Ann. N.Y. Acad. Sci. ISSN 0077-8923

ANNALS OF THE NEW YORK ACADEMY OF SCIENCES
Issue: *The Year in Cognitive Neuroscience*
REVIEW

On the research of time past: the hunt for the substrate of memory

Bridget N. Queenan,[1,a] Tomás J. Ryan,[2,3,a] Michael S. Gazzaniga,[4] and Charles R. Gallistel[5]

[1]Neuroscience Research Institute, Department of Mechanical Engineering, University of California Santa Barbara, Santa Barbara, California. [2]School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, and Trinity College Institute of Neuroscience, Trinity College Dublin, Dublin, Ireland. [3]Picower Institute for Learning and Memory, Department of Biology and Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, Massachusetts. [4]Department of Psychological & Brain Sciences, University of California Santa Barbara, Santa Barbara, California. [5]Rutgers Center for Cognitive Science, Rutgers University, Piscataway, New Jersey

Address for correspondence: Bridget N. Queenan, queenan@brain.ucsb.edu; Tomás J. Ryan, tomas.ryan@tcd.ie

The search for memory is one of the oldest quests in written human history. For at least two millennia, we have tried to understand how we learn and remember. We have gradually converged on the brain and looked inside it to find the basis of knowledge, the trace of memory. The search for memory has been conducted on multiple levels, from the organ to the cell to the synapse, and has been distributed across disciplines with less chronological or intellectual overlap than one might hope. Frequently, the study of the mind and its memories has been severely restricted by technological or philosophical limitations. However, in the last few years, certain technologies have emerged, offering new routes of inquiry into the basis of memory. The 2016 Kavli Futures Symposium was devoted to the past and future of memory studies. At the workshop, participants evaluated the logic and data underlying the existing and emerging theories of memory. In this paper, written in the spirit of the workshop, we briefly review the history of the hunt for memory, summarizing some of the key debates at each level of spatial resolution. We then discuss the exciting new opportunities to unravel the mystery of memory.

Keywords: memory; engram; memory encoding; memory localization; optogenetics; neural labeling; engram cell labeling; neural plasticity; synaptic plasticity; history of memory

Introduction

How are experiences stored in the brain? The search for memory is considerably older than cognitive science, neuroscience, or psychology. It predates modern medicine and anatomy, the theory of evolution, and our belief in a heliocentric universe. For at least 2400 years, humans have wondered what enables perception and thought and what accounts for learning and memory. The search for the mind has been led by priests and physicians, philosophers and physicists. The lines of inquiry have diverged and the theories have differed, but the central goal has remained the same: to understand the world and how we understand it.

How has the hunt for memory changed in the last 2000 years? The modern scientific search for memory is critically dependent on the following two fundamental assumptions: (1) the memory trace is physical and (2) the trace will be found in the brain. A modern reader may find both of these issues trivial. If someone proposed to look for your memories in your bone marrow, most would consider them a lunatic. However, the search for memory originated in philosophy and divinity, not in science and certainly not in neuroanatomy. Our memories were not always considered a physical entity, nor were they suspected to lie in the brain.

[a]These authors contributed equally to this work.

doi: 10.1111/nyas.13348

"Of something or of nothing:"[b] the physical basis of memory

Modern science is less than 500 years old, and almost all of our modern discussions of the mind show vestiges of these prescientific theories. Three theories of human knowledge, all generated before the first century AD, have been particularly influential over the last two millennia: (1) knowledge originates in a foreign entity—humans can accept knowledge in the form of food or light created by this external source;[c] (2) knowledge is an innate property of the human mind—we know and remember things that are preembedded and accessed through intelligence;[d] and (3) knowledge is built up systematically through iterations of the mind experiencing the world. The model of accumulated sensory experience dominates today, having been resuscitated at the beginning of the most recent scientific revolution.[e]

These theories have profound implications for where and how we would look for memory. However, when each theory was first articulated, no experiments would have been designed at all. Historically, the idea of peering inside you to find memory or the mind was generally considered ridiculous. Many have maintained that the physical material of the body could *never* give rise to the processes of the mind.[f] Others have considered the mind and body as interdependent physical entities.[g] These concerns are referred to as the "mind–body problem," the (unsolved) mystery of how the physical material of our bodies could generate our mental states.

Only in the last 500 years have investigations into the basis of human knowledge and memory migrated into the realm of science. With the transition comes the demand for theories to be tested with evidence acquired through experimentation. If the mind and its memories have no physical basis, then there cannot be a scientific discipline to study them. We can relegate the conversations forever to the territory of philosophy. So the question is: do we believe we will ever find the physical evidence of memory?

At the beginning of the 20th century, Richard Semon[1,2] invented the term *engram* to signify "the enduring though primarily latent modification in the irritable substance produced by a stimulus (from an experience)."[3] In this theory, interaction with the world leaves a physical residue within the excitable substance of the nervous system. Some internal modifications may manifest directly as a behavioral change or memory; others accumulated over time into a learned behavior.[4,5] The first internal modification will not necessarily produce the full behavioral readout of memory (the first handful of sand does not a castle make), but the residues will, past some threshold, result in a robust, observable

[b] Plato's *Theaetetus* (369 BC) is a dialogue on the nature of knowledge in which Socrates coaxes a theory of epistemology from the hapless Theaetetus. It contains the following exchange: "*Socrates*: And you would admit that there is such a thing as memory? *Theaetetus*: Yes. *Socrates*: And is memory of something or of nothing? *Theaetetus*: Of something, surely."

[c] One of the most beloved theories maintains that knowledge is found in the fruit trees of the Garden of Eden: "In the day ye eat thereof, then your eyes shall be opened, and ye shall be as gods, knowing good and evil" (*King James Bible* (1611), Genesis 3:5). Augustine of Hippo was a notable proponent of the theory of divine illumination. Just as the earth cannot light itself, but requires the sun, "The mind needs to be enlightened by light from outside itself, so that it can participate in truth, because it is not itself the nature of truth" (*Confessions* (397–400), IV.xv)

[d] Plato favored the theory that truth was an inborn property of the human soul (see *Theaetetus* (369 BC)), as did René Descartes: "[W]e come to know [truths] by the power of our own native intelligence, without any sensory experience" (1643 letter, AT 8b: 166–167).

[e] The theory was popularized by Aristotle, who maintained that information was written onto the substance of the mind: "What the mind thinks must be in it in the same sense as letters are on a tablet which bears no actual writing; that is just what happens in the case of the mind" (*On the Soul* (350 BC), 3.4 430a1). John Locke and David Hume are widely credited with disinterring Aristotle's notions and overthrowing Descartes' regime.

[f] Descartes is widely credited with the formalized theory of mind–body dualism (*Meditations* (1641), II.6–8), though he is generally misinterpreted (see Noam Chomsky's 2014 talk at the Vatican for discussion[192]). Descartes maintained that the material of the body (*res extensa*), subject to purely mechanical laws, could not account for the mind. The mind must therefore have a different substrate (*res cogitans*), a fairly legitimate scientific theory before the discovery of electromagnetism and electrochemical transmission.

[g] Supporters of this theory include the Roman physician Galen, the Buddha, David Hume, Charles Darwin, Francis Crick, and Cristof Koch.

behavior that signifies that the internal memory is present. Semon's theory of a physical form of memory was in direct opposition to the "psychical" form successfully advocated well into the 20th century by William McDougall, William James's successor as chair of psychology at Harvard, among others.[6,7]

The stomach contains evidence of food, and the liver certainly contains evidence of drink. Why wouldn't the brain contain evidence of thoughts? Why are we still so mystical about the physical basis of memory?

"I am a brain, Watson. The rest of me is a mere appendix:"[h] discovering the brain

The putative organ of thought has migrated quite a lot over the centuries. Numerous structures or cavities were proposed as the seat of knowledge. In eras where hydraulics were the height of technological sophistication, the suitable organ of thought was a hydraulic pump, the most obvious being the heart circulating blood. Later, the ventricles were observed to be circulating cerebrospinal fluid and the pineal was theorized to be the source of the mechanical propulsion. In light of these technological metaphors, the ancient Egyptians and Aristotle (335 BCE) hailed the heart as the organ of intelligence, Leonardo da Vinci voted for the ventricles (1506), and Descartes preferred the pineal (1649). Once the era of electricity began, the most suitable organ of thought would obviously have to be electrical. In our current era, the height of technological sophistication is the computer and, unsurprisingly, the brain is now almost exclusively described as such.

Metaphors can occasionally prove useful, but the natural world is not obliged to abide by them. As is so often the case in science, things progress when people use their eyes. The latest scientific revolution, precipitated by Nicolaus Copernicus and Andreas Vesalius, placed the sun and brain at the center of their respective universes.[i] Both realignments occurred as a consequence of a suddenly enhanced ability to see. The creation of the telescope let people peer into the heavens, while the decriminalization of human dissection allowed people to peer into the body. Beginning in the Renaissance, artists, scientists, and surgeons began to systematically look for the physical basis of perception. How does the body extract information from the world? First and foremost, the eye was established as the organ of sight. Other senses followed and, suspiciously, all the sensory organs were in direct communication with the brain. The most reasonable conclusion was that the nerve hub was, somehow, important.

"The Hollow Men:"[j] discovering brain areas

By the mid-19th century, thought and memory had been localized in the brain, and many were content to stay at organ-level resolution. Those supporting the "holist" view of brain function maintained that brain functions were performed by the brain as a whole, an organ that could not be divided into components. The holist theory of whole-brain function ended not with a whimper but with a bang, or rather a series of them. Throughout the 19th century, the pioneers of cognitive psychology, including Paul Broca and Carl Wernicke, used the damages of stroke, seizure, and head injury to pinpoint distinct brain functions. Through this careful mapping, the misfortunes of a select few were used to create a theory of many. At the beginning of the 20th century, the Great War supplied the many unfortunates to paint a high-resolution picture of brain function.[k] Localized brain damage did not

[h] Arthur Conan Doyle, *The Adventure of the Mazarin Stone* (1921).

[i] Copernicus' *De revolutionibus orbium coelestium* (1543) and Vesalius' *De humani corporis fabrica* (1543) are widely considered the beginning of the modern scientific era.

[j] T.S. Eliot, "The Hollow Men" (1925).

[k] The ancient Egyptians and Greeks, most notably Hippocrates (see *On Injuries of the Head* (400 BC)), had also noticed the connection between head trauma and specific behavioral deficits. However, at the beginning of the 20th century, the scale of slaughter during World War I, the Victorian obsession with categorization, and the advancements in septic technique in the late 1800s combined to yield an unprecedentedly large population of individuals who survived head injury and could be catalogued. In 1919 alone, the *British Journal of Surgery* published G. Horrax, "Observations on a series of gunshot wounds of the head" 7(25): 10–54; A. Newton & A. Brown, "A study of gunshot wounds of the brain" 7(25): 72–94; and G. Jefferson, "The physiological pathology of gunshot wounds of the head" 7(26): 262–289. However, the injuries are still generally classified according to type (e.g., scalp vs. penetrating vs. perforating vs. fracture),

indiscriminately destroy all cognitive capabilities as one might expect under the holist theory. Instead, specific damages produced specific and remarkably reproducible deficits. Brain functions were not all distributed broadly throughout the entire organ, but instead resided in distinct locations.

By the mid-20th century, localism had long overthrown holism: the brain had identifiable areas that performed distinct functions, and some were even being removed therapeutically.[l] However, in the field of memory research, holism was alive and well. After a systematic search for memory through a series of cortical lesion studies in rats, Karl Lashley famously concluded: "It is not possible to demonstrate the isolated localization of a memory trace anywhere within the nervous system . . . The engram is represented throughout the region."[8]

Loss-of-function lesion studies allow brain functions to be inferred by subtraction. However, complementary evidence of positive functions is also needed. Intractable epilepsy, to this day, is one of the only conditions that justifies invasive recording from or stimulation of the human brain. While evaluating an epileptic patient for surgery, Wilder Penfield and Theodore Rasmussen were allowed to stimulate the temporal cortex of a living human. In so doing, they happened to elicit the recall of random episodic memories.[9] These findings were tantalizing, but it was the temporal cortex of Henry Molaison that altered memory research most profoundly. Having identified the medial temporal lobe as the source of Molaison's seizures, the neurosurgeon William Scoville removed the anterior hippocampus, the amygdala, and the surrounding cortex from both sides of the brain. The consequences were severe memory loss in the form of both anterograde and retrograde amnesia for episodic memories.[10] Molaison's overall intelligence was not negatively affected, and his ability to learn motor tasks was intact. Brenda Milner's and Suzanne Corkin's subsequent work with "patient HM" launched the modern era of human memory research.[10–12] New memories could not be formed without the medial temporal lobe, and the recall of previously stored episodic memories was heavily impaired (for review, see Refs. 13–15). In subsequent years, distinct memory systems have been described and localized in humans.[16,17] Not all memories are created equal, nor are they created in the same place. Different types of learning occur in different brain areas. But what material actually learns?

One by one blossomed the stars:[m] creating the neuron doctrine

By the beginning of the 20th century, the holist–localist debate had been settled at the organ level: the brain had distinct regions with distinct functions. The cortex *alone* could be reproducibly grouped into 52 territories.[18,19,n] However, while losing on the organ front, the holist theory had retreated to a different level of resolution, down to the cell.

By the end of the 19th century, cellular theory[20,21] was widely accepted. However, many scholars of the brain maintained that it was exempt from such rules. Joseph von Gerlach described the brain as an indivisible "protoplasmic network" acting as a functional whole.[22] Early supporters of this "reticular theory" included Franz Nissl and the highly respected Camillo Golgi, who devoted his Nobel acceptance speech to a refutation of the neuron doctrine. In a cruel twist of fate, the father of modern neuroscience, Santiago Ramon y Cajal, used Golgi's own invention (silver staining) to create the images that convinced the world that the brain was

complication (e.g., fungal infection, embedded shrapnel), and/or general symptoms (e.g., headache vs. paralysis vs. ability to work) as the preoccupation was with reducing mortality, not conducting science. Gordon Holmes emerged as arguably the greatest neurological hero of the Great War, carefully piecing together delicate maps of brain function while he treated the horrific brain injuries of his fellow soldiers. One hundred years later, the modern researchers of the Human Connectome Project are continuing this work, creating maps from the thankfully intact human brain.

[l] Antonio Egas Moniz won the 1949 Nobel Prize in Physiology & Medicine for the "discovery of the therapeutic value of leucotomy [lobotomy] in certain psychoses." See http://www.nobelprize.org.

[m] "Silently, one by one, in the infinite meadows of heaven, blossomed the lovely stars, the forget-me-nots of the angels."—Henry Wadsworth Longfellow, *Evangeline, A Tale of Acadie* (1847).

[n] This year, David van Essen and colleagues, as part of the Human Connectome Project, provided evidence for 100 new cortical areas. See Ref. 185.

made of cells. The resulting neuron doctrine formally extended cell theory into the nervous system. The brain was built from discrete areas built from discrete cells. Localism won this hand, too.

The next logical step would be to extend the holist–localist debate to perception and memory, to information processing and storage. The brain perceives the world, creates and deploys concepts, and orchestrates behavior. Are these internal representations located in one place or throughout the brain? Again, we find a split decision. In the localist corner, we have the intellectual powerhouse William James, who theorized that "every brain-cell has its own individual consciousness, which no other cell knows anything about."[23] The theory was later developed into the notion of the "grandmother cell," the idea that an individual neuron can represent a concept[24,25] and will be activated by multimodal stimuli associated with that concept. In the holist corner are proponents of the distributed representation theory, the notion that each stimulus, concept, or behavior is represented in a pattern of neural activity across neurons.[26,27]

Once again, epilepsy provides the only substantial insights to date. The most spectacular observations come from Fried and colleagues, who performed recordings on epileptic patients and found brain cells that reliably responded to pictures of popular actors, athletes, and movie characters, among others.[28] The results have been interpreted to support both the localist (grandmother) and holist (distributed representation) theories. The debate has not been settled.[29] The distributed representation theory cannot be tested, because we cannot see. We cannot collect the patterns of individual neural firing from the brains of living humans. However, the scientific community just recently developed the ability to do this in mice.

"She would give them order. She would create constellations:"[o] discovering network engrams

When the technology does not exist to extract the requisite evidence, the hypothesis cannot be conclusively tested. If information is encoded in patterns of neural firing across neurons, we have to examine the firing of individual neurons during information encoding. In healthy humans, we cannot currently do this. A 1-mm^3 voxel in a functional magnetic resonance imaging scan contains thousands to tens of thousands of neurons. We can observe none of their individual spiking behaviors. From a depth recording in an epileptic patient, we can extract the spiking of a random subset of a few hundred neurons. Neither method is sufficient to conclusively determine the mechanism of neuronal information encoding.

Recently, new technical developments have made it possible to examine the neural mechanisms of memory encoding in mice.[30] The tremendous body of work can be concisely summarized in the following four technical strategies.

Technical strategies

Transgene expression. It is now possible to induce the artificial expression of proteins in mammals through transgenic technology[31–34] (for review, see Ref. 35). Genes can be artificially introduced into or deleted from cells, including brain cells.[36–38] Using the template of the transplanted gene (transgene), the host cell will create the desired protein capable of performing a function or labeling the cell for visualization purposes.

Activity-dependent expression. It is possible to limit the expression of the transgene to specific populations of cells. For example, the transgene can be expressed only in neurons that are active. Immediate early genes (IEGs) naturally turn on immediately after neuronal activity[39–43] (for review, see Ref. 44). The IEG promoters can be used to control an introduced transgene.[30,45–47] The protein of interest will only show up in neurons that fired, producing activity-dependent labeling of cells.

Time-dependent expression. It is possible to limit the expression of transgenes to specific time windows. A transgene attached directly to an IEG promoter would be promiscuously expressed any time the cell fired. However, in a two-component transgenic system, the time window of transgene expression can be controlled, with the aid of a third, exogenous molecule (often an ingested antibiotic (e.g., doxycycline (DOXX)).[48,49] An IEG promoter is attached to a transgene that can itself induce gene expression (a transactivator). Neuronal activity produces the transactivator protein, which binds the promoter of a second transgene. Crucially, the transactivator protein is engineered to be either repressed

[o] Thomas Pynchon, *The Crying of Lot 49* (1965).

or induced by a systemically administered molecule (e.g., rendered inactive by DOX). When the subject is fed a DOX-rich diet, neuronal activity will cause expression of the first but not the second transgene. Only when the subject is taken off DOX is the second transgene expressed, thus labeling the target cells. This method permits labeling to be restricted to a 24-h period during which learning occurs.[30]

Nonelectrical control of neural firing. It is now possible to activate neurons with light, a technique referred to as *optogenetics*.[50,51] Neuronal firing is controlled by ion channels. These ion gates admit charged molecules (mostly sodium, potassium, and chloride) under the direction of certain amino acids and their derivatives (usually glutamate or GABA) acting on synaptic receptors. Other species, including algae, have ion channels that open and close based not on amino acids but on certain wavelengths of light. It is now quite common to express these light-controlled ion channels in neurons.[52,53] Specific colors of light can then turn the neurons on or off as needed.[54,55] Critically, a nonelectrical strategy provides a way of targeting only certain populations. All cells will be affected by electrical stimulation in a distance-dependent manner. The restricted expression of light-sensitive molecules allows for optical stimulation of neurons selected by activity status, cell type, or timeframe.[p]

These four strategies can be combined in the following way to reveal the neurons participating in a memory.[56] An IEG promoter is used to express a transactivator protein in an activity-dependent manner. The transactivator causes expression of a light-sensitive ion channel (e.g., channelrhodopsin[53]) tethered to a visual marker (e.g., green fluorescent protein). The animal is fed a DOX-rich diet to keep the expression of the opsin turned off, even if the host neuron is active. For a brief period of time, the animal no longer receives the antibiotic. During this time window, the animal learns something (e.g., the features of a novel context and/or that the context is pleasant or dangerous). The animal is then fed the antibiotic to stop the artificial expression of the transgenes. Within a few hours of training, the light-sensitive ion channel (tethered to the cell marker) will be expressed, but only in neurons that were active while the animal was learning.[2,30,57] It is therefore possible to see which neurons participated in the learning.[56,58–61] Importantly, light can be used to reversibly turn neural ensembles on[56,58,59,62–65] (with the excitatory channelrhodopsin) or off[60,61,66] (with the inhibitory halorhodopsin). In this way, it can be empirically determined whether those particular neural ensembles encoded the memory.

What has been learned from mapping memory in mice? Firstly, when a mouse learns a task, a subset of neurons is activated, not the whole brain, nor a whole brain area.[30,58,59] If these neurons are directly reactivated (i.e., with light), the memory, or more specifically the behavioral readout of the target memory, can be elicited.[56,59] If the cells are inhibited, the natural cue-induced recall of the target memory is impaired.[60,61,66–68] Control experiments have shown that activation and inhibition of these cell ensembles affects the targeted memory specifically, not other similar but nonlabelled memories.[56,58–60] If the labelled neural ensembles are destroyed, so too is the memory.[69] However, if the cells are not destroyed, they can be manipulated to update an existing memory,[58,59,62] including to switch the emotional valence of a negative experience to a positive one.[62] These findings, arising independently from a number of research groups, have established that the hippocampal cells that are active during a defined training episode are both sufficient and necessary for the recall of a particular contextual memory, including social memory.[70] The labelled cellular ensembles represent a component of a bona fide, specific memory engram. It is now possible to search for the mechanism of memory storage.

The persistence of memory and plasticity of synapses

Engram-labeling studies have shown that certain populations of neurons encode specific memories in mice. How, exactly, do the cells encode the memory? Does each neuron contain a memory? Is the memory in the location of the neurons, the firing order, or the relative spike rates? The answer might be none of the above. Ramon y Cajal speculated that structural changes within neurons were the mechanism of learning:

> "The organ of thought is, within certain limits, malleable and capable of perfection. . . . The cerebral cortex

[p] Note: It is also possible to remotely control neurons with chemicals and engineered receptors.[194]

> is similar to a garden filled with trees, the pyramidal cells, which, thanks to an intelligent culture, can multiply their branches, sending their roots deeper and producing more and more varied and exquisite flowers and fruits."[71,72]

Synapses, the remarkably dynamic connections between neurons, have always seemed particularly suspicious in the study of memory.[73,74] Each neuron has thousands of connections, and they are not static. Almost 70 years ago, Donald Hebb theorized that synaptic connections between coactivated cells change in an activity-dependent manner,[75] leading to the postulate that neurons that "fire together, wire together"[76] into cellular assembles. Early studies in sea slugs demonstrated that synapses could be directly conditioned,[77] bringing synaptic plasticity to the forefront as a plausible subcellular learning mechanism. Since the original Hebbian postulate, an enormous amount of evidence has been compiled to suggest that neurons do, in fact, alter synaptic connectivity in an activity-dependent manner. Neurons can reinforce synaptic connections following rules of spike timing-dependent plasticity.[78–80] When an action potential is fired, synapses that were active just before the action potential (i.e., relevant to the generation of the action potential) are reinforced.[81] Meanwhile, synapses that were active after the action potential (i.e., irrelevant to its generation) are weakened.[81] Synapses can become transiently depressed or overly enthusiastic, through processes known as short-term plasticity.[82] The effectiveness of synaptic transmission can also be enhanced or diminished in a semipermanent fashion through processes known as long-term potentiation and long-term depression, respectively.[83] These persistent forms of synaptic strengthening and weakening, known as associative (or Hebbian) synaptic plasticity, establish patterns of connectivity that are generally thought to encode information.[84–86]

However, decades later, the coding scheme remains elusive.[87] At the cellular level, neuronal codes have been observed: neuronal firing patterns can signal various components of space and time.[q] At the subcellular level, the code has not yet been cracked. How exactly is acquired information (e.g., the location of a food source or the duration of a wait interval) mapped to a pattern of synaptic alterations? How is the encoded information entered into the neuronal operations that underlie goal-directed behavior?[87] Until recently, the problem has been intractable. There are thousands of synapses per neuron, and each can be changed through a wide variety of mechanisms: more neurotransmitter can be released;[88,89] more receptors can be expressed;[90–94] and the properties of the receptors[95,96] or the release machinery[97–99] can be altered. Additionally, new synaptic junctions can be formed,[100,101] and structurally intact but functionally silent synapses can be revealed.[97,102–104] It has been difficult to pinpoint which of these mechanisms, if any, results in which "memory." Confounding the problem is the manner in which synaptic plasticity is often induced. Electrical stimulation is widely used to mimic the natural learning experience and induce synaptic change.[105,106] Comparatively few studies have examined the consequences of ethologically relevant learning on synaptic function.[107–110] Plasticity studies have focused on artificial stimulation protocols, which recapitulate some but not all aspects of learning-induced plasticity,[87] rather than actual learning-induced changes, because it was not previously possible to identify the cells allocated to a particular experience. With these new technologies, we now know where to look.

By comparing the physiological properties of engram and non-engram cells *ex vivo*, it was found that both synaptic strength and specificity of connectivity increased between certain neurons during learning, forming functional coalitions of cells across brain areas that manifest the memory.[59] These findings are compelling, but alone they are correlates of actual learning. Importantly, it is also now possible to test candidate plasticity mechanisms by interfering with plasticity and determining the consequences on both engram cells and memory function in a unitary experimental preparation. For example, it has been established for decades that

[q] Place cells,[195] grid cells,[196] head-direction cells,[197] boundary-vector cells,[198] and time cells[199] have all been proposed to represent components of space and time through neuronal firing patterns. Neuronal firing patterns within the motor cortices also signal the temporal and spatial components of motor movement of a limb reliably enough that these signals can be decoded and used to guide prosthetic limb movement after injury.[200–202]

long-term memory formation requires new gene expression[111,112] (for review, see Refs. 113 and 114): when a protein synthesis inhibitor (e.g., puromycin or anisomycin) is delivered to mice immediately after a training experience, amnesia for the target memory results.[59,111] Only recently has it become possible to test the mechanisms underlying amnesia, induced either by protein synthesis inhibitors or mouse models of early-stage Alzheimer's disease. Anisomycin treatment and early Alzheimer's produced amnesia that correlated with reduced synaptic strength and number.[59,65] However, the specific connectivity pattern between engram cells was altered, and the resulting neural ensembles survived the amnesia.[59,65] Importantly, when the amnesic engram cells were directly stimulated with light, memory retrieval occurred, demonstrating the persistence of memory in a latent but reactivatable state.[59,65] Thus, a plasticity program including new gene expression is activated specifically in cells undergoing learning, and disruption of this process impairs both synaptic strengthening and the manifestation of the long-term memory as a behavior.[59] Alzheimer's pathology appears to attack these synaptic targets,[65] as has long been hypothesized.[115] However, the memory remained in the amnesic brain, presumably in the altered pattern of neural connectivity, and could be reactivated by direct stimulation of the engram cells.[59,65]

Using these new techniques, it is for the first time possible to dissociate the mechanisms underlying memory retrieval and those underlying memory storage. When enhanced synaptic strength between engram cells is abolished, the memory is not.[59,65] Gross synaptic strengthening can be excluded as a candidate mechanism for memory storage, though it seems essential for natural memory retrieval.[1,59] Indeed, a corroborating study showed that amnesia could be induced in rats by depressing amygdala synapses *in vivo*.[116] Remarkably, the artificial re-potentiation of those synapses restored the behavioral response, though such stimulation did not create the conditioned behavioral response in naive animals because no relevant memory was present.[116] Similar findings have been reported in sea slugs, where reminder training seems to reverse amnesia through the restoration of the learned synaptic structure.[117,118]

These findings collectively implicate synaptic strength as a crucial determinant of memory accessibility or retrievability. When synapses are not operational, information—however stored—cannot be extracted from cells. Permanent or widespread disruption of synaptic structure and function would be devastating, producing cognitive deficits from autism[119] to late-stage Alzheimer's[120,121] to clinical depression.[122] However, *subtle* disruption of synaptic transmission may be necessary for brain function: recent work has shown that excitatory neurotransmitter receptors are removed and modified during sleep,[123] while much more widespread dendritic changes have been found in hibernating animals.[124] These results suggest that the brain may adaptively modulate the accessibility of stored information by altering the strength of transmission at synapses. Indeed, several types of systematic synaptic alteration have been extensively studied and characterized, including associative plasticity (mechanisms that strengthen or weaken individual synapses during learning[125]), homeostatic plasticity (negative feedback mechanisms that act more globally across dendritic trees to ensure stability[126,127]), and metaplasticity (mechanisms that alter the threshold for subsequent plasticity[128]). These processes of synaptic regulation, operational in excitatory and inhibitory[129,130] networks, may be part of a sophisticated and dynamic control system by which information is extracted from or hidden within neurons according to the electrical, chemical, or metabolic state of the network. Determining how the various forms of synaptic control are deployed across synapses, dendrites, neurons, and brain regions may reveal how the brain rapidly and transiently accesses information.

However, the empirical findings clearly show that permanently enhanced synaptic transmission does not and cannot store the memory information itself.[1] How then is the memory stored?

"To see a World in a Grain of Sand:"[r] is there a memory molecule?

The substrate of memory has been at least partially localized within the brain, to certain brain areas, and now to constellations of neurons. We can

[r] "To see a World in a Grain of Sand/And Heaven in a Wild Flower/Hold Infinity in the palm of your hand/And Eternity in an hour."—William Blake, *Auguries of Innocence* (1803).

now address the long-neglected question: how do the residues of past experience encode that experience? Where within those constellations are the memories? Conventionally, the answer has been: at the synapse itself.[131] The proteins forming the neurotransmitter receptors change with learning, thus encoding information in their enhanced density, function, and affiliation with synaptic scaffolds, all regulated by the receptor's phosphorylation status.[132–134] Synaptic weight changes can now be excluded as a means of information storage, though they appear to dictate engram retrieval.[59,65] What alternate mechanisms could store the information itself?

The most intuitive answer would be long-lived molecules. Candidates for molecular substrates of information storage include the particularly long-lived proteins associated with DNA (i.e., nucleoporins and histones[135]). These proteins could make permanent changes in how often or under what circumstances a protein is expressed. Alternately, some have suggested that DNA (or the epigenetic modifications on it[136,137]) is the most suitable candidate for memory, being the cellular storage mechanism for other (lifelong) information.[s] A more radical speculation is that information might be a stored in genetic material itself,[138] though how that could happen at a sufficiently fast rate remains to be described.

The physical connectivity within neural ensembles is a plausible new candidate substrate for memory information storage, with many merits, including robustness to insult,[59,65] bioenergetic efficiency, stability of information storage in a potentially binary format,[139,140] and a high capacity for informational content (see Refs. 1 and 141 for review). If physical connectivity mediates memory storage, how is it maintained? The late Roger Tsien recently proposed the notion that perineuronal nets, the extracellular matrices around neurons and synapses, provide the architecture for information.[142] Structural changes in the extracellular matrix, mediated by matrix metalloproteases and other enzymes, could provide long-lasting information storage,[142] delineating the neural constellations that are formed during learning and maintained as connectivity patterns even under amnesia.[59,65] This hypothesis addresses a fundamental problem with any theory of channel- or receptor-based encoding: proteins are not long for this world.[142,143] Most mammalian proteins live for only a few days[144,145] while synaptic proteins can manage up to a week.[146] Individual synaptic signaling proteins simply cannot "remember" for very long, on either side of the synapse, making them a suitable substrate for short- but not long-term memory. If the engram requires a protein, it should arguably be a fairly long-lived one. The longest-lived proteins are generally structural,[143] especially those in the extracellular matrix.[142] Despite these theoretical advantages, little is currently known about how neural ensembles are formed and maintained. Even less is known about the code that maps the elements of experience to the pattern of cellular and synaptic connectivity assumed to encode those facts.

An alternative to storage supported by very long-lasting molecules is engram maintenance in short-lived molecules, or molecular states, which exists as part of a long-lived arrangement. Antoine Danchin has famously used the metaphor of the Delphic boat to describe how life emerges from dynamic cellular components. The metaphor comes from a riddle posed by the oracle at Delphi: "If we consider a boat made of planks, what is it that makes a boat a boat?" The question seems simple enough: looks like a boat, acts like a boat. However, "as time passes, some of the planks begin to rot and have to be replaced. There comes a time when not one of the original planks is left. Is it still the same boat? The owner would certainly say yes, this is my boat. Yet none of the material it was originally built from is still there."[t] Structures composed of short-lived components could constitute a long-term memory if the configurations were preserved by normal homeostatic replenishment of steady-state components. For example, synapses are built from actin polymers, which restructure with learning.[147,148] Embedded within the actin network are scaffolding proteins,[149] the sites into which neurotransmitter receptors are inserted. Synaptic proteins could hop

[s] It should be noted that many of these theories work within the more conservative paradigm whereby genetic changes operate solely through the modulation of gene expression and its slow effect on late-phase synaptic plasticity.

[t] Antoine Danchin, *The Delphic Boat* (2003).

in and out of the synaptic lattices just like electrons move within semiconductors. In this model, consistent with the engram connectivity model, the structure of the actin–scaffold lattice (semiconductor) would dictate the behavior of the synapses,[84,150–152] not the receptors (electrons) that occupy them with some probability. Other theories involve information storage or processing in microtubules,[153] the long polarized helices of tubulin subunits that compose the cellular skeleton.[154,155] New microtubule tracks are laid directly toward the synapse during activity,[156–158] possibly encoding a change in status. The tubulin subunits or helical structure could be modified directly,[159,160] or the pairing between microtubules and actin structures could be altered by cytoskeletal binding proteins,[161,162] changing the likelihood of synaptic proteins being recruited to the synapse.[163] Posttranslational modification of particular synaptic proteins has also been suggested to mediate memory storage by modifying the trafficking and behavior of the molecules.[164] The short-term modification could be maintained in the long term by active positive feedback that preserves the altered state. While such a mechanism would certainly be rapid and plastic, it also carries the weakness of vulnerability to disruption and the thermodynamic cost of any necessarily active mechanism of maintenance. Substantial changes in synaptic behavior could also be achieved by altering the biophysical properties of membranes (e.g., the relative compositions of fatty acids and cholesterol would alter the behavior of membrane-embedded receptors) or the dimensions of the synapse itself. Importantly, many synaptic processes are controlled by glia,[165] making non-neuronal brain cells potential mediators of memory encoding, maintenance, or extraction.

None of the above potential mechanisms need to function exclusively in isolation, and indeed some of them may represent different manifestations of the same substrate. Early studies of memory engram cells have shown that much work remains to done in order to discriminate between the enabling mechanism of memory retrieval and the essential mechanisms of information storage. Recent technological advances, including multiphoton brain imaging,[166,167] may permit us to look inside cells *during* learning to see the cellular events as they occur. Indeed, a tantalizing new model of cellular learning has recently been provided. Behavioral results long ago established that animals learn to tell time, specifically the durations of intervals.[168,169] A simple instance of interval-duration learning occurs in Pavlovian conditioning paradigms, where a neutral conditioned stimulus (CS) predicts an aversive unconditioned stimulus (US), which occurs at some delay. Regardless of species, subjects do not learn to simply respond to the CS alone, they learn to respond at the right latency. In eye-blink conditioning, the encoding of the CS–US interval has been tracked to Purkinje cells in the cerebellum. When developing an appropriately timed response, the cerebellar neurons establish new sequences of neural firing[170,171] by learning to pause for particular time intervals,[172–174] just as we learn to play the piano by perfecting the timing between individual finger strikes. Recently, these cells have been artificially conditioned by direct stimulation of their immediate inputs to learn the CS–US interval, so that the entirety of the paradigm (encoding of CS, association to US, timed response, etc.) has been reduced to a manipulatable experiential fact—the CS–US interval. Using this experimental paradigm, it was revealed that the learned firing pause is initiated by metabotropic signaling cascades that terminate by acting on inhibitory ion channels.[175] The results provide a means of dissecting the components of the cascade between the metabotropic receptor and the inhibitory ion channels to determine if and how the previously experienced CS–US interval is encoded and decoded.[174,175]

Intracellular neurochemical processes are attractive as bioenergetically efficient, local, and flexible tools for neural computation (eloquently discussed in Ref. 176). Cheap, dynamic, and local chemical computation could provide a perfect complement to a one-time expensive investment in infrastructural change (e.g., in reorganization of extracellular or cytoskeletal components) across brain areas. By examining the intra- and extracellular processes that create and maintain neural ensembles, it may be possible to unite the molecular, neuronal, and circuit mechanisms of neural learning. However, at the behavioral level, learning differs significantly across brain regions,[16] so it will be important to determine whether conditioned or procedural learning within the cerebellum and striatum, for example, proceed via distinct cellular or molecular mechanisms from learning within the cortex and hippocampus. Indeed, plasticity processes that seem to be crucial

for information storage have been characterized in mammalian visual cortex cells and in perceptual regions as early as olfactory sensory neurons.[177,178] Moreover, synaptic dysfunction in peripheral nerves has been shown to produce learning deficits.[179] Tracing the engram ensembles from their peripheral origins may be necessary to understand how information is stored, accessed, and reconfigured over time.

It has long been believed that, in addition to any cellular or synaptic consolidation, systems-level consolidation of certain memories occurs via gradual transfer of the representation from one brain area to another,[180] notably from the hippocampus to cortex[181] or from cortex to striatum.[182] However, optogenetic studies have challenged this pipeline hypothesis of consolidation. Recently, neuronal representations of a single contextual memory were shown to form simultaneously in the hippocampus and prefrontal cortex.[183] Over time, the preferred retrieval pathway under standard recall conditions shifted from hippocampal to prefrontal engram neurons, resulting in the apparent "transfer" of the memory to cortical circuits even though the representation persisted in both areas.[183,184] Circuit-level redundancies in the initial representation might enhance the robustness of memory, increasing the likelihood of subsequent retention, consolidation, or recall through whichever pathway is available. Strong evidence for such redundancy was first provided by chronic optogenetic inhibition of hippocampal CA1, which demonstrated that contextual fear memory recall can be diverted to other less cannonical structures, including the anterior cingulate cortex.[185] Alternatively, the highly distributed nature of the engram may represent a hierarchy of informational content rather than a true redundancy, with certain aspects of a global representation becoming more salient depending on the remoteness of the memory or conditions of recall.[186]

Human functional imaging studies have indicated that the flexibility of modular network organization is predictive of subsequent learning[187] and rodent optogenetic systems now offer the ability to watch and manipulate the brain as it flexibly reconfigures. If memory performance depends, at least in part, on the systems-level redundancy and plasticity of representations, not the fidelity of encoding within a specific area, it will place useful constraints on the search for the biological substrate. These are speculations, but they are suddenly testable speculations. After centuries of suspecting, doubting, hoping, and wishing for a trace of past thoughts, modern neuroscientists have been able to map the constellations of neurons forming a memory. It is now possible to look inside and between those cells for residues of past experience.

"There is no easy way from the earth to the stars:" the future of memory research

In the practice of science, we demand empirical evidence to distinguish facts from speculations and to draw meaningful conclusions. We can now see more clearly into the brains of mice and, accordingly, we can now see where their memories are housed. If memories are encoded in cellular-resolution patterns of connectivity, we may soon be able to decode them. If they are not, we will have to look more closely, inventing new ways of noninvasively seeing dynamic synapses, organelles, or subcellular structures at finer resolutions or inventing new computational approaches to handle the behavior of these networks. Many proposed substrates of memory will remain in the realm of speculation until technologies capable of testing their suitability emerge. Hippocrates once speculated on the future of epilepsy, the "sacred disease:" "It appears to me to be nowise [sic] more divine nor more sacred than other [things], but has a natural cause from which it originates. Men regard its nature and cause as divine from ignorance and wonder . . . And this notion of its divinity is kept up by their inability to comprehend it."[u] Over the last several millennia, the symptoms of epilepsy have remained roughly constant. What has changed is the conviction that causes can be determined, mechanisms mapped, and solutions engineered.

Because the mind is part of the natural world, there is no reason to believe that it will be any less tangible and ultimately comprehensible than other components. However, there is ample reason to suspect that the answers (or the interpretations) lie outside the current radius of neuroscience. There are three major sources of progress available. The first is the expansion of knowledge through basic research. Perhaps the critical discoveries have simply not been made yet. For example, the formulation of

[u] Hippocrates, *On the Sacred Disease* (400 BC).

gravitation and later electromagnetism as fundamental interactions crystallized centuries of observations that had previously lacked a certain coherence. The second is the infusion of existing knowledge from other fields. For example, the discovery of rhodopsin is not recent,[v] and the rhodopsins are quite commonplace, found in algae, bacteria, and viruses, as well as the retina of mammals, including humans. However, when channelrhodopsin was strategically imported from single-celled algae into mammalian neurons,[50,52,53] neuroscience was endowed with a new power to manipulate individual neurons at will. The third is the reorientation of existing knowledge. Certain answers may be sitting right in front of us, in the territory of conventional biochemistry and physiology. However, a paradigm shift may be required to look at the existing data from a more productive angle.

Neuroscience is currently dominated by the metaphor of the mind as a computer. Under this regime, we implicitly look for "bits" that "encode" "information," terms and concepts that were invented or formalized within the last century.[w] It may be that an organ that arose through 200 million years of cephalization, composed of specialized cells with synaptic connections that originated at least a billion years ago,[188] behaves in a manner similar to our human-made machines. However, the brain is not obliged to compute in a digital fashion. The representations that mediate basic perception and motor action may be stored and transmitted in analog format,[189] or the "computations" may be non-algorithmic.[190] The brain may be a nonlinear system that bears no resemblance to a computer at all. The mind has previously been conceptualized as other fashionable technologies—it has been propelled by hydraulics, driven by mechanical gears, and powered by combustion; it has been imagined as a system of telegraph wires and a GPS system. It will be reimagined as other popular tools and toys that capture our imaginations. Our minds will presumably continue to function regardless of which technological transformation we demand next.

According to Thomas Kuhn, "To be accepted as a paradigm, a theory must seem better than its competitors, but it need not, and in fact never does, explain all the facts with which it can be confronted."[191] By adhering too strictly to any dominant model, we severely limit the questions that can be asked and the acceptable answers that can be produced. Any anomalies that arise—observations that do not fit the theory—will be ignored or suppressed, subconsciously as well as systematically. This is because "assimilating a new sort of fact demands a more than additive adjustment of theory, and until that adjustment is completed—until the scientist has learned to see nature in a different way—the new fact is not quite a scientific fact at all."[183]

If past progress is any indication, future developments will happen not by subscribing to a theory and pursuing data to support it but by pursuing the central question and respecting anomalies as they arise. The invention of novel experimental techniques and methodologies is allowing researchers to pursue anomalies in the neuroscience of a memory in a way not previously possible, dissecting mechanisms of memory retrieval from those of memory encoding. These investigations will generate useful predictions on the nature of memory storage and novel therapeutic tactics. Sustained progress may soon result in a new theory of memory with enough explanatory value to temporarily satisfy the original question: How are experiences stored in the brain?

Acknowledgments

The authors thank all participants in the 2016 Kavli Futures Symposium, in particular Hessam Akhlaghpour, David Glanzman, Steven Grant, Germund Hesslow, Fredrik Johansson, Dan Margoliash, and John McGann. We also thank Luke Theogarajan and Karl Deisseroth for helpful discussion. The symposium was made possible by support from the SAGE Center for the Study of the Mind, the Kavli

[v] Beginning in the 1850s, early investigators of the retina, including Heinrich Müller, Franz Boll, and Willy Kühne, pieced together an understanding of the light-sensitive molecules of the visual system, identifying "visual purple," which later became rhodopsin.[203]

[w] "Information" has only recently become a precise term rather than a hazy concept. In 1928, Hartley attempted a "quantitative measure of 'information' ... which is based on physical as contrasted with psychological considerations."[204] Two decades later, Claude Shannon produced "A Mathematical Theory of Communication,"[205] which coined the term "bit" and formalized information theory as a field of study, rather than an offshoot of electrical engineering. See James Gleick's *The Information* for a full history.[206]

Foundation and the Kavli Summer Institute for Cognitive Neuroscience, which also received support from the National Institute of Mental Health and the National Institute on Drug Abuse. We thank Ron Mangun and Jayne Kelly for their help.

Competing interests

The authors declare no competing interests.

References

1. Tonegawa, S., M. Pignatelli, D.S. Roy & T.J. Ryan. 2015. Memory engram storage and retrieval. *Curr. Opin. Neurobiol.* **35:** 101–109.
2. Tonegawa, S., X. Liu, S. Ramirez & R. Redondo. 2015. Memory engram cells have come of age. *Neuron* **87:** 918–931.
3. Semon, R. 1904. *Die Mneme.* Allen & Unwin.
4. Muller, G. & A. Pilzecker. 1900. Experimentelle Beiträge zur Lehre vom Gedächtnis. *Z. Psychol. Ergänzungsband* **1:** 1–300.
5. McGaugh, J.L. 2000. Memory—a century of consolidation. *Science* **287:** 248–251.
6. Schacter, D.L., J.E. Eich & E. Tulvlng. 1978. Richard Semon's theory of memory. *J. Verbal Learn. Verbal Behav.* **17:** 721–743.
7. Greenwood, M. & M. Smith. 1940. William McDougall, 1871–1938. *Obit. Not. Fellows R. Soc.* **3:** 39–62.
8. Lashley, K. 1950. In search of the engram. In *Experimental Biology Symposium No. 4: Physiological Mechanisms in Animal Behaviour*. J.F. Danielli & R. Brown, Eds.: 454–482. New York: Academic Press.
9. Penfield, W. & T. Rasmussen. 1950. *The Cerebral Cortex of Man.* Macmillan Publishers Limited.
10. Scoville, W. & B. Milner. 1957. Loss of recent memory after bilateral hippocampal lesions. *J. Neurol. Neurosurg. Psychiatry* **20:** 11–21.
11. Penfield, W. & B. Milner. 1958. Memory deficit produced by bilateral lesions in the hippocampal zone. *AMA Arch. Neurol. Psychiatry* **79:** 475–497.
12. Squire, L.R. 2009. The legacy of patient H.M. for neuroscience. *Neuron* **61:** 6–9.
13. Bird, C.M. & N. Burgess. 2008. The hippocampus and memory: insights from spatial processing. *Nat. Rev. Neurosci.* **9:** 182–194.
14. Kim, J.J. & D.M. Diamond. 2002. The stressed hippocampus, synaptic plasticity and lost memories. *Nat. Rev. Neurosci.* **3:** 453–462.
15. Neves, G., S.F. Cooke & T.V.P. Bliss. 2008. Synaptic plasticity, memory and the hippocampus: a neural network approach to causality. *Nat. Rev. Neurosci.* **9:** 65–75.
16. Squire, L.R. 2004. Memory systems of the brain: a brief history and current perspective. *Neurobiol. Learn. Mem.* **82:** 171–177.
17. Tulving, E. 1972. Episodic and semantic memory. In *Organization of Memory.* E. Tulving & W. Donaldson, Eds.**:** 381–403. New York; London: Academic Press.
18. Brodmann, K. 1909. Vergleichende Lokalisationslehre der Großhirnrinde: in ihren Prinzipien dargestellt auf Grund des Zellenbaues. Leipzig: Barth.
19. Zilles, K. & K. Amunts. 2010. Centenary of Brodmann's map—conception and fate. *Nat. Rev. Neurosci.* **11:** 139–45.
20. Schleiden, M. 1838. *Contributions to Phytogenesis.* Trans. By H. Smith. London: Syndenham Society, 1847; 231–263.
21. Schwann, T. 1839. *Microscopical Researches into the Accordance in the Structure and Growth of Animals and Plants.* London: Sydenham Society.
22. Shepherd, G.M. 1991. *Foundations of the Neuron Doctrine.* Oxford University Press.
23. James, W. 1911. *Principles of Psychology.* Henry Holt & Co.
24. Gross, C.G. 2002. Genealogy of the 'grandmother cell'. *Neuroscience* **8:** 512–518.
25. Konorski, J. 1967. *Integrative Activity of the Brain: An Interdisciplinary Approach.* University of Chicago Press.
26. Rumelhart, D.E., G.E. Hinton & R.J. Williams. 1986. Learning internal representations by error propagation. In *Parallel Distributed Processing: Explorations in the Microstructure of Cognition, Volume 1: Foundations.* D.E. Rumelhart & J.L. McClelland, Eds.: 318–362. Cambridge, MA: MIT Press.
27. Kanerva, P. 1988. *Sparse Distributed Memory.* MIT Press.
28. Quiroga, R.Q., L. Reddy, G. Kreiman, *et al.* 2005. Invariant visual representation by single neurons in the human brain. *Nature* **435:** 1102–1107.
29. Quiroga, R.Q., I. Fried & C. Koch. 2013. Brain cells for grandmother. *Sci. Am.* **308:** 30–35.
30. Reijmers, L.G., B.L. Perkins, N. Matsuo & M. Mayford. 2007. Localization of a stable neural correlate of associative memory. *Science* **317:** 1230–1233.
31. Gordon, J.W., G.A. Scangos, D.J. Plotkin, *et al.* 1980. Genetic transformation of mouse embryos by microinjection of purified DNA. *Proc. Natl. Acad. Sci. U.S.A.* **77:** 7380–7384.
32. Brinster, R.L. *et al.* 1981. Somatic expression of herpes thymidine kinase in mice following injection of a fusion gene into eggs. *Cell* **27:** 223–231.
33. Costantini, F. & E. Lacy. 1981. Introduction of a rabbit beta-globin gene into the mouse germ line. *Nature* **294:** 92–94.
34. Wagner, E.F., T.A. Stewart & B. Mintz. 1981. The human beta-globin gene and a functional viral thymidine kinase gene in developing mice. *Proc. Natl. Acad. Sci. U.S.A.* **78:** 5016–5020.
35. Palmiter, R.D. & R.L. Brinster. 1986. Germ-line transformation of mice. *Annu. Rev. Genet.* **20:** 465–499.
36. Mayford, M. *et al.* 1996. Control of memory formation through regulated expression of a CaMKII transgene. *Science* **274:** 1678–1683.
37. Naldini, L., U. Blömer, F.H. Gage, *et al.* 1996. Efficient transfer, integration, and sustained long-term expression of the transgene in adult rat brains injected with a lentiviral vector. *Proc. Natl. Acad. Sci. U.S.A.* **93:** 11382–11388.
38. Tsien, J.Z. *et al.* 1996. Subregion- and cell type-restricted gene knockout in mouse brain. *Cell* **87:** 1317–1326.
39. Greenberg, M.E. & E.B. Ziff. 1984. Stimulation of 3T3 cells induces transcription of the c-*fos* proto-oncogene. *Nature* **311:** 433–438.

40. Link, W. *et al.* 1995. Somatodendritic expression of an immediate early gene is regulated by synaptic activity. *Proc. Natl. Acad. Sci. U.S.A.* **92:** 5734–5738.
41. Lyford, G.L. *et al.* 1995. Arc, a growth factor and activity-regulated gene, encodes a novel cytoskeleton-associated protein that is enriched in neuronal dendrites. *Neuron* **14:** 433–445.
42. Müller, R., R. Bravo, J. Burckhardt & T. Curran. 1984. Induction of c-*fos* gene and protein by growth factors precedes activation of c-*myc*. *Nature* **312:** 716–720.
43. Curran, T. & J. Morgan. 1985. Superinduction of c-*fos* by nerve growth factor in the presence of peripherally active benzodiazepines. *Science* **229:** 1265–1268.
44. West, A.E., E.C. Griffith & M.E. Greenberg. 2002. Regulation of transcription factors by neuronal activity. *Nat. Rev. Neurosci.* **3:** 921–931.
45. Smeyne, R.J. *et al.* 1992. Fos-IacZ transgenic mice: mapping sites of gene induction in the central nervous system. *Neuron* **8**, 13–23.
46. Wang, K.H. *et al.* 2006. *In vivo* two-photon imaging reveals a role of Arc in enhancing orientation specificity in visual cortex. *Cell* **126:** 389–402.
47. Barth, A.L., R.C. Gerkin & K.L. Dean. 2004. Alteration of neuronal firing properties after *in vivo* experience in a FosGFP transgenic mouse. *J. Neurosci.* **24:** 6466–6475.
48. Gossen, M. & H. Bujard. 1992. Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. *Proc. Natl. Acad. Sci. U.S.A.* **89:** 5547–5551.
49. Gossen, M. *et al.* 1995. Transcriptional activation by tetracyclines in mammalian cells. *Science* **268:** 1766–1769.
50. Zhang, F. *et al.* 2007. Multimodal fast optical interrogation of neural circuitry. *Nature* **446:** 633–639.
51. Deisseroth, K. 2011. Optogenetics. *Nat. Methods* **8:** 26–29.
52. Boyden, E.S., F. Zhang, E. Bamberg, *et al.* 2005. Millisecond-timescale, genetically targeted optical control of neural activity. *Nat. Neurosci.* **8:** 1263–1268.
53. Zhang, F., L.-P. Wang, E.S. Boyden & K. Deisseroth. 2006. Channelrhodopsin-2 and optical control of excitable cells. *Nat. Methods* **3:** 785–792.
54. Cardin, J.A. *et al.* 2010. Targeted optogenetic stimulation and recording of neurons *in vivo* using cell-type-specific expression of Channelrhodopsin-2. *Nat. Protoc.* **5:** 247–254.
55. Zhang, F. *et al.* 2010. Optogenetic interrogation of neural circuits: technology for probing mammalian brain structures. *Nat. Protoc.* **5:** 439–456.
56. Liu, X. *et al.* 2012. Optogenetic stimulation of a hippocampal engram activates fear memory recall. *Nature* **484:** 381–385.
57. Matsuo, N., L. Reijmers & M. Mayford. 2008. Spine-type-specific recruitment of newly synthesized AMPA receptors with learning. *Science* **319:** 1104–1107.
58. Ramirez, S. *et al.* 2013. Creating a false memory in the hippocampus. *Science* **340:** 216–219.
59. Ryan, T.J., D.S. Roy, M. Pignatelli *et al.* 2015. Engram cells retain memory under retrograde amnesia. *Science* **348:** 1007–1013.
60. Tanaka, K.Z.Z. *et al.* 2014. Cortical representations are reinstated by the hippocampus during memory retrieval. *Neuron* **84:** 347–354.
61. Denny, C.A. *et al.* 2014. Hippocampal memory traces are differentially modulated by experience, time, and adult neurogenesis. *Neuron* **83:** 189–201.
62. Redondo, R.L. *et al.* 2014. Bidirectional switch of the valence associated with a hippocampal contextual memory engram. *Nature* **513:** 426–430.
63. Cowansage, K.K.K. *et al.* 2014. Direct reactivation of a coherent neocortical memory of context. *Neuron* **84:** 432–441.
64. Ramirez, S. *et al.* 2015. Activating positive memory engrams suppresses depression-like behaviour. *Nature* **522:** 335–339.
65. Roy, D.S. *et al.* 2016. Memory retrieval by activating engram cells in mouse models of early Alzheimer's disease. *Nature* **531:** 508–512.
66. Trouche, S. *et al.* 2016. Recoding a cocaine-place memory engram to a neutral engram in the hippocampus. *Nat. Neurosci.* **19:** 564–567.
67. Nomoto, M. *et al.* 2016. Cellular tagging as a neural network mechanism for behavioural tagging. *Nat. Commun.* **7:** 12319.
68. Yokose, J. *et al.* 2017. Overlapping memory trace indispensable for linking, but not recalling, individual memories. *Science* **355:** 398–403.
69. Jan, J.-H. *et al.* 2009. Selective erasure of a fear memory. *Science* **323:** 1492–1496.
70. Okuyama, T., T. Kitamura, D.S. Roy, *et al.* 2016. Ventral CA1 neurons store social memory. *Science* **353:** 1536–1541.
71. Jones, E.G. 1994. Santiago Ramon y Cajal and the Croonian lecture, March 1894. *Trends Neurosci.* **17:** 190–192.
72. Ramon y Cajal, S. 1894. Croonian lecture: la fine structure des centres nerveux. *Proc. R. Soc. Lond.* **44:** 444–468.
73. Harris, K.M. & S. Kater. 1994. Dendritic spines: cellular specializations imparting both stability and flexibility to synaptic function. *Annu. Rev. Neurosci.* **17:** 341–371.
74. Yuste, R. & T. Bonhoeffer. 2001. Morphological changes in dendritic spines associated with long-term synaptic plasticity. *Annu. Rev. Neurosci.* **24:** 1071–1089.
75. Hebb, D.O. 1949. *The Organization of Behavior: A Neuropsychological Theory*. Wiley.
76. Shatz, C.J. 1992. The developing brain. *Sci. Am.* **267:** 60–67.
77. Kandel, E.R. & L. Tauc. 1965. Heterosynaptic facilitation in neurones of the abdominal ganglion of *Aplysia depilans*. *J. Physiol.* **181:** 1–27.
78. Dan, Y. & M.-M. Poo. 2004. Spike timing-dependent plasticity of neural circuits. *Neuron* **44:** 23–30.
79. Harvey, C.D. & K. Svoboda. 2007. Locally dynamic synaptic learning rules in pyramidal neuron dendrites. *Nature* **450:** 1195–200.
80. Magee, J.C. & D. Johnston. 1997. A synaptically controlled, associative signal for Hebbian plasticity in hippocampal neurons. *Science* **275:** 209–213.
81. Zhang, L.I., H.W. Tao, C.E. Holt, *et al.* 1998. A critical window for cooperation and competition among developing retinotectal synapses. *Nature* **395:** 37–44.
82. Zucker, R.S. & W.G. Regehr. 2002. Short-term synaptic plasticity. *Annu. Rev. Physiol.* **64:** 355–405.
83. Malenka, R.C. & M.F. Bear. 2004. LTP and LTD: an embarrassment of riches. *Neuron* **44:** 5–21.

84. Malinow, R. & R.C. Malenka. 2002. AMPA receptor trafficking and synaptic plasticity. *Annu. Rev. Neurosci.* **25:** 103–126.
85. Martin, S.J., P.D. Grimwood & R.G. Morris. 2000. Synaptic plasticity and memory: an evaluation of the hypothesis. *Annu. Rev. Neurosci.* **23:** 649–711.
86. Kandel, E.R. 2001. The molecular biology of memory storage: a dialogue between genes and synapses. *Science* **294:** 1030–1038.
87. Gallistel, C.R. & L.D. Matzel. 2013. The neuroscience of learning: beyond the Hebbian synapse. *Annu. Rev. Psychol.* **64:** 169–200.
88. Dobrunz, L.E. & C.F. Stevens. 1999. Response of hippocampal synapses to natural stimulation patterns. *Neuron* **22:** 157–166.
89. Stevens, C.F. & T. Tsujimoto. 1995. Estimates for the pool size of releasable quanta at a single central synapse and for the time required to refill the pool. *Proc. Natl. Acad. Sci. U.S.A.* **92:** 846–849.
90. Lu, W.-Y. *et al.* 2001. Activation of synaptic NMDA receptors induces membrane insertion of new AMPA receptors and LTP in cultured hippocampal neurons. *Neuron* **29:** 243–254.
91. Ju, W. *et al.* 2004. Activity-dependent regulation of dendritic synthesis and trafficking of AMPA receptors. *Nat. Neurosci.* **7:** 244–53.
92. Shi, S.-H., Y. Hayashi, J.A. Esteban & R. Malinow. 2001. Subunit-specific rules governing AMPA receptor trafficking to synapses in hippocampal pyramidal neurons. *Cell* **105:** 331–343.
93. Hayashi, Y. *et al.* 2000. Driving AMPA receptors into synapses by LTP and CaMKII: requirement for GluR1 and PDZ domain interaction. *Science* **287:** 2262–2267.
94. Matsuzaki, M., N. Honkura, G.C.R. Ellis-Davies & H. Kasai. 2004. Structural basis of long-term potentiation in single dendritic spines. *Nature* **429:** 761–766.
95. Derkach, V., A. Barria & T.R. Soderling. 1999. Ca^{2+}/calmodulin-kinase II enhances channel conductance of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate type glutamate receptors. *Proc. Natl. Acad. Sci. U.S.A.* **96:** 3269–3274.
96. Benke, T.A., A. Lüthi, J.T. Isaac & G.L. Collingridge. 1998. Modulation of AMPA receptor unitary conductance by synaptic activity. *Nature* **393:** 793–797.
97. Choi, S., J. Klingauf & R.W. Tsien. 2000. Postfusional regulation of cleft glutamate concentration during LTP at 'silent synapses'. *Nat. Neurosci.* **3:** 330–336.
98. Emptage, N.J., C.A. Reid, A. Fine & T.V.P. Bliss. 2003. Optical quantal analysis reveals a presynaptic component of LTP at hippocampal Schaffer-associational synapses. *Neuron* **38:** 797–804.
99. Zakharenko, S.S., L. Zablow & S.A. Siegelbaum. 2001. Visualization of changes in presynaptic function during long-term synaptic plasticity. *Nat. Neurosci.* **4:** 711–717.
100. Moser, M.B., M. Trommald & P. Andersen. 1994. An increase in dendritic spine density on hippocampal CA1 pyramidal cells following spatial learning in adult rats suggests the formation of new synapses. *Proc. Natl. Acad. Sci. U.S.A.* **91:** 12673–12675.
101. Black, J.E., K.R. Isaacs, B.J. Anderson, *et al.* 1990. Learning causes synaptogenesis, whereas motor activity causes angiogenesis, in cerebellar cortex of adult rats. *Proc. Natl. Acad. Sci. U.S.A.* **87:** 5568–5572.
102. Malenka, R.C. & R.A. Nicoll. 1997. Silent synapses speak up. *Neuron* **19:** 473–476.
103. Poncer, J.C. & R. Malinow. 2001. Postsynaptic conversion of silent synapses during LTP affects synaptic gain and transmission dynamics. *Nat. Neurosci.* **4:** 989–996.
104. Kerchner, G.A. & R.A. Nicoll. 2008. Silent synapses and the emergence of a postsynaptic mechanism for LTP. *Nat. Rev. Neurosci.* **9:** 813–825.
105. Bliss, T.V.P. & T. Lomo. 1973. Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J. Physiol.* **232:** 331–356.
106. Dudek, S.M. & M.F. Bear. 1993. Bidirectional long-term modification of synaptic effectiveness in the adult and immature hippocampus. *J. Neurosci.* **13:** 2910–2918.
107. Rioult-Pedotti, M.S., D. Friedman & J.P. Donoghue. 2000. Learning-induced LTP in neocortex. *Science* **290:** 533–536.
108. Whitlock, J.R., A.J. Heynen, M.G. Shuler & M.F. Bear. 2006. Learning induces long-term potentiation in the hippocampus. *Science* **313:** 1093–1097.
109. Rogan, M.T., U.V. Stäubli & J.E. LeDoux. 1997. Fear conditioning induces associative long-term potentiation in the amygdala. *Nature* **390:** 604–607.
110. Clem, R.L. & R.L. Huganir. 2010. Calcium-permeable AMPA receptor dynamics mediate fear memory erasure. *Science* **330:** 1108–1112.
111. Flexner, J.B., L.B. Flexner & E. Stellar. 1963. Memory in mice as affected by intracerebral puromycin. *Science* **141:** 57–59.
112. Flexner, L.B., J.B. Flexner & R.B. Roberts. 1967. Memory in mice analyzed with antibiotics. *Science* **155:** 1377–1383.
113. Davis, H.P. & L.R. Squire. 1984. Protein synthesis and memory: a review. *Psychol. Bull.* **96:** 518–559.
114. Sutton, M.A. & E.M. Schuman. 2006. Dendritic protein synthesis, synaptic plasticity, and memory. *Cell* **127:** 49–58.
115. Selkoe, D.J. 2002. Alzheimer's disease is a synaptic failure. *Science* **298:** 789–791.
116. Nabavi, S. *et al.* 2014. Engineering a memory with LTD and LTP. *Nature* **511:** 348–352.
117. Chen, S. *et al.* 2014. Reinstatement of long-term memory following erasure of its behavioral and synaptic expression in *Aplysia*. *Elife* **3:** e03896.
118. Jacobson, R. 2015. Memories may not live in neurons' synapses. *Sci. Am.* **312:** 14–16.
119. Monteiro, P. & G. Feng. 2017. SHANK proteins: roles at the synapse and in autism spectrum disorder. *Nat. Rev. Neurosci.* **18:** 147–157.
120. Sheng, M., B.L. Sabatini & T.C. Sudhof. 2015. Synapses and Alzheimer's disease. *Cold Spring Harb. Perspect. Biol.* **4:** 1–18.
121. DeKosky, S.T. & S.W. Scheff. 1990. Synapse loss in frontal cortex biopsies in Alzheimer's disease: correlation with cognitive severity. *Ann. Neurol.* **27:** 457–464.

122. Duman, R.S. & G.K. Aghajanian. 2012. Synaptic dysfunction in depression: potential therapeutic targets. *Science* **338:** 68–72.
123. Diering, G.H. *et al.* 2017. Homer1a drives homeostatic scaling-down of excitatory synapses during sleep. *Science* **355:** 511–515.
124. von der Ohe, C.G., C.C. Garner, C. Darian-Smith & H.C. Heller. 2007. Synaptic protein dynamics in hibernation. *J. Neurosci.* **27:** 84–92.
125. Huganir, R.L. & R.A. Nicoll. 2013. AMPARs and synaptic plasticity: the last 25 years. *Neuron* **80:** 704–717.
126. Turrigiano, G.G. 2008. The self-tuning neuron: synaptic scaling of excitatory synapses. *Cell* **135:** 422–435.
127. Davis, G.W. 2006. Homeostatic control of neural activity: from phenomenology to molecular design. *Annu. Rev. Neurosci.* **29:** 307–323.
128. Abraham, W.C. 2008. Metaplasticity: tuning synapses and networks for plasticity. *Nat. Rev. Neurosci.* **9:** 387.
129. Ramaswami, M. 2014. Network plasticity in adaptive filtering and behavioral habituation. *Neuron* **82:** 1216–1229.
130. Letzkus, J.J., S.B.E. Wolff & A. Lüthi. 2015. Disinhibition, a circuit mechanism for associative learning and memory. *Neuron* **88:** 264–276.
131. Mayford, M., S.A. Siegelbaum & E.R. Kandel. 2016. Synapses and memory storage. *Cold Spring Harb. Perspect. Biol.* **4:** 1–18.
132. Lee, H.-K. *et al.* 2003. Phosphorylation of the AMPA receptor GluR1 subunit is required for synaptic plasticity and retention of spatial memory. *Cell* **112:** 631–643.
133. Lee, H.K., M. Barbarosie, K. Kameyama, *et al.* 2000. Regulation of distinct AMPA receptor phosphorylation sites during bidirectional synaptic plasticity. *Nature* **405:** 955–959.
134. Barria, A., D. Muller, V. Derkach, *et al.* 1997. Regulatory phosphorylation of AMPA-type glutamate receptors by CaM-KII during long-term potentiation. *Science* **276:** 2042–2045.
135. Toyama, B.H. *et al.* 2013. Identification of long-lived proteins reveals exceptional stability of essential cellular structures. *Cell* **154:** 971–982.
136. Day, J.J. & J.D. Sweatt. 2010. DNA methylation and memory formation. *Nat. Neurosci.* **13:** 1319–1323.
137. Marshall, P. & T.W. Bredy. 2016. Cognitive neuroepigenetics: the next evolution in our understanding of the molecular mechanisms underlying learning and memory? *NPJ Sci. Learn.* **1:** 16014.
138. Gallistel, C.R. & P.D. Balsam. 2014. Time to rethink the neural mechanisms of learning and memory. *Neurobiol. Learn. Mem.* **108:** 136–144.
139. Petersen, C.C.H., R.C. Malenka, R.A. Nicoll & J.J. Hopfield. 1998. All-or-none potentiation at CA3–CA1 synapses. *Proc. Natl. Acad. Sci. U.S.A.* **95:** 4732–4737.
140. O'Connor, D.H., G.M. Wittenberg & S.S.-H. Wang. 2005. Graded bidirectional synaptic plasticity is composed of switch-like unitary events. *Proc. Natl. Acad. Sci. U.S.A.* **102:** 9679–9684.
141. Poo, M. *et al.* 2016. What is memory? The present state of the engram. *BMC Biol.* **14:** 40.
142. Tsien, R.Y. 2013. Very long-term memories may be stored in the pattern of holes in the perineuronal net. *Proc. Natl. Acad. Sci. U.S.A.* **110:** 12456–12461.
143. Toyama, B.H. & M.W. Hetzer. 2012. Protein homeostasis: live long, won't prosper. *Nat. Rev. Mol. Cell Biol.* **14:** 55–61.
144. Cambridge, S.B. *et al.* 2011. Systems-wide proteomic analysis in mammalian cells reveals conserved, functional protein turnover. *J. Proteome Res.* **10:** 5275–5284.
145. Price, J.C., S. Guan, A. Burlingame, *et al.* 2014. Analysis of proteome dynamics in the mouse brain. *Proc. Natl. Acad. Sci. U.S.A.* **111:** 3644–3644.
146. Cohen, L.D. *et al.* 2013. Metabolic turnover of synaptic proteins: kinetics, interdependencies and implications for synaptic maintenance. *PLoS One* **8:** e63191.
147. Kim, C.H. & J.E. Lisman. 1999. A role of actin filament in synaptic transmission and long-term potentiation. *J. Neurosci.* **19:** 4314–4324.
148. Matus, A. 2000. Actin-based plasticity in dendritic spines. *Science* **290:** 754–758.
149. Sheng, M. & E. Kim. 2011. The postsynaptic organization of synapses. *CSH Perspect. Biol.* **3:** a005678.
150. Nithianantharajah, J. *et al.* 2013. Synaptic scaffold evolution generated components of vertebrate cognitive complexity. *Nat. Neurosci.* **16:** 16–24.
151. Migaud, M. *et al.* 1998. Enhanced long-term potentiation and impaired learning in mice with mutant postsynaptic density-95 protein. *Nature* **396:** 433–439.
152. Kim, E. & M. Sheng. 2004. PDZ domain proteins of synapses. *Nat. Rev. Neurosci.* **5:** 771–781.
153. Hameroff, S. & R. Penrose. 1996. Orchestrated objective reduction of quantum coherence in brain microtubules—a model for consciousness? In *Toward a Science of Consciousness: The First Tucson Discussions and Debates.* S.R. Hameroff, A.W. Kazniak & A.C. Scott, Eds.: 507–540. Cambridge, MA: The MIT Press.
154. Dent, E.W. & P.W. Baas. 2014. Microtubules in neurons as information carriers. *J. Neurochem.* **129:** 235–239.
155. Brown, J.A. & J.A. Tuszyn. 1997. Dipole interactions in axonal microtubules as a mechanism of signal propagation. *Phys. Rev. E* **56:** 5834–5840.
156. Hu, X., C. Viesselmann, S. Nam, *et al.* 2008. Activity-dependent dynamic microtubule invasion of dendritic spines. *J. Neurosci.* **28:** 13094–13105.
157. Merriam, E.B. *et al.* 2011. Dynamic microtubules promote synaptic NMDA receptor-dependent spine enlargement. *PLoS One* **6:** e27688.
158. Jaworski, J. *et al.* 2009. Dynamic microtubules regulate dendritic spine morphology and synaptic plasticity. *Neuron* **61:** 85–100.
159. Sahu, S., S. Ghosh, K. Hirata, *et al.* 2013. Multi-level memory-switching properties of a single brain microtubule. *Appl. Phys. Lett.* **102:** 123701.
160. Craddock, T.J.A., J.A. Tuszynski & S. Hameroff. 2012. Cytoskeletal signaling: is memory encoded in microtubule lattices by CaMKII phosphorylation? *PLoS Comput. Biol.* **8:** e1002421.
161. Coles, C.H. & F. Bradke. 2015. Coordinating neuronal actin–microtubule dynamics. *Curr. Biol.* **25:** R677–R691.

162. Martel, G. *et al.* 2016. Genetic demonstration of a role for stathmin in adult hippocampal neurogenesis, spinogenesis, and NMDA receptor-dependent memory. *J. Neurosci.* **36:** 1185–1202.
163. Kim, C.-H. & J.E. Lisman. 2001. A labile component of AMPA receptor-mediated synaptic transmission is dependent on microtubule motors, actin, and N-ethylmaleimide-sensitive factor. *J. Neurosci.* **21:** 4188–4194.
164. Routtenberg, A. & J.L. Rekart. 2005. Post-translational protein modification as the substrate for long-lasting memory. *Trends Neurosci.* **28:** 12–19.
165. Eroglu, C. & B.A. Barres. 2010. Regulation of synaptic connectivity by glia. *Nature* **468:** 223–231.
166. Göppert-Mayer, M. 1931. Über Elementarakte mit zwei Quantensprüngen. *Ann. Phys.* **401:** 273–294.
167. Denk, W. & K. Svoboda. 1997. Photon upmanship: why multiphoton imaging is more than a gimmick. *Neuron* **18:** 351–357.
168. Balsam, P.D., M.R. Drew & C.R. Gallistel. 2010. Time and associative learning. *Comp. Cogn. Behav. Rev.* **5:** 1–22.
169. Gibbon, J., C. Malapani, C.L. Dale & C. Gallistel. 1997. Toward a neurobiology of temporal cognition: advances and challenges. *Curr. Opin. Neurobiol.* **7:** 170–184.
170. Hesslow, G. 1994. Inhibition of classically conditioned eyeblink responses by stimulation of the cerebellar cortex in the decerebrate cat. *J. Physiol.* **476:** 245–256.
171. Hesslow, G. 1994. Correspondence between climbing input and motor output in eyeblink-related areas in cat cerebellar cortex. *J. Physiol.* **476:** 229–244.
172. Jirenhed, D.-A. & G. Hesslow. 2011. Time course of classically conditioned Purkinje cell response is determined by initial part of conditioned stimulus. *J. Neurosci.* **31:** 9070–9074.
173. Rasmussen, A., R. Zucca, F. Johansson, *et al.* 2015. Purkinje cell activity during classical conditioning with different conditional stimulus explains central tenet of Rescorla–Wagner model. *Proc. Natl. Acad. Sci. U.S.A.* **112:** 14060–14065.
174. Johansson, F., D. Jirenhed, A. Rasmussen, *et al.* 2014. Memory trace and timing mechanism localized to cerebellar Purkinje cells. *Proc. Natl. Acad. Sci. U.S.A.* **111:** 14930–14934.
175. Johansson, F., H.A.E. Carlsson, A. Rasmussen, *et al.* 2015. Activation of a temporal memory in Purkinje cells by the mGluR7 receptor. *Cell Rep.* **13:** 1741–1746.
176. Sterling, P. & S. Laughlin. 2015. *Principles of Neural Design.* The MIT Press.
177. Cooke, S.F., R.W. Komorowski, E.S. Kaplan, *et al.* 2015. Visual recognition memory, manifested as long-term habituation, requires synaptic plasticity in V1. *Nat. Neurosci.* **18:** 262–271.
178. Kass, M.D., M.C. Rosenthal, J. Pottackal & J.P. McGann. 2013. Fear learning enhances neural responses to threat-predictive sensory stimuli. *Science* **342:** 1389–1392.
179. Orefice, L.L.L. *et al.*2016. Peripheral mechanosensory neuron dysfunction underlies tactile and behavioral deficits in mouse models of ASDs. *Cell* **166:** 299–314.
180. McGaugh, J.L. 1966. Time-dependent processes in memory storage. *Science* **153:** 1351–1358.
181. Frankland, P.W. & B. Bontempi. 2005. The organization of recent and remote memories. *Nat. Rev. Neurosci.* **6:** 119–130.
182. Packard, M.G. & B.J. Knowlton. 2002. Learning and memory functions of the basal ganglia. *Annu. Rev. Neurosci.* **25:** 563–593.
183. Kitamura, T., S.K. Ogawa, D.S. Roy & T. Okuyama. 2017. Engrams and circuits crucial for systems consolidation of a memory. **78:** 73–78.
184. Lesburgueres, E. *et al.* 2011. Early tagging of cortical networks is required for the formation of enduring associative memory. *Science* **331:** 924–928.
185. Goshen, I. *et al.* 2011. Dynamics of retrieval strategies for remote memories. *Cell* **147:** 678–689.
186. Bussey, T.J. & L.M. Saksida. 2007. Memory, perception, and the ventral visual-perirhinal-hippocampal stream: thinking outside of the boxes. *Hippocampus* **17:** 898–908.
187. Bassett, D.S. *et al.* 2010. Dynamic reconfiguration of human brain networks during learning. *Learning* **108:** 7641–7646.
188. Ryan, T.J. & S.G.N. Grant. 2009. The origin and evolution of synapses. *Nat. Rev. Neurosci.* **10:** 701–712.
189. Dennett, D.C. 2017. *From Bacteria to Bach and Back: The Evolution of Minds.* W.W. Norton & Company.
190. Penrose, R. & M. Gardner. 1989. *The Emperor's New Mind: Concerning Computers, Minds, and the Laws of Physics.* Oxford University Press.
191. Kuhn, T.S. 1962. *The Structure of Scientific Revolutions.* University of Chicago Press.
192. Chomsky, N. 2014. Science, mind, and limits of understanding. In Science, Theology and the Ontological Quest Foundation, The Vatican. Available at: https://chomsky.info/201401__/. Accessed 30 January 2017.
193. Glasser, M.F. *et al.* 2016. A multi-modal parcellation of human cerebral cortex. *Nature* **536:** 171–178.
194. Roth, B.L. 2016. DREADDs for Neuroscientists. *Neuron* **89:** 683–694.
195. O'Keefe, J. 1979. A review of the hippocampal place cells. *Prog. Neurobiol.* **13:** 419–439.
196. Moser, M.-B., D.C. Rowland & E.I. Moser2015. Place cells, grid cells, and memory. *Cold Spring Harb. Perspect. Biol.* **7:** a021808.
197. Zhang, K. 1996. Representation of spatial orientation by the intrinsic dynamics of the head-direction cell ensemble: a theory. *J. Neurosci.* **16:** 2112–2126.
198. Barry, C. *et al.* 2006. The boundary vector cell model of place cell firing and spatial memory. *Rev. Neurosci.* **17:** 71–97.
199. Eichenbaum, H. 2014. Time cells in the hippocampus: a new dimension for mapping memories. *Nat. Rev. Neurosci.* **15:** 732–744.
200. Velliste, M., S. Perel, M.C. Spalding, *et al.* 2008. Cortical control of a prosthetic arm for self-feeding. *Nature* **453:** 1098–1101.
201. Donoghue, J.P. 2002. Connecting cortex to machines: recent advances in brain interfaces. *Nat. Neurosci.* **5:** 1085–1088.
202. Hochberg, L.R. *et al.* 2006. Neuronal ensemble control of prosthetic devices by a human with tetraplegia. *Nature* **442:** 164–171.

203. Marmor, M.F. & L.J. Martin. 1978. 100 years of the visual cycle. *Surv. Ophthalmol.* **22:** 279–285.
204. Hartley, R.V.L. 1928. Transmission of information. *Bell Syst. Tech. J.* **7:** 535–563.
205. Shannon, C.E. 1948. A mathematical theory of communication. *Bell Syst. Tech. J.* **27:** 379–423.
206. Gleick, J. 2011. *The Information: A History, A Theory, A Flood.* Vintage Books.