cell culture, with gene regulatory pathways associated with top hits in glucose metabolism, ribonucleoprotein structures (important for regulating gene expression), and primary cilia (critical for regulating key signaling pathways). Most of the aforementioned pathways have previously not been associated with NSC activation and/or regulation, highlighting the power of this approach[150].

Identifying genetic interventions to rejuvenate the adult brain would be the "holy grail" of adult neurogenesis research. Such an approach could greatly impact the rejuvenation potential of old tissues, improving cognitive and motor functions, and reverse overall brain age.

An alternative approach to endogenous cell activation via the SVZ and/or SGZ is to directly transdifferentiate pre-existing glial cells into neurons in vivo. Such an approach would avoid the need to produce clinical grade, pure cell populations; the trauma associated with direct transplantation; and the risks of graft rejection. Work in this area has already established direct conversion from astrocytes to neurons via transduction of various neurogenic factors[151,152], and conversion of pericytes (cells that line blood vessels) into neurons in-vitro[153]. However, due to the complex and dynamic environment of the brain, direct conversion into neurons is a

challenge. Some controversy exists, however, with some data suggesting that reprogrammed astrocytes into neurons in-vivo were actually just targeted neurons which stayed as neurons[154].

Initial work in this area has shown that glial cells are able to convert into neurons following injury in vivo, however, the subsequent neuronal populations were immature, the conversion rates low, and newly born neurons died early[155]. However, with the expression of neurogenin 2 (a neurogenic factor), vitamin D and E, around 90% of glial cells were able to convert into mature, morphologically complex neurons following injury. Interestingly, both astrocytes and oligodendrocytes are able to convert into glutamatergic deep layer neurons following injury, whereas oligodendrocytes can only generate GABAergic neurons[156,157], suggesting that starting cell-type plays a key role.

In a mouse model of Alzheimer's disease, an increase in conversion efficiency for astrocytes to neurons in old vs. young mice was observed, indicating the important of the microenvironment on cell fate[157]. Furthermore, the brain region can also greatly affect direct reprogramming. In the striatum, glial cells converted into mature neurons following Sox2 and BDNF expression, whereas cortical derived glia converted into immature neurons or failed to convert altogether[158–160]. Similar observations have been made in other cases[161,162].

Altogether, it is evident that while direct conversion is possible, producing subtype-specific cell types remains a challenge. Conversion and maturation of new neurons may be dependent on the local circuitry for reprogramming[163,164], which is lost in aging and disease. Work to elucidate the complex signaling factors that can efficiently convert glia into neurons, and robust targeting of those factors to target brain regions would make for an effective therapeutic approach to brain injury and age associated disease.

5. **Concluding remarks:**

The understanding of adult neurogenesis in humans would be revolutionary. Such a discovery would fundamentally transform our healthcare system. The way we treat neurodegeneration or traumatic brain injury would be “proactive,” rather than “reactive” to symptoms. It is not clear how this issue should be addressed; but three major avenues seem promising.

First, we must get better at generating desired cell types for cell replacement therapies. As covered in section 2, production of over 25 different cell types to a high degree of purity has already been achieved. By “road-mapping” to a higher degree of specificity, it is possible to chart the differentiation and production of currently unattainable cell types – hindbrain derived neurons, glia, and the hundreds of neuronal subtypes that extend throughout the brain. Second, as covered in section 1 and 3, the avenues to cell and/or tissue replacement as a means to treat neurodegeneration, injury, or aging offer distinct clinical outcomes. It is vital that the quality of cells transplanted be of a high degree of purity to enable suitable migration and integration. Previous research has explored the potential of neuronal transplantation as cell replacement, with varying results in both animal models and humans.

Much of neurodegeneration, injury, and aging is mediated by inflammation; in turn, inflammation is thought to be controlled to a large extent by glia. Glia have emerged as having paramount roles in brain health and disease, and therefore glial transplantation offers an alternative approach to treat aging and disease. By transplanting glia, we gain the opportunity to modulate inflammation and ultimately brain health. However, our ability to produce glia safe for cell replacement therapy remains underdeveloped. Tissue replacement, in contrast to cell replacement, is a strategy that replaces damaged cells as well as the supportive ECM. As we age, the ECM changes and stiffens, likely accelerating decline. Tissue replacement encompassing both cells and ECM offers the opportunity to reverse the effects of neurodegeneration and aging in general.

Finally, section 4 covers the “holy grail” in the neurogenesis field - namely, creating new neurons or glia endogenously within the brain itself - and consequently, developing ways to activate the stem cell niche contained within the SVZ and SVG as a means of cell replacement. This would be the most ideal clinical outcome. However, our understanding of how we can activate and guide these stem cells remains poor. Recent technologies, using CRISPR, have developed ways to screen a large number of genes that are implicated in neural stem cell regulation. Using these technologies, we can discover new ways to activate and control cell fate for treatment of disease and old age.

Appendix 1.

Brief profiles of scientific participants:

- Dr Kyle Loh
  Kyle Loh is a stem cell and developmental biologist, currently an Assistant Professor and The Anthony DiGenova Endowed Faculty Scholar at Stanford. Kyle's laboratory has developed methods to rapidly and efficiently generate 25+ human cell-types—ranging from brain to bone to blood vessels—from pluripotent stem cells, providing a platform for basic research and future cell replacement therapies.

- Dr Xin Jin
  Xin Jin is an Assistant Professor in the Department of Neuroscience at Scripps Research, where her team develops and applies scalable genetic screening tools, *in vivo* Perturb-seq, to study the gene functions in brain disease and aging with single-cell and spatial resolution. Previously, Xin was a Junior Fellow at the Society of Fellows at Harvard University and obtained her Ph.D. in neuroscience from Rockefeller University and her B.Sc. in chemistry from MIT.

- Dr Tyson Ruetz
  Tyson Ruetz is a cell and molecular biologist who is focused on the fundamental question of how old adult cells and tissues can be reprogrammed to regain a youthful state. He built his foundation with a PhD focused on induced pluripotent stem cells and Postdoctoral training in neural stem cells and advanced genome engineering techniques. He is developing *in vivo* screening technologies to rejuvenate neural stem cells in the aging brain.

- Prof. Jean Hébert
  Jean Hébert obtained his PhD from the University of California San Francisco studying how embryonic stem cells generate the early cell lineages that make up mammalian embryos. He then moved to Stanford University for postdoctoral studies on neural stem cells and how they generate the neocortex. These studies continued after he joined the faculty at the Albert Einstein College of Medicine, where his lab works on establishing tissue replacement strategies to reverse all forms of age-related brain damage.

- Dr Nicole Coufal
  Nicole Coufal is a physician scientist with research interests in neuroimmunology and innate immune therapeutics and a clinical practice in pediatric critical care. Her background combines clinical training with research in stem cell biology, translational neuroscience, and epigenetics.

- Prof. Timothy Gardner
  Tim Gardner did his PhD in Physics and Biology at Rockefeller University and is currently an Associate Professor at the Knight Campus, University of Oregon. His lab's research is focused on the systems neuroscience of sensory-motor learning in songbirds. Before the University of Oregon, Tim was a founding team member of Neuralink, a company focussed on brain-computer interface development.

Appendix 2.

Brief profiles of workshop organizers:

- James Fickel
  James is an early Ethereum investor and founded Amaranth to accelerate research in longevity.

  He assembled Amaranth's scientific advisory board, a group of young leaders in longevity biotech, to source and evaluate ambitious research covering the aggregome, centenarian genetics, cryopreservation, germline rejuvenation and brain aging. He has backed the Norn Group, Impetus Grants, the SuperAgers Family Initiative, the Time Initiative Undergraduate Fellows Program, the MBL Aging Course, as well as labs from Harvard, MIT, and Stanford.

- Joanne Peng
  Joanne is currently at Princeton and the Boyden Lab at MIT, where she works on tools for brain aging including spatial proteomics and whole brain modeling. Previously, she was a Thiel Fellow, where she worked on mitochondrial dynamics at the Biomedical Institute at MaRS, COVID testing at Curative, and cancer research at the Buck Institute.

  Joanne has been supported by the Thiel Fellowship, Interact Fellowship, Day One Project, the Institute for Progress, the Davis UWC Foundation, and VitaDAO.

- Rohan Krajeski
  Rohan is currently a PhD student in Biotechnology (Molecular Neuroscience) at the University of Cambridge where he develops brain organoid models to study neurodegenerative diseases. He is co-advised by Prof. Gabriele Kaminski Schierle and Dr Madeline Lancaster.

  Rohan has an extensive scientific background in genetic engineering and neuroscience, having previously worked at MIT in the Abudayyeh-Gootenberg Lab, the University of Oxford and University College London (UCL). At Amaranth, Rohan focuses on brain aging.

- Dr Alex Colville
  Alex joined Laura Deming to build the next evolution of the Longevity Fund, called age1, as General Partner, to invest in and catalyze ambitious founder-led longevity biotech companies.

Previously he was Chief of Staff at the Amaranth Foundation where he built out the longevity focus of the family office. Alex completed his PhD in Genetics at Stanford University in Tom Rando's lab studying the biology of aging after having worked in Boston in management consulting at Putnam Associates, a boutique life sciences consulting firm.

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