

# The Brain Preservation Foundation's Aspirational Neuroscience Prize

## Introduction:

The Aspirational Neuroscience Prize is designed to spark debate within the neuroscience community about the long-term promise of neuroscience for humanity. Its chief goal is to inspire the neuroscience community to openly consider and debate the following deep and controversial questions:

- 1.) What will 22<sup>nd</sup> century neuroscience and technology look like?

Is it possible that we will have, by that time:

- 2.) Succeeded in obtaining a comprehensive computational understanding of the brain?
- 3.) Developed the technology to map whole preserved human brains at a level which shows neuronal connectivity and synaptic ultrastructure, and at a level which shows the densities of receptors, ion channels, and other key proteins?
- 4.) Developed the understanding needed to decode memories based on such maps?
- 5.) Developed the technology to computationally simulate a brain's functioning based on such maps?

And most controversial:

- 6.) Is it possible that 22<sup>nd</sup> century neuroscience will have the understanding and technology needed to 'revive', through digital, biological or other methods, the mind of a person whose brain was preserved in the early 21<sup>st</sup> century?

These are deep and controversial questions indeed. If the answer to question #2 is 'No', then it might imply that a computational understanding of the brain is fundamentally unobtainable. On the other hand, if the answer to question #6 is 'Yes' then it may have momentous ramifications, not just for future generations but for us today as well. Only the neuroscience community can knowledgeably debate these questions. And these questions can only be answered by neuroscience research targeted directly at their core premises.

The Aspirational Neuroscience Prize is designed to highlight and reward research that clearly addresses these questions, and in the process it is intended to raise awareness among neuroscientists of the larger, longer-term ramifications of their research. Specifically it is designed to raise awareness that the brain preservation techniques used in today's laboratories to research the synaptic and molecular basis of memory might already be sufficient to preserve the unique information content of a human brain—the preserved brain acting as a 'time capsule' of sorts that can only be opened by 22<sup>nd</sup> century neuroscience and technology.

In effect, the Aspirational Neuroscience Prize is designed to reward outstanding research that offers a glimpse into what neuroscience might be like in the 22<sup>nd</sup> century so that society can better appreciate the promise neuroscience research has for all of us today.

## Structure of the Prize:

It is planned that a total of four \$25,000 Aspirational Neuroscience Prizes will be awarded each year for 10 years, a total of \$1,000,000 of prize awards in total. Management of the Aspirational Neuroscience Prize will be handled by the nonprofit Brain Preservation Foundation (BPF). Each year's final selection will be determined by the Brain Preservation Foundation after input from a committee of prominent neuroscience experts. Months prior to the award a list of at least ten official nominees (finalists) will be announced and their research will be highlighted on the Brain Preservation Foundation's website to elicit public comment. The final awarding ceremony will be planned to coincide with a prominent neuroscience conference, e.g. the annual Society for Neuroscience conference.

Outstanding research contributions in three broad categories will be considered:

### Breakthroughs in the Neuroscience of Memory

The Aspirational Neuroscience Prize will honor basic neuroscience research that significantly advances our understanding of the structural and molecular encoding of memory. We seek a definitive answer to the question: *"What ultrastructural and molecular features must be preserved to allow for the possibility of future mind uploading with memories and personality intact?"* This is a wide ranging question that touches many areas of research including cognitive and computational neuroscience, but most centrally it is a question regarding the synaptic and molecular basis of memory.

The brain contains a number of distinct memory systems (e.g. declarative, procedural, perceptual, emotional, etc.) supported by distinct brain regions (e.g. medial temporal lobe structures, basal ganglia, neocortex, amygdala, etc.) (Squire 2004). A person's conscious mind is thought to emerge from interactions among these different memory systems; and their mind's uniqueness is thought to be fundamentally traceable to synaptic and molecular changes in these brain regions which have occurred over a lifetime of learned experiences.

The neuroscience community has made incredible progress toward understanding the synaptic and molecular changes that underlie learning and memory in each of these systems. In fact, a tentative consensus has emerged that they all may share a common core mechanism—long-term potentiation/depression (LTP/LTD) at glutamatergic synapses onto dendritic spines (Lamprecht & LeDoux 2004; Yuste 2010). There is now a body of research showing that the functional strengthening of a synapse is accompanied by clear, long-term ultrastructural changes which are readily visible, via electron microscopy, in glutaraldehyde-preserved brain tissue (Bailey, Kandel & Harris 2015; Kasai et al. 2003; Matus 2000). This chain of logic implies a provisional answer to our original question: *It is the ultrastructural details of the neuronal processes and their synaptic contacts (e.g. the pattern of neuronal connectivity and the sizes of dendritic spines) that must be preserved to allow for the possibility of future mind uploading.*

Unfortunately existing evidence, although compelling, is nowhere near sufficient to prove this statement, and possible exceptions have already been identified (e.g. Johansson et al. 2014). The Aspirational Neuroscience Prize challenges the neuroscience community to design new experiments which can offer clear support, or refutation, of this provisional answer—experiments that get to the core question of how memories are physically encoded. Ultimately we hope to award prizes to neuroscientists whose research clearly demonstrates how simple, but non-trivial, memories (e.g. an

auditory fear memory in a mouse) can be decoded based only on ultrastructural and molecular images of an animal's preserved brain.

### **Breakthroughs in Brain Preservation**

The Aspirational Neuroscience Prize will honor the development of improved methods for preserving whole mammalian brains at the ultrastructural and molecular levels. This includes methods designed to prepare whole brains for electron microscopic and immunofluorescent imaging, and methods designed to stabilize neural tissue for extremely long-term storage. Research seeking to transition laboratory preservation techniques to human clinical settings will receive special consideration, especially research that demonstrates robustness to varied patient conditions and research demonstrating clinically-viable quality control checks.

### **Breakthroughs in Connectomics**

Advances in the automation of electron microscopic and other ultrastructural imaging techniques over the last decade have finally provided neuroscientists the ability to comprehensively map out the neural circuits they study, at least over small (<1mm<sup>3</sup>) volumes (Briggman & Bock 2012). These new 'connectome' mapping techniques have demonstrated their worth in a number of high-profile studies (e.g. Briggman, Helmstaedter & Denk 2011; Kim et al. 2014; Kasthuri et al. 2015; Lee et al. 2016), but volume, speed, reliability, and resolution limitations have so far constrained their usefulness. The Aspirational Neuroscience Prize will honor technological advances in connectomics that dramatically increase the sizes of connectomes that can be mapped. The Aspirational Neuroscience Prize will also honor researchers that apply these new connectomic mapping techniques to tackle fundamental questions such as whether learned memories can be decoded based on the structural connectome alone (Seung 2009). If the answer proves to be 'Yes' then large-scale automated electron microscopy could be a route to future mind uploading. If the answer proves to be 'No' then this will motivate the invention of new techniques that can provide 'molecularly-annotated' structural connectomes—techniques specifically geared toward preserving, labeling, and imaging those biomolecules that are crucial to decoding memory.

Detailed descriptions of exemplary research from each of these categories are given in the 'Background Information' section below. That section is designed to briefly summarize the state-of-the-art in each research category as well as to give clear examples of the types of research that will be considered for Aspirational Neuroscience Prize nomination.

# Background Information

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## 1. The Brain Preservation Foundation

The ultimate goal of neuroscience is to obtain a complete computational understanding of how brain circuits give rise to mental functions. The neuroscience community has made tremendous strides towards this lofty goal over the past century, and the pace of progress accelerates every year. But given the incredible complexity of the brain, final success may well take yet another century or more. The Brain Preservation Foundation's mission is grounded in this key assumption: We expect that the neuroscience community *will* eventually succeed, likely sometime late this century or early in the 22<sup>nd</sup> century, and from this scientific knowledge will flow the technological ability to scan a preserved human brain at the synaptic level, decode its neural connectivity, and simulate its functioning computationally. In other words, we find it likely that 22<sup>nd</sup> century neuroscience will have perfected methods to upload the mind of an individual based on a high-resolution scan of their chemically-preserved brain.

It is already possible to preserve the precise synaptic connectivity of an entire large mammalian brain for indefinitely long-term storage using a combination of glutaraldehyde perfusion fixation and cryopreservation (McIntyre & Fahy 2015). This fact opens up the possibility of preserving the brains of terminally-ill patients today with the hope that they can be revived by brain scanning and mind uploading perhaps 100 years from now. Under this perspective, brain preservation should be viewed as a potentially life-saving medical procedure and bridge to the future for patients who have run out of more traditional medical options. Of course not every terminally ill patient would desire this option, but informal surveys show that a significant fraction of individuals would choose brain preservation over the grave, for themselves and their loved ones, if such a procedure was available in hospitals and regulated to ensure that each patient received the highest-quality preservation possible. The Brain Preservation Foundation's mission is to spur the development and deployment of just such a medical option.

The Brain Preservation Foundation has already stimulated research through the Brain Preservation Prize challenge (launched in 2011, awarded 2016/2018) and through targeted research grants to laboratories developing brain preservation techniques. These efforts resulted in the development of an

entirely new brain preservation procedure called Aldehyde Stabilized Cryopreservation (ASC) –the first ever to demonstrate ultrastructural preservation of an entire large mammal brain in a manner compatible with indefinitely long-term cryostorage (McIntyre & Fahy 2015). We feel that this result, along with the rapidly advancing progress in our understanding of the synaptic basis of memory (Bailey, Kandel & Harris 2015; Poo et al. 2016), connectomic imaging technologies (Briggman & Bock 2012; Mikula 2016), and computational simulation of neural circuits (Markram et al. 2015; Hassabis et al. 2017), has set the stage for a new interdisciplinary field of scientific and medical research, a field focused both on perfecting brain preservation techniques and on developing the technologies to eventually revive preserved individuals computationally or by other means.

## 2. The Aspirational Neuroscience Prize

The Aspirational Neuroscience Prize has been designed to help foster the development of this new interdisciplinary field, honoring its most significant breakthroughs and raising awareness among neuroscientists of the larger, longer-term ramifications of their research. Each year the Aspirational Neuroscience Prizes will be awarded to the individuals, groups or institutions that best demonstrates advancement toward full brain preservation and cognitive restoration through digital, biological or other methods. Of course no one is expecting a breakthrough demonstrating revival of an individual in the near-term. As previously noted, such a demonstration requires so many fundamental advancements in neuroscience and technology that a complete demonstration is likely many decades, perhaps even centuries away. Instead, the Aspirational Neuroscience Prize is designed to honor, reward, and thereby accelerate precisely those fundamental advancements in neuroscience and technology that may one day add up to make revival possible.

## 3. Examples of the kinds of research that will be considered for the Aspirational Neuroscience Prize

### 3.1 Breakthroughs in the Neuroscience of Memory

As already stressed, we obviously do not currently understand enough to successfully simulate a brain based on structural and molecular maps of its neural circuitry. Many further decades of neuroscience research, along with advances in brain imaging, will be required to accomplish that feat. Instead the pertinent questions are:

- “Does our current understanding of the brain support the hypothesis that future uploading is possible in principle?”
- “Do we understand enough to determine which structural and molecular features must be preserved to allow for the possibility of future uploading with memories and personality intact?”

If the answer to both of these questions is ‘Yes’, and if there exists a brain preservation technique (like ASC) that can demonstrate preservation of these key structural and molecular features, then it would seem to imply that there is a moral argument that such an option be developed and made available to terminal patients. Only the neuroscience community can provide definitive answers to these questions as they are fundamentally questions regarding the neurobiology of memory encoding. As such, any basic neuroscience research that significantly advances our understanding of the structural and molecular encoding of memory will be considered for nomination for an Aspirational Neuroscience Prize.

For illustration purposes, we review here several broad categories of memory research that are prime candidates for the types of breakthroughs the Aspirational Neuroscience Prize is meant to reward:

### **3.1.1 Research on Memory Systems:**

Intelligent behavior is dependent upon the interaction between many different specialized memory systems (McDonald & White 1993). Squire (2004) offers a taxonomic review of these memory systems. A brief overview:

**Hippocampus:** Neural circuits within the hippocampus and other medial temporal lobe structures support the initial learning of what is colloquially referred to as memory: specifically declarative or episodic memories (Squire, Stark & Clark 2004). This memory system specializes in rapid ‘one-shot’ learning with little generalization in order to provide maximal discriminability among distinct episodes (Atallah, Frank & O’Reilly 2004).

**Striatum:** Circuits within the striatum support the initial phase of procedural learning (Ashby, Ennis & Spiering 2007) -the learning of sequences of motor or cognitive actions (Aldridge et al. 1993). Learning in the striatum is modulated by dopaminergic inputs from the brain’s reward system, which in turn is modulated by the striatum itself. This arrangement is thought to create a joint system optimized for reinforcement and temporal difference learning (O’Reilly et al. 2007).

**Cortex:** The knowledge initially learned within both of the above systems is, over time, thought to be consolidated in the cortex which is specialized for generalization (Pasupathy & Miller 2005; Kitamura et al. 2017). For example, learning within cortical sensory hierarchies (visual, auditory, etc.) can be thought of as creating ‘perceptual memories’. Repeated exposures to sensory stimuli train these cortical hierarchies to categorize the raw sensory signals along a myriad of different perceptual dimensions (e.g. shape, size, orientation, movement, color, texture, etc.) (Kanwisher 2010; DiCarlo, Zoccolan & Rust 2012).

**Amygdala:** Circuits within the amygdala support basic emotional memories—learning that associates high-level cortical states with more primary motivational inputs (Janak & Tye 2015).

The amazing flexibility of human cognition can be traced to interactions among these different memory systems (Anderson 2009). For example, relying only on reinforcement learning in the striatum would be inefficient, but the cortex’s sensory hierarchies provide the striatum with highly-informative representations making reinforcement learning much more efficient (e.g. Mnih et al. 2015). Cortical circuits are good at generalization but require many repeated stimulus presentations; one-shot learning in the hippocampus followed by replay to cortex and striatum is hypothesized to solve this problem (Lansink et al. 2008). Cognitive architecture models like ACT-R (Anderson et al. 2008), Lebra (O’Reilly, Hazy & Herd 2012), and Spaun (Eliasmith et al. 2012) offer evidence that computational systems employing these types of interacting memory systems can model some complex human behaviors and learning.

**Breakthrough research that increases our understanding of how these memory systems work individually and interactively will be considered for nomination for an Aspirational Neuroscience Prize.** Such research directly addresses the fundamental question “How do computations in the brain give rise to mind?” Such research also provides a taxonomy of the most important types of information encoding in the brain. Any brain preservation procedure that is offered to human patients must demonstrate that it preserves *all* of these different types of information encoding.

### **3.1.2 Research on morphological changes that correlate with learning and synapse strength:**

Current models of learning in each of the above memory systems suggest that long-term potentiation and long-term depression (LTP/LTD) at specific classes of synapses is the primary method of encoding knowledge in each system:

**Striatum** – Thought to rely on dopamine modulated LTP/LTD in glutamatergic synapses onto the dendritic spines of striatal medium spiny neurons (Kreitzer & Malenka 2008; Yagishita et al. 2014).

**Hippocampus** – Thought to rely on LTP/LTD in glutamatergic synapses onto the dendritic spines of hippocampal dentate, CA1, and CA3 cells (Lisman 2015; Rolls & Kesner 2006).

**Cortex** – Thought to rely on LTP/LTD in glutamatergic synapses onto the dendritic spines of cortical pyramidal cells (Holtmaat & Svoboda 2009; Matsuzaki et al. 2004).

**Amygdala** - Thought to rely on LTP/LTD in glutamatergic synapses onto the dendritic spines of lateral amygdala pyramidal cells (Maren 2005; Johansen et al. 2010).

At a minimum, any proposed brain preservation procedure must demonstrate that it preserves these classes of synaptic connections<sup>1</sup> along with sufficient ultrastructural and molecular details to allow each synapse's functional strength to, in principle, be determinable by future neuroscientists. As the above list implies, there is considerable similarity in the way these systems encode learned knowledge at the synaptic level. Importantly, all of these systems are thought to rely on *dendritic spines* for memory encoding.

Dendritic spines are small, motile protrusions that extend up to a few microns from a main dendritic branch, and are thought to be the key 'morphological building blocks of memory' (Yuste 2010; Segal 2016; Lamprecht & LeDoux 2004). Both during development and during life-long learning these spines can retract to eliminate an existing synapse that is no longer beneficial, and can then reach out to form a trial connection with another axon in its local neighborhood. Such 'learning spines' are smaller than usual, just sufficient to test the timing of pre- and post-synaptic firing (Bourne & Harris 2011). This attribute is, in fact, exactly what is hypothesized to drive synaptic weight changes in neural learning algorithms (O'Reilly Hazy & Herd 2012; Bengio et al. 2017). If the pre- and post-synaptic firing history is appropriate for strengthening the connection, then the thin 'learning spine' will stabilize and grow in volume to become a mushroom spine (Bourne & Harris 2007). If not, it will retract and explore other potential synaptic partners. The additional wiring flexibility provided by these motile dendritic spines increases the brain's memory capacity many times over what would be possible without them (Chklovskii, Mel & Svoboda 2004), which is why they are ubiquitous across the brain's memory systems.

Once a mushroom spine is stabilized it can potentially remain constant for the rest of the animal's life (Zuo et al. 2005; Trachtenberg et al. 2002) potentially stabilized by molecular feedback loops (Rossetti et al. 2017). Life-long memories can also, theoretically, be stabilized even in the face of continual, gradual spine turnover via the collective dynamic reinforcement inherent in attractor neural

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<sup>1</sup> This list is of course not meant to be comprehensive in terms of unique information encoding brain regions (e.g. thalamus, cerebellum should certainly be listed), nor in terms of cell and synapse types.



networks having Hebbian plasticity (Fauth et al. 2017). These two mechanisms in tandem potentially explain how learning and memories are retained over a lifetime by changes to spiny synapses.

Stabilized spiny synapses can still change their effectiveness for transmitting signals between the pre- and post-synaptic neurons, and new research suggests that synaptic strength can be finely tuned over a range of strengths (Bartol et al. 2015). The strength of transmission is related to the number of AMPA-type glutamate receptors on the dendritic spine. Crucially much evidence now exists showing a relationship between the volume of an individual dendritic spine and the number of AMPA receptors it contains –the larger a spine’s volume the stronger its functional connection (Kasai et al. 2003). This structure-function correlation has now been demonstrated in a wide range of experiments including:

- Two photon imaging of dendritic spines along with local ‘photorelease’ glutamate uncaging to determine the amount of AMPA receptors expressed (Matsuzaki et al. 2001; Noguchi et al. 2011). **Result:** Spines with larger volumes had more AMPA receptor response and thus a larger functional strength.
- Quantitative immunogold labeling and electron microscopy of dendritic spines (Nusser et al. 1998). **Result:** Spines within a given class (e.g. mossy fiber to CA3 spines) show a correlation between the size of the post synaptic density and the number of AMPA receptors.
- Two photon imaging of dendritic spines along with local photorelease glutamate uncaging (in external solution lacking magnesium ions, or holding post synaptic cell at depolarized potential) to induce LTP on an individual spine basis (Matsuzaki et al. 2004; Harvey & Svoboda 2007). **Result:** Photoreleased glutamate induced both LTP *and* long-lasting enlargement of the stimulated spine.
- Optogenetic co-stimulation of dopaminergic and glutamatergic inputs onto medium spiny neuron spines in the striatum (Yagishita et al. 2014). **Result:** Dopamine promoted the spines to increase their volume only when this dopamine ‘reward’ was delivered in a narrow time window following glutamatergic stimulation. This is evidence that striatal reinforcement learning occurs at the individual synapse level and that it results in clear structural changes to synapses.
- Electrophysiological induction and measurement of LTP at hippocampal synapses followed by electron microscopic measurements of spine morphology (Bourne & Harris 2011). **Result:** Clear ultrastructural changes, including spine volume, distinguished the LTP-strengthened synapses from others.
- Electron microscopic reconstructions of dendritic spines having the same pre- and post-synaptic partners, and which therefore had shared histories of presynaptic and postsynaptic activity (Bartol et al. 2015). **Result:** Dendritic spines with shared histories were ‘nearly identical’ in size. The shared learning history implies similar learned functional strength which, in turn, predicts the similar structural size seen.

**The Aspirational Neuroscience Prize will promote and reward basic neuroscience research, like the above, which seeks to determine the structural correlates of memory:** Are dendritic spines really the main site of learned knowledge in the cortex, striatum, hippocampus, and amygdala? Can a synapse’s functional strength really be estimated by imaging its glutaraldehyde-fixed ultrastructure? Are molecular level details required as well to estimate functional strength? Are there significant exceptions to the synaptic basis of memory? For example, do the known changes in a neuron’s intrinsic excitability that occur during initial memory formation (Lisman et al. 2018) also provide a means of long-term memory storage? **Breakthrough research addressing any of these questions will be considered for nomination for an Aspirational Neuroscience Prize.**

### **3.1.3 Research that tags a neural representation to manipulate memory encoding and retrieval:**

Some of the most powerful research into the structural basis of memory encoding has been studies that manipulate memories in living animals optogenetically. An example is the landmark experiment out of the Tonegawa laboratory (Liu et al. 2012) in which it was demonstrated that the population of hippocampal cells representing the spatial context component of a fear memory could be tagged optogenetically in a mouse. This tagging allowed the whole memory, along with its behavioral response, to be later recalled by photostimulation alone<sup>2</sup>.

This experiment tested one of the most fundamental assumptions in neuroscience: the assumption that information in the brain is represented in the firing rates of sparse populations of neurons (Amit 1996), also referred to as neuronal ensembles (Buzsaki 2010; Carrillo-Reid et al. 2017) or cell assemblies (Harris 2005). In the Liu et al. (2012) case, the information that the mouse was in a particular cage (spatial context) was presumed to be represented via a particular population of hippocampal cells. That hypothesis was tested by raising the firing rates of that population of neurons in a different context and seeing if the animal would, in effect, think it was back in the original cage. Given the coarse nature of such optogenetic stimulation (i.e. its inability to provide anything more than firing rate increases) it is significant that it was able to reactivate the fear memory.

These studies, along with hundreds of others, have narrowed in on the general principles of information representation in the brain—the neural code. A growing, but still debated, consensus is that different brain areas learn to represent different aspects of the internal and external world by means of neuronal ensembles and ‘rate coding’. I.e. a cortical area will signal a particular representation to downstream areas when a particular ensemble of its neurons increase their firing rates above other cells in that same cortical area. This increase in firing rate within the time constant of downstream neurons (~10-30ms) is particularly effective in driving ‘decoding’ neurons in these downstream areas which results in the activation of neuronal ensembles in these downstream areas (Buzsaki 2010). Cortical representation and computation can, to some extent, be viewed as sequences of neuronal ensemble activations providing a robustness of computation far beyond what would be expected at the single neuron level (Buzsaki 2010). A recent study of the neural representation of face identity in primate visual cortex by Chang and Tsao (2017) summarizes this idea nicely:

“By formatting faces as points in a high-dimensional linear space, we discovered that each face cell’s firing rate is proportional to the projection of an incoming face stimulus onto a single axis in this space, allowing a face cell ensemble to encode the location of any face in the space.”  
(Chang & Tsao 2017)

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<sup>2</sup> This technique has subsequently been used to demonstrate the inception of a false memory: A mouse that is made to believe (through photostimulation of a tagged neural population) that it is in cage#1 when it is, in fact, being shocked in cage#2. The mouse mistakenly learns to fear cage#1 but not cage#2 (Liu, Ramirez & Tonegawa 2014). This technique has also begun to be used to explore the synaptic connectivity between separately tagged populations of cells (Ryan et al. 2015; Roy et al. 2017). These are experiments that have the potential to probe deeper into the synaptic basis of memory than ever before.

Such a rate coded neuronal ensemble representation is likely used in many brain regions even in situations where temporal sequences are being represented. For example, a recent study by Pfeiffer and Foster (2015) provided evidence that sequence replay in the hippocampus is actually composed of a sequence of discrete rate-coded representations:

“During sharp-wave ripple (SWR) events, hippocampal neurons express sequenced reactivations, which we show are composed of discrete attractors. Each attractor corresponds to a single location, the representation of which sharpens over the course of several milliseconds, as the reactivation focuses at that location. Subsequently, the reactivation transitions rapidly to a spatially discontinuous location. This alternation between sharpening and transition occurs repeatedly within individual SWRs and is locked to the slow-gamma (25 to 50 hertz) rhythm. These findings support theoretical notions of neural network function and reveal a fundamental discretization in the retrieval of memory in the hippocampus...” (Pfeiffer & Foster 2015)

What is the significance of this growing body of evidence for ‘rate coding’ to brain preservation and neural simulation? It is potentially significant because it lends credence to a wide range of neural network models which are explicitly based upon this rate coding assumption. For example, deep learning neural network models of visual object recognition (Cadieu et al. 2014) use this rate coding assumption throughout and, if accurate, show how the brain’s object recognition ‘algorithm’ is encoded by the pattern and strengths of synaptic connections.

Similarly, neural network models of hippocampus sequence learning and retrieval (Jensen & Lisman 2005; Rolls & Kesner 2006; Lisman 2015) use this rate coding assumption throughout and, if accurate, show how sequences are encoded by the pattern and strengths of synaptic connections among the different regions of the hippocampus.

In short, evidence that the brain uses rate coded neuronal ensemble representations lends substantial support to a wide range of existing neural network models of brain systems (e.g. Lisman 2015; Rolls & Treves 1998; Hassabis et al. 2017). In turn, these models are explicitly based on the idea that all of the brain’s learned knowledge and memory is fundamentally encoded in the pattern and strengths of synaptic connections (i.e. the brain’s connectome). Put succinctly:

“[E]verything you know is encoded in the patterns of your synaptic weights...” (O'Reilly 2012)

**Such studies straightforwardly address one of the most fundamental assumptions underlying the proposal for long-term brain preservation—the assumption that preserving the structural synaptic connectivity of the brain will simultaneously preserve a record of the information content of that brain. Because of this, studies like these are clearly worthy of nomination for an Aspirational Neuroscience Prize.**

#### **3.1.4 Research that tags and manipulates the subset of synapses encoding a particular memory:**

The optogenetic ‘memory engram’ (Tonegawa et al. 2015) tagging technique described above really only tags part of what constitutes a memory. At its most basic neural-implementation level a memory purportedly consists of three things:

- 1.) An ensemble of active cells in brain region (A).
- 2.) An ensemble of active cells in a separate brain region (B).
- 3.) A strengthened set of synapses connecting the ensemble in (A) to the ensemble in (B).

When a new memory is formed, it is the set of strengthened synapses that actually encodes a new association between two previously existing neural representations. The Tonegawa lab experiments described above focused on tagging an ensemble of active cells in one brain region (A), and showing that subsequent reactivation of that ensemble can also reactivate the ensemble in region (B). This indirectly showed that there was a strengthening of synapses between the two ensembles. But we can ask: Are there experimental techniques that could more directly ‘tag’ and manipulate the subset of *synapses* that putatively constitutes a memory?

In fact a technique has recently been developed in the laboratory of Haruo Kasai that appears to do just that (Hayashi-Takagi et al. 2015). They developed a genetic probe that localizes specifically to dendritic spines which have recently undergone LTP. This allowed them to visualize, via fluorescent labeling, the subset of synapses involved in a particular memory. Further, by adding a photoactivatable version of the Rac1 protein, they were able to induce, via photostimulation, that specific subset of spines to shrink. This technique was used to literally count the number of synapses forming a memory in motor cortex and to selectively ‘erase’ that new memory by photostimulation. The paper’s abstract summarizes this result and makes clear that this is some of the most direct evidence to date supporting the theory that dendritic spines are the key structural correlates of memory:

“Dendritic spines are the major loci of synaptic plasticity and are considered as possible structural correlates of memory. Nonetheless, systematic manipulation of specific subsets of spines in the cortex has been unattainable, and thus, the link between spines and memory has been correlational. We developed a novel synaptic optoprobe, AS-PaRac1 (activated synapse targeting photoactivatable Rac1), that can label recently potentiated spines specifically, and induce the selective shrinkage of AS-PaRac1-containing spines. In vivo imaging of AS-PaRac1 revealed that a motor learning task induced substantial synaptic remodeling in a small subset of neurons. The acquired motor learning was disrupted by the optical shrinkage of the potentiated spines, whereas it was not affected by the identical manipulation of spines evoked by a distinct motor task in the same cortical region. Taken together, our results demonstrate that a newly acquired motor skill depends on the formation of a task-specific dense synaptic ensemble.”  
(Hayashi-Takagi et al. 2015)

The technique and results described in the Hayashi-Takagi et al. (2015) paper are simply breathtaking—so breathtaking that enthusiasm should be constrained until the results are thoroughly replicated by other labs. However **it seems clear that techniques like this that allow the tagging and manipulation of ensembles of synapses are now on the horizon** (Hoshihira et al. 2017), and they are sure to advance our fundamental understanding of the structural and molecular encoding of memory. Because of this, **studies like these are clearly worthy of nomination for an Aspirational Neuroscience Prize.**

### 3.2 Breakthroughs in Brain Preservation

Research breakthroughs considered for nomination will include improved methods of long-term brain preservation. These include chemical fixation methods (e.g. Mikula & Denk 2015, Hua et al. 2015), cryofixation methods (e.g. Fahy & Wowk 2015), and hybrid methods like ASC (e.g. McIntyre and Fahy 2015), all of which competed to demonstrate structural connectome preservation in the BPF's original Brain Preservation Prize challenge.

ASC has demonstrated structural connectome preservation of a large mammalian brain (a pig) but there remains considerable room for improvement. For example it would be desirable to reduce or eliminate a range of ultrastructural artifacts seen in the published ASC results including myelin figures and loss of extracellular space. Of greater import would be research that clearly determines the degree of biomolecular preservation in ASC, for example by applying a battery of glutaraldehyde-compatible immunofluorescence assays (Murray et al. 2015; Collman et al. 2015). There is also considerable research necessary to transition ASC from a laboratory demonstration into a reliable medical procedure suitable for human application. This might include research exploring the application of ASC in non-optimal circumstances (e.g. in cases with prolonged post-mortem ischemia), and research to develop methods for case-by-case quality control and evaluation. For example, whole brain X-ray, MRI, or angiography might be performed during or following the ASC procedure for quality control (Jackowski et al. 2005). These could be followed by CT-targeted needle biopsies for final verification that the patient's neural ultrastructure has been preserved (Aghayev et al. 2007). **A clinical trial demonstrating ASC on human volunteers and including a full range of quality checks would certainly warrant nomination for an Aspirational Neuroscience Prize.**

Dr. Mikula's entry in our first prize challenge involved glutaraldehyde perfusion fixation of the brain followed by chemical lipid stabilization and plastic embedding (Mikula & Denk 2015). His method of brain preservation has two potential advantages over ASC. First, the BROPA (brain-wide reduced-osmium staining with pyrogallol-mediated amplification) method allows for indefinitely long-term storage at room temperature which would represent a considerable reduction in the cost and complexity of long-term storage. Second, this method produces a brain that is directly compatible with whole-brain electron microscopy (Mikula 2016). Unfortunately the BROPA protocol has so far been limited to mouse brain volumes. **If a chemical fixation and room-temperature storage technique like his could be made applicable to the human brain it would clearly represent an advance worthy of nomination for an Aspirational Neuroscience Prize.**

Both ASC and BROPA methods rely on glutaraldehyde perfusion fixation of the brain which almost instantly halts metabolic decay processes and reliably stabilizes neural and synaptic structures by covalently crosslinking proteins in place. This is desirable when one is focused on provably preserving the information content of the brain for scientific research or in hopes of revival via mind uploading. However glutaraldehyde fixation is obviously a 'dead end' when considering biological revival. A truly reversible method like that used to cryopreserve human embryos would clearly be preferable. Unfortunately the science of cryobiology has yet to come anywhere close to demonstrating reversible long-term cryopreservation of whole mammals. A more realistic goal being worked on today is cryopreservation of mammalian organs which, if perfected, would revolutionize the organ transplant supply chain (Fahy, Wowk & Wu 2006).

The research laboratory 21<sup>st</sup> Century Medicine, which competed in the initial brain preservation prize, has published promising results showing that rabbit kidneys that were loaded with high concentrations of cryoprotectants and lowered in temperature to -45°C could be rewarmed, cleared of cryoprotectant, and transplanted successfully (Fahy et al. 2004). And they have published results demonstrating that 0.5 mm thick hippocampal slices can be vitrified (i.e. solidified at low temperature into a glass-like solid without the formation of ice crystals) and stored at -130°C while retaining viability and even the ability to undergo classic LTP electrophysiology experiments (Pichugin et al. 2006; Fahy et al. 2013). They have even published one case of a rabbit kidney that was vitrified and then successfully transplanted (Fahy et al. 2009).

All of these cryopreservation-for-revival experiments were challenging and have so far been difficult to replicate, let alone extend. A delicate balance must be achieved. If the final cryoprotectant concentration is not sufficiently high then ice crystals will form. If too high a concentration is used then its own toxicity will kill the cells. In practice this means cryoprotectant concentration must be ramped up rapidly even as temperature is lowered. When applied to whole brains this balance has never been demonstrated successfully. Even when the goal of revival is replaced with the lesser goal of structural preservation, the procedures tested resulted in severely shrunken brains (due to osmotic dehydration) with distorted and presumably damaged ultrastructure as seen under the electron microscope<sup>3</sup>.

Nonetheless these cryopreservation-for-revival experiments are promising and certainly should be pursued as a method for brain preservation. **A reasonable near-term goal would be to demonstrate that such a technique can preserve the structural connectome of a whole mammalian brain. Such a milestone would be a significant step forward on the path to true reversible cryopreservation of the brain and would certainly be worthy of nomination for an Aspirational Neuroscience Prize.**

### 3.3 Breakthroughs in Connectomics

The term ‘connectome’ has several connotations. In the context of the Human Connectome Project (Van Essen et al. 2013) it simply refers to a map of the coarse axonal tracts among brain regions as can be gleaned using magnetic resonance imaging (MRI) techniques. In contrast, what we have been referring to as the brain’s ‘structural connectome’ is the hypothetical map showing all of the synaptic connections in the brain annotated with whatever additional information (neuronal types, dendritic branching morphologies, synaptic strengths, etc.) that could reasonably be determined based on ~10nm resolution volume electron microscopy (e.g. Xu et al. 2017). Brain preservation techniques employing glutaraldehyde fixation are known to preserve more than just this ‘structural’ connectome and are potentially open to high-resolution molecular as well as structural imaging techniques (e.g. Chen, Tillberg & Boyden 2015; Chung & Deisseroth 2013; Murray et al. 2015). As such, we could also discuss the concept of a ‘**molecularly-annotated structural connectome**’ which would annotate the structural connectome with information on the densities of potentially all key membrane proteins like receptors and ion channels (e.g. Collman et al. 2015). From a brain preservation perspective we are clearly only interested in definitions of the connectome that, at a minimum, include a map of all structural synaptic connections since learned knowledge and memories are presumed to be encoded at this synaptic level.

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<sup>3</sup> It should be noted that ASC avoids these issues mainly because its (deadly) glutaraldehyde fixative stabilizes the brain sufficiently to allow room temperature perfusion of the cryoprotectants.

The section '**Breakthroughs in the Neuroscience of Memory**' addressed the question: "Is learned knowledge and memory encoded in the connectome?" The section '**Breakthroughs in Brain Preservation**' addressed the question: "Can a human brain's connectome be preserved for long-term storage?" In this section, '**Breakthroughs in Connectomics**', we will address the question: "Will it likely be possible in the future to map and interpret the connectomes of entire preserved human brains?"

Mapping an entire human brain with 10nm resolution is well beyond our technological ability today. In fact, the largest contiguous volume mapped at such resolution so far is less than a cubic millimeter in size. A reasonable argument against developing human brain preservation would be an argument that it is simply impossible to map the structural connectome of a human brain. It is certainly impossible for us today, but will it likely also be impossible for 22<sup>nd</sup> century neuroscientists? For 23<sup>rd</sup> century neuroscientists? The best way to approach this question is to look at the current state of connectomics today, its current rate of progression, and to look for any fundamental roadblocks that might be on the horizon.

Mapping small pieces of brain tissue in 3D at the synaptic level using serial section electron microscopy has been a staple of neuroscience research at least since the pioneering work of Sjöstrand in the 1950s (Sjöstrand 1958). However until very recently 3D electron microscopic reconstruction of brain tissue has been a manual, laborious, and error-prone process suitable only for tiny tissue volumes (reviewed in Harris et al. 2006). That changed in 2004 with the invention of Serial Block-Face Scanning Electron Microscopy (SBF-SEM) by Denk & Horstmann (2004). Since that 2004 demonstration of truly automated connectome imaging technology the field has simply exploded. There are now many automated techniques for acquiring volume electron microscope datasets suitable for connectome mapping. Four of these techniques are reviewed by Briggman & Bock (2012). These techniques have proven their ability to map neural circuits at the synapse level in dozens of high-profile publications (e.g. Briggman, Helmstaedter & Denk 2011; Kim et al. 2014; Kasthuri et al. 2015; Lee et al. 2016; Takemura et al. 2015; Kornfeld et al. 2017; Schmidt et al. 2017). Each of these studies was based on an imaged volume less than 1mm<sup>3</sup>, however recent advances in automation and electron imaging are poised to push volumes substantially above 1mm<sup>3</sup>. For example, the IARPA MICrONS Project ([www.iarpa.gov/index.php/research-programs/microns](http://www.iarpa.gov/index.php/research-programs/microns)) is funding teams that are well on their way to imaging and tracing the connectome of a 1mm<sup>3</sup> volume of mouse cortex tissue. One is utilizing a suite of transmission electron microscopes operating in parallel (<https://www.youtube.com/watch?v=LO8xCLBv6j0>), the other is using a new 61 beam scanning electron microscope to perform the imaging (Schalek et al. 2016), and both have automated the process of serial section collection. An even more ambitious project utilizing a 91 beam scanning electron microscope (Kemen, Garbowski & Zeidler 2015) is pursuing the goal of imaging an entire mouse brain using the SBF-SEM technique (Mikula 2016). Hayworth (2012) addresses the question of whether a fundamental roadblock exists that would prevent human brain-scale connectomics, and concludes that there is none as long as a brain can be reliably subdivided and imaged in parallel, and as long as mass production and economies of scale can be applied to the new multibeam scanning electron microscopes.

**The Aspirational Neuroscience Prize will highlight and reward technological breakthroughs that substantially advance the field of Connectomics.** This will include rewarding technological breakthroughs in automated 3D electron microscopy techniques demonstrating how larger volumes can be acquired more reliably, at faster rates, and with higher image quality. For example this might include faster electron microscopes (e.g. Eberle et al. 2015), new and improved automated imaging techniques (e.g. Denk & Horstmann 2004; Knott et al. 2008; Hayworth et al. 2014; Horstmann et al. 2012; Briggman

& Bock 2012; Xu et al. 2017), and improved tissue staining and processing techniques (Mikula & Denk 2015; Hayworth et al. 2015; Hua, Laserstein & Helmstaedter 2015).

**The Aspirational Neuroscience Prize will also highlight and reward computational breakthroughs demonstrating substantially improved methods for interpreting raw electron microscope data**—methods that automatically identify synapses and sizes (e.g. Merchan-Perez et al. 2009; Kreshuk et al. 2011), methods that automatically trace neuronal processes (e.g. Jain, Seung & Turaga 2010; Plaza et al. 2014; Kaynig et al. 2015; Januszewski et al. 2017), and methods that might eventually be able to convert raw electron microscope volumes directly to a format suitable for simulation (e.g. Gornet & Scheffer 2017).

**The Aspirational Neuroscience Prize will also highlight and reward neuroscience research which utilizes these connectomic imaging technologies to study neural function**, especially studies which demonstrate how biological function is related to structural connectivity. Some recent significant examples include: Mouse retina (Briggman, Helmstaedter & Denk 2011; Helmstaedter et al. 2013; Kim et al. 2014), mouse cortex (Bock et al. 2011; Kasthuri et al. 2015; Lee et al. 2016), mouse thalamus (Morgan et al. 2016), zebra finch (Kornfeld et al. 2017), zebrafish (Wanner et al. 2016; Hildebrand et al. 2017), and insect (Takemura et al. 2015; Takemura et al. 2017; Zheng et al. 2017).

The above research is solely focused on the structural connectome as mapped by electron microscopy. It is not yet universally accepted that learned knowledge and memory can be decoded based solely on this structural connectome, therefore it is important to pursue avenues that might generate a ‘molecularly-annotated structural connectome’. This has proved to be a difficult technological problem but nonetheless several promising avenues of research are being explored today (e.g. Micheva et al. 2010; Collman et al. 2015; Zador et al. 2012; Chen, Tillberg & Boyden 2015; Marblestone et al. 2014; Yoon et al. 2017). If one of these technologies achieves a breakthrough which makes possible **the acquisition of molecularly-annotated structural connectomes**, then it **will certainly be considered for nomination for an Aspirational Neuroscience Prize**.

Finally, **one of the ‘holy grails’ of connectomics research is to demonstrate that a non-trivial ‘memory’ can be recovered from a map of the structural connectome alone** (Seung 2009). Such a clear demonstration has proved elusive for now, but it is being hotly pursued by many labs. **Such a clear demonstration will certainly receive immediate nomination for an Aspirational Neuroscience Prize as it would offer the clearest evidence to date that future revival may be possible following brain preservation**.

## 4. Summary

In summary, the Aspirational Neuroscience Prize has been designed to reward outstanding research that offers a glimpse into what neuroscience might be like in the 22<sup>nd</sup> century. Many of the publications described above do just that:

- Research that explores the different memory systems of the brain and how they interact to create an intelligent, unified mind (e.g. Anderson 2009; Lansink et al. 2008; Kitamura et al. 2017; Lisman 2015).



- Research that clearly shows how neural representations code information (e.g. Cadieu et al. 2014; Chang & Tsao 2017; Pfeiffer & Foster 2015).
- Research into the synaptic basis of memory, showing how learning literally changes the structure of the brain (e.g. Kasai et al. 2003; Matsuzaki et al. 2004; Yagishita et al. 2014; Bourne & Harris 2011).
- Methods which can invoke, in living animals, particular neural representations at will through optogenetics (Tonegawa et al. 2015).
- Methods that can visualize and manipulate precisely those synapses which make up a new memory (Hayashi-Takagi et al. 2015).
- New brain banking techniques that should be able to preserve the ultrastructural and molecular details of entire intact human brains for indefinitely long-term storage (McIntyre & Fahy 2015).
- Connectomic imaging techniques that can map neural circuits in their entirety at the synaptic level (Briggman, Helmstaedter & Denk 2011; Kim et al. 2014; Kasthuri et al. 2015; Lee et al. 2016; Takemura et al. 2015).
- The invention of new instruments and techniques that promise to extend connectome mapping to entire mammalian brains in the near future (Eberle et al. 2015; Mikula 2016)

This is just a taste of what is possible with early 21<sup>st</sup> century neuroscience. **The Aspirational Neuroscience Prize will inspire the neuroscience community as a whole to imagine what might be possible with 22<sup>nd</sup> century neuroscience and to contemplate neuroscience's long-term promise for humanity.**

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