

Preface

OVER THE LAST DECADE, the neurobiological study of learning and memory has progressed dramatically, fueled in part by the availability of new technologies such as gene knockouts, multi-unit recordings, human and animal brain imaging, and optogenetics. These biological methods have been supplemented by behavioral insights about the reliability and stability of memory. For example, the reconsolidation model of memory suggests that recall itself may evoke or reinstate an active neuronal plasticity process that is required for memory maintenance. Human psychological studies have highlighted that false memories can develop quite easily—a finding that has important societal implications.

With these advances in mind, Cold Spring Harbor Laboratory Press has invited us to bring together an updated review of the field, which we have attempted to do in the following 19 chapters, written by many of the leaders in the field. We have asked our authors to cover the four major areas of the field outlined below:

- encoding of experience-dependent information
- consolidation and off-line transformation of information
- maintenance, reconsolidation, and retrieval
- novel approaches to the study of plasticity and memory

For readers outside the field it might be useful to place the study of learning and memory into a broader context.

Learning and memory are two of the most magical capabilities of our mind. Learning is the biological process of acquiring new knowledge about the world, and memory is the process of retaining and reconstructing that knowledge over time. Most of our knowledge of the world and most of our skills are not innate, but learned. Thus, we are who we are in large part because of what we have learned and what we remember and forget.

During the last four decades, neuroscience, the biological study of the brain, has established a common, broad conceptual framework that extends from cell and molecular biology, on the one hand, to brain systems biology, psychophysics, psychology, and neuronal modeling, on the other. Within this new, interdisciplinary structure, the scope of memory research ranges from genes to cognition, from molecules to mind.

Throughout this volume we will emphasize that memory storage is not the result of a linear sequence of events that culminates in an indelible, long-term memory. Rather, it is the dynamic outcome of several interactive processes: encoding or acquisition of new information, short-term memory, intermediate-term memory, consolidation of long-term memory, maintenance of long-term memory, and destabilization and restabilization of memory in the course of retrieving, updating, and integrating a given memory with other memories. We can see these dynamics at work in multiple levels of analysis and brain organization and in varying degrees, from simple to complex memory systems. These dynamics are initiated by molecular and cellular modifications at the level of individual synaptic connections and the neuronal cell bodies to which these synapses relate. These dynamics then extend to more distributed changes through multiple synaptic connections

of many neurons embedded in larger neuronal networks whose interactions are expressed at the behavioral level.

We have organized the book around these basic temporal components of memory. In Section I, we focus on information encoding or learning; in Section II, we focus on postlearning consolidation where the molecular and in some cases the anatomical nature of the memories change. In Section III, we cover the maintenance, stability, and retrieval of memories. Finally, in Section IV, we discuss several emerging technologies that we think will have a significant impact on future studies of memory. In each section we discuss studies in species ranging from invertebrates to human beings. This is an evolutionary perspective that is based on the finding that memory is implemented in and subserved by cellular and molecular plasticity processes that are conserved and that insights can be gained from studying these basic processes across species.

Even simple forms of reflex learning in invertebrates show encoding, consolidation, and retrieval of memory that are reflected in synaptic and cell-wide plastic changes that control the behavior. In higher organisms there is an increase in circuit complexity of several orders of magnitude. With this increased complexity we see increased anatomical segregation of sensory processing streams and functional specialization of brain regions. Nevertheless, mammalian neurons show synaptic plasticity with some molecular similarities to invertebrates, as well as some differences. At the behavioral level mammals show the same basic, generic processes of encoding, consolidation, and retrieval. The added circuit complexity of the mammalian brain allows for greater diversity in behavior but also makes a full description of the information processing from sensory input to motor output much more difficult.

This can be seen throughout in the approaches applied and the nature of the questions asked in the various systems. In human beings, our knowledge necessarily comes primarily from noninvasive, functional imaging techniques and from study of patients who have damage to specific brain areas that affect memory. Our molecular understanding of memory in human beings is thus quite limited, but with the advent of functional magnetic resonance imaging (fMRI), our knowledge of the anatomy and of activity patterns is sometimes quite detailed. In mice and rats, the availability of genetic and pharmacological tools has allowed a detailed dissection of molecular signaling events important in learning and memory. However, the complexity of the mammalian brain has made mapping this knowledge onto the precise neurons and circuits that control behavior and its modification difficult, although new approaches offer some promise in this area. Finally, in simple systems like *Aplysia*, a deep understanding of the molecular mechanisms operating in neurons to modify behavior can be obtained and mapped directly onto the circuits that control the behavior. By integrating studies from multiple systems and varied approaches, we hope to convey some general principles and theoretical frameworks that operate in the field that can be useful in whatever level of analysis or species most captures the reader's interest.

SECTION I: ENCODING

In this section we cover the principles of encoding, the initial modification of behavior with experience. We begin with an examination of circuit complexity, anatomical specialization, and memory subtypes initially identified in humans. Squire and Zola-Morgan discuss the two major memory systems postulated for the mammalian brain: (1) explicit (also called declarative) memory, for facts and events, people, places, and objects; and (2) implicit (also called nondeclarative) memory, for perceptual and motor skills. The critical neurobiological insight for this distinction came from studies of patient H.M. (Scoville and Milner 1957), which revealed that the major aspects of explicit memory require the hippocampus and adjacent cortex—and in humans normally involve conscious awareness—whereas implicit memory does not require conscious awareness and relies mostly on other brain systems: namely, the cerebellum, the striatum, the amygdala, and simple reflex pathways themselves. We discuss these memory subsystems, and some more recent developments in their conceptualization, in subsequent chapters of this section.

Although it was accepted by the early 1970s that there are two major types of memory, little was known about how either type is formed. We could not distinguish experimentally, for example, between two leading—and conflicting—approaches to the mechanisms of memory storage: the aggregate field approach advocated by Lashley in the 1950s and by Adey in the 1960s, which assumed that information is stored in the bioelectric field generated by the aggregate activity of many neurons; and the cellular connectionist approach, which derived from Cajal's idea that memory is stored as an anatomical change in the strength of synaptic connections (Cajal 1894). In 1948, Konorski (1948) renamed Cajal's idea synaptic plasticity (the ability of neurons to modulate the strength of their synapses as a result of use).

To distinguish between these disparate neurobiological approaches to memory storage it soon became clear that one needed to develop tractable behavioral systems. Such systems would make it more likely to see how specific changes in the neuronal components of a behavior cause modifications of that behavior during learning and memory storage. From 1964 to 1979 several simple model systems of implicit memory emerged: the flexion reflex of cats, the eye-blink response of rabbits, and a variety of simple forms of reflex learning in invertebrates—namely, the defensive gill-withdrawal reflex of *Aplysia*, olfactory learning in *Drosophila*, the escape reflex of *Tritonia*, and various behavioral modifications in *Hermisenda*, *Pleurobranchaea*, *Limax*, crayfish, and honeybees (see Kandel 1976 for more detailed discussion).

Hawkins and Byrne, in the second and fifth chapters, discuss the insights that emerged from this reductionist approach in *Aplysia*. The first was purely behavioral and revealed that even animals with relatively few nerve cells, approximately 20,000 in the central nervous system of *Aplysia*, have remarkable learning capabilities. This simple nervous system can give rise to a variety of elementary forms of learning: habituation, dishabituation, sensitization, classical conditioning, and operant conditioning. Each form of learning, in turn, gives rise to short- or long-term memory.

Single-trial learning and the formation of short-term memory in the gill and siphon-withdrawal reflex of *Aplysia* result in part from changes in the strength of a sensorimotor synapse that mediates a major component of the reflex behavior. This synaptic plasticity is due to the modulation of the release of chemical transmitters from the presynaptic sensory neuron. A decrease in the amount of transmitter released was found to be associated with short-term habituation, whereas an increase was associated with short-term dishabituation and sensitization.

Further studies of the synaptic connections between the sensory and motor neurons that control the withdrawal reflex in *Aplysia* revealed that a single sensitizing stimulus to the tail increases the strength of the synaptic connections between the sensory and motor neurons by activating modulatory neurons that release serotonin onto the sensory neuron. Serotonin, in turn, increases the concentration of cyclic adenosine monophosphate (cAMP) in the sensory cell. The cAMP molecules activate the cAMP-dependent protein kinase (PKA), which signals the sensory neuron to release more of the transmitter glutamate into the synaptic cleft, thus temporarily strengthening the connection between the sensory and motor neuron. In fact, simply injecting cAMP or PKA directly into the sensory neuron produces temporary strengthening of the sensorimotor connection.

Next, Hawkins and his colleagues (1983) and Walters and Byrne (1983) succeeded in producing classical conditioning of *Aplysia*'s withdrawal reflex and began to analyze the mechanisms underlying this form of learning. Paired training, in which the conditioned stimulus (stimulation of the siphon) is applied just before the unconditioned stimulus (a shock to the tail), produces a greater increase in the gill-withdrawal reflex than either stimulus alone or unpaired stimuli. This is in part because the firing of an action potential by the sensory neuron just before the tail shock causes an influx of Ca^{2+} into the sensory neuron that enhances the response of the adenylyl cyclase to serotonin. This, in turn, increases the amount of cAMP in the sensory neuron, which causes greater facilitation of the synaptic connection between sensory and motor neurons, an action also known as activity-dependent enhancement of synaptic facilitation. These studies support a synaptic plasticity model for memory encoding and describe some of the molecular underpinnings of this plasticity, thereby

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providing a conceptual and experimental framework for subsequent studies of encoding in mammalian systems, some of which have revealed similar mechanisms (Yagishita et al. 2014; Johansen et al. 2014).

In the third chapter, De Zeeuw and Ten Brinke examine eye-blink conditioning, a form of implicit memory in the mammalian brain. This is produced by pairing a tone (the conditioned stimulus [CS]) with an aversive air puff to the eye (the unconditioned stimulus [US]), resulting in a learned eye blink response to the CS alone (Thompson et al. 1983). The circuit mechanisms underlying this form of learning are one of the best characterized in a mammalian system. Theoretical and experimental studies suggest that before learning, activation of cerebellar Purkinje neurons in response to the CS leads to an inhibition of neurons in the interpositus nucleus (one of the deep nuclei of the cerebellum), thereby inhibiting motor output. With conditioning there is a decrease in the activity of the Purkinje cell in response to the CS, resulting in disinhibition of the neurons of the interpositus nucleus, leading to an eye blink. This model is consistent with findings that Purkinje cell activity can be reduced as a result of long-term depression (LTD), a form of synaptic plasticity, at the parallel fiber excitatory synaptic input onto the Purkinje neurons (Ito 2001). This decrease in the strength of the parallel fibers occurs when the climbing fiber inputs to the cerebellum are activated in appropriate temporal proximity to parallel fiber activity. The Purkinje cells become less responsive to input, as a result of a down-regulation of AMPA receptors at the parallel fiber to Purkinje cell synapse.

In the fourth chapter, Graybiel and Grafton examine the basal ganglia, a group of subcortical nuclei that includes the striatum, which are important in the implicit learning of skills and habits. The ventral striatum contains dopaminergic circuitry that is a critical component of reward and is a major target for drugs of abuse. The dorsal striatum, which is the primary focus of this chapter, is involved in the control of movement and skill and habit learning. These are primarily stereotyped motor programs that may begin as explicit memories but eventually progress to unconscious skills. For example, a child learns to tie her shoes in an explicit manner following specific instructions, but over many repetitions this common task becomes an implicit skill or habit involving the striatum. This progression can be seen in the activity pattern of striatal neurons that bracket the habitual motor behavior, firing before and after completion, suggesting a signal for the initiation and successful completion of the task. This principal can be extended to the individual steps of a complex motor task as well as to higher cognitive functions in the process of chunking. In chunking, the task is broken up into smaller motor programs with bracketing neural activity signaling the initiation of the appropriate behavioral chunk and feedback reward activity indicating the degree of successful completion of the behavior.

In the sixth chapter, Fanselow and Wassum discuss the mechanisms of Pavlovian conditioning in vertebrates, focusing primarily on fear conditioning. As in invertebrates, Pavlovian fear conditioning is also a behavioral model widely used in rodent experiments where it is considered to have both implicit and explicit forms (see also LeDoux 2015). When an animal is presented with a tone that is followed by a shock to the foot, the animal exhibits a learned fear response that can be gauged by freezing (a natural fear behavior) in response to the tone alone. This is an implicit form of learning that involves the amygdala but not the hippocampus. However, animals also develop a fear response to the test chamber in which they received the shocks (the context). This contextual learning is dependent on the hippocampus and is a model of explicit learning accessible in rodents.

This important paradigm in mammalian learning and memory research offers the richness in behavioral features of Pavlovian conditioning (e.g., blocking, interference, second-order conditioning). It has been well studied at the anatomical and circuit level and provides some of the strongest evidence linking synaptic plasticity and learning in a mammalian system. Finally, the contextual version of the task is one of the very few hippocampus-dependent tasks in rodents that, in some cases, shows the temporal gradient of retrograde amnesia that was reported in studies of human hippocampal patients.

The hippocampus was also the brain area where a form of synaptic plasticity thought to be important for memory, long-term potentiation (LTP), was first described by Lomo (1966) and more extensively by Bliss and Lomo (1973). They found that high-frequency electrical stimulation of the perforant path input to the hippocampus resulted in an increase in the strength of the stimulated synapses that lasted for many days. Subsequent studies found that some forms of LTP displayed the elementary properties of associability and specificity formulated by Hebb (1949), according to which (a) only synapses that are active when the postsynaptic cell is strongly depolarized are potentiated and (b) inactive synapses were not potentiated. Thus, groups of synapses that are coordinately active and contribute together to the firing of the target postsynaptic neuron will be strengthened, providing a plausible mechanism for linking ensembles of neurons encoding different environmental features that are presented together and thereby forming memory associations.

These properties of LTP have made it the focus of intense interest as a model synaptic mechanism for memory encoding in mammals. Angelakos and Able discuss the use of genetically modified mice in neurobiology with a focus on attempts to test the role of LTP in learning and memory. Then Basu and Siegelbaum explore the molecular signaling and cellular mechanisms that underlie LTP and the complementary form of plasticity, LTD, as well as the studies of their role in learning and memory.

The mechanism for initial induction of LTP varies in different regions of the hippocampus and in the same region with different patterns of stimulation. In the CA1 region, 100-Hz stimulation induces a form of LTP that is dependent on *N*-methyl-D-aspartate (NMDA) receptor activation. Moreover the properties of this receptor were proposed to explain the associative and activity-dependent properties of LTP. NMDA receptors are both voltage- and ligand-gated, and, to become active, they require both depolarization of the postsynaptic membrane, in which they reside, and concurrent release of glutamate from the presynaptic terminal. Activated NMDA receptors produce a strong Ca^{2+} influx into the postsynaptic cell that is required to induce LTP. This Ca^{2+} signal can activate a wide range of signaling pathways in the postsynaptic cell including calcium/calmodulin protein kinase II (CaMKII), protein kinase C (PKC), PKA, and mitogen-activated protein kinase (MAPK), each of which has been implicated in the induction of LTP as well as in its later stabilization.

Attempts to understand the role of LTP in memory storage have focused on genetic and pharmacological interventions designed to block LTP and consequent examination of the impact of this LTP blockade on memory formation. Many of these studies have used pharmacological or genetic blockade of the NMDA receptors required for LTP initiation or of other molecular signaling components in the LTP pathway. The results of these studies have been inconclusive. Some studies show learning deficiencies in animals lacking LTP and others do not. These results highlight some of the difficulties in mammalian studies that attempt to relate synaptic plasticity to behavior. First, LTP is not a unitary phenomenon and its natural induction is not well understood so that in some cases of apparent inhibition of LTP, other behaviorally relevant forms of LTP may still be induced. Also, many of the studies have focused on one brain region, usually the hippocampus, and it is possible that other brain circuits may compensate when LTP in the hippocampus is impaired or that animals may solve the behavioral tasks in a hippocampus-independent manner. These are difficulties inherent in the study of complex behaviors in the absence of a complete understanding of the underlying processing circuits.

SECTION II: CONSOLIDATION

Over the years, the term “memory consolidation” has been used to define two different yet inter-related processes (Dudai and Morris 2000). Synaptic, cellular, or immediate consolidation refers to the gene expression–dependent transformation of information into a long-term form in the neural circuit that encodes the memory. Systems consolidation refers to a slower postencoding reorganization of long-term memory over distributed brain circuits into remote memory lasting months to years, and it is commonly studied within the context of the corticohippocampal system that subserves explicit memory.

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In the first chapter in this section, Alberini and Kandel examine the mechanisms of cellular and synaptic consolidation. The idea of a cellular memory consolidation process derives from the observation that inhibitors of protein synthesis, when given during or shortly after initial learning, do not disrupt short-term memory at 1 hour, but impair 24-hour memory. These and subsequent studies suggest that a transcriptional/translational program, initiated with learning, is required for the stabilization or consolidation of memories at later time points. This chapter focuses on a substantial body of work characterizing the molecular switch from short-term to long-term memory. A canonical pathway found in both *Aplysia* and mammals involves the activation of the transcription factor cAMP response element-binding protein 1 (CREB-1). The CREB-mediated response to external stimuli can be activated by a number of kinases (PKA, CaMKII, CaMKIV, RSK2, MAPK, and PKC) and phosphatases, which suggests that CREB integrates signals from these various pathways. The activation of CREB-dependent transcription in turn leads to the expression of a variety of downstream effector molecules that help to stabilize synaptic changes and memory.

More recent studies have uncovered a rich regulatory complexity in the control of this memory-related transcriptional program. In addition to positive factors such as CREB-1, there are inhibitory transcription factors such as CREB-2, which act as suppressors to oppose the formation of long-term memory. The transcription required for consolidation is also controlled by epigenetic factors. Regulated covalent modifications of histones and DNA exert a strong effect on this transcriptional cascade. Finally, small regulatory RNAs contribute the control of the transcription required for memory consolidation.

One of the consequences of the transcriptional signaling for long-term memory consolidation is the formation and stabilization of new synaptic connections, which is discussed by Bailey, Kandel, and Harris. The authors examine structural changes in the *Aplysia* sensorimotor synapse and in the mammalian hippocampus with synaptic plasticity and memory storage. Long-term sensitization training in *Aplysia* results in an increase in the number of anatomical synaptic connections between the sensory and motor synapses controlling the behavior. This form of structural plasticity requires the CREB-based transcriptional cascade for memory consolidation interacting with local changes in various cell surface and cytoskeletal molecules to stabilize the new synapses.

In rodents, similar types of structural plasticity have been observed in the hippocampus with learning and with the induction of LTP. The current inability to identify the precise neurons involved in a given learning task in the mammalian brain makes it difficult to study these effects with behavior; however, new *in vivo* imaging techniques that allow individual neurons to be followed longitudinally are facilitating behavioral studies. There are now a series of studies showing that activity-dependent synaptic plasticity (LTP) produces increases in synapse number and size. There are also important developmental components to this regulation. Taken together, these results suggest that physical changes in synaptic connections are a major contributor to the long lasting stabilization of memory.

Finally, Squire, Genzel, Wixted, and Morris deal with proposed mechanisms of systems consolidation. Systems consolidation is a concept originally used to explain the temporal gradient of amnesia that was reported following hippocampal damage in humans and some animal models. Although hippocampal damage in humans produces a retrograde amnesia for explicit memories that were acquired within a year or two before the injury, older memories are reported to stay intact. Thus, a new memory requires the hippocampus for retrieval but some process of consolidation allows older memories to be accessed independently of the hippocampus. The “standard model” for this process posits that shortly after learning the hippocampus is required to recruit distributed cortical elements of the memory into a coherent recall event, but over time persistent spontaneous activity, or replay, within the cortical elements strengthens their synaptic connectivity to allow the memory to be retrieved independently of the hippocampus. This model and other, competing models developed to explain this fascinating characteristic of hippocampal amnesia are discussed.

SECTION III: MAINTENANCE, RECONSOLIDATION, AND RETRIEVAL

In this section we cover the mechanisms of memory retrieval and its use in guiding behavior. Retrieval is critical in memory research, and in the current state of memory research, there is in fact no evidence of a memory's existence until it is retrieved. Retrieval is generally thought to involve the reactivation or reconstruction of components of the neural ensembles activated during the initial learning. In some behavioral paradigms it has become clear that retrieval is not a passive process but engages molecular signaling to maintain the memory in an active process called reconsolidation. The overall picture is that of a dynamic process involving circuit activity as well as molecular signaling to maintain stable memories.

In the first chapter in this section, Si and Kandel explore molecular mechanisms for the persistent maintenance of memory. As we have seen, the consolidation and stabilization of memories require a transcriptional and translational program initiated in the nucleus, but the actual physical substrate of memory is at the synapse. Because individual neurons can have thousands of individual synaptic connections carrying different information, how do the synapses relevant to a specific memory selectively make use of the products of this transcriptional program? Prions are a class of self-aggregating proteins in which a conformational shift from the native, nonaggregated form to the prion form can be transferred from one protein molecule to the other. Si and Kandel have found that a naturally occurring protein, CPEB, has this self-aggregating prion-like property. CPEB is localized to synapses where it regulates the translation of mRNA for specific target proteins. Evidence is presented in this chapter that during memory maintenance CPEB is converted at the relevant synapses to an active prion-like state, which is self-maintaining, and that this conformational change allows the translation of proteins required specifically at those synapses. This mechanism, first identified in *Aplysia*, has now also been found in *Drosophila* and mice and provides a candidate mechanism for long-term synapse-specific gene expression changes.

The dynamic molecular changes evoked with memory retrieval are considered in the chapter on reconsolidation by Nader. A major development in research on consolidation in the past decade has been the revitalization of the idea (Misanin et al. 1968) that consolidation does not occur just once per item, but that under some circumstances it can be actively recruited during later retrieval of that same item. In many cases, when inhibitors of protein synthesis are given in a short time window after memory retrieval, they disrupt the subsequent storage of the memory, similar to what is seen with consolidation of initial learning—hence the term reconsolidation. This chapter covers the current state of this research and discusses the similarity and differences between consolidation and reconsolidation. There is a great deal of clinical interest in reconsolidation as a potential means of disrupting pathological memories that are a hallmark of posttraumatic stress disorder (PTSD).

Ben-Yakov, Dudai, and Mayford discuss the current state of research on the retrieval process itself in mice and men. Our brain can retrieve complex information and act on it within a fraction of a second, but we still do not know how it does so. Behavioral models (Tulving 1983; Roediger et al. 2007) lead us to expect that the brain does this through a combination of sequential and parallel distributed processes that involve multiple brain circuits. Human fMRI studies examining activity during retrieval provide snapshots of this distributed retrieval network, which recruits prefrontal and parietal cortices as well as medial temporal lobe structures such as the hippocampus. Electrophysiological observations in human patients undergoing seizure monitoring are also contributing to better understanding of the fast distributed spatiotemporal configurations that could allow fast retrieval of complex information. In mice, new techniques that allow the observation of activity in large populations of neurons with single-cell resolution has revealed that specific neural ensembles are recruited during retrieval. This is consistent with the idea that the brain represents specific memories in the spatiotemporal pattern of neurons that are activated. This idea has been tested in several recent studies showing that artificial stimulation of the precise pattern of neurons activated during learning can directly produce apparent memory recall.

Moser, Rowland, and Moser explore the nature of information coding and processing in medial temporal lobe structures. Because the hippocampus was identified as critical for explicit memory based on studies of human amnesic patients, animal studies of the hippocampus focused on the nature of the information that the hippocampus is concerned with. Electrophysiological recording of hippocampal activity in freely behaving rats demonstrated that the most striking feature of hippocampal neurons is their spatially specific firing (O'Keefe and Dostrovsky 1971). When animals are allowed to move freely in an open space or on more restrictive tracks, individual hippocampal pyramidal neurons are "place cells"; that is, they are active only when the animal passes through a limited region of the environment, their place field, suggesting that the hippocampal neurons encode a map of the animal's spatial location. In 2005, Edvard and May-Britt Moser extended this idea when they found a precursor of the spatial map in the entorhinal cortex that is formed by a new class of cells known as "grid cells." Each of these space-encoding cells has a grid-like, hexagonal receptive field and conveys information to the hippocampus about position, direction, and distance (Fyhn et al. 2004).

Explicit memory involves recall of people, places and events, and the properties of this entorhinal-hippocampal network serves as a model for studying the encoding and retrieval of place at the systems level. The chapter focuses on the properties of these neurons and how they form their unique firing properties and could contribute to spatial navigation and memory. This is one of the most well-studied and tractable models of higher-order sensory coding where the neurons respond not to specific features of the environment but in an integrated manner to a determination of where they are in the environment. How this occurs at a circuit level and how this information is conveyed to other brain areas is critical for understanding explicit forms of memory.

Working memory refers to the memory system that holds information in temporary storage (seconds) during the planning and execution of a task (Baddeley 2012). It is considered to engage multiple components that combine perceptual information, short- and long-term memory, intentions, and decision making (D'Esposito and Postle 2015). Nyberg and Eriksson, who cover the topic of working memory and its neurobiological underpinnings in this book, emphasize that working memory underlies many basic cognitive abilities that far exceed mnemonic performance per se. They focus on human imaging studies and conclude that working-memory maintenance involves frontal-parietal regions and distributed representational areas, which can be based on persistent activity in reentrant loops, synchronous oscillations, or changes in synaptic strength. Manipulation of the content of working memory depends on dorsal frontal cortex, and updating is apparently realized by a frontostriatal gating function, which involves dopaminergic modulation. Goals and intentions are considered to be represented as cognitive and motivational contexts in the rostral frontal cortex. Working memory has rather limited capacity, and it appears that variations in this capacity as well as working memory deficits can largely be accounted for by the effectiveness and integrity of the basal ganglia and dopaminergic neurotransmission.

SECTION IV: NOVEL APPROACHES

The final three chapters discuss newly developed techniques that we think will have a significant impact on the study of memory in complex nervous systems. The most difficult problem in mammalian systems is identifying the precise neurons within a circuit that participate in the processing of information in a specific behavioral task, how these neural ensembles encode that information and convey it from one brain region to the next to ultimately control motor behavior, and where within this dispersed circuitry the physical changes with experience occur to produce memories. The focus of much of this research, reflected for example in the goals of the U.S. BRAIN Initiative, is on the recording and manipulation of large ensembles of neurons in behaving animals and people. The larger the data set of neural activity recorded during behavior, the more likely it is to reveal principles of information encoding, particularly if combined with modeling.

In combination with optogenetics, these principles can then be directly tested, as we will begin to see in the remaining chapters.

In the first chapter in this section, Jercog, Rogerson, and Schnitzer discuss new optical methods for the simultaneous recording of activity in large ensembles of neurons repeatedly over long time periods. Classical electrophysiological techniques suffer from difficulties in recording activity from large numbers of neurons over prolonged time periods and with certainty that the same neuron is identified in repeated recording trials. With the advent of chemical and genetic voltage and calcium sensors that reflect neural activity with an optical signal, these difficulties can be overcome to allow the recording of hundreds of neurons repeatedly over weeks to months in intact behaving animals. This chapter covers these approaches with a specific focus on a form of microscopy that allows the imaging of deep brain structures in these freely moving animals. This combination of techniques opens the possibility of recording neural ensemble activity in any brain region during any behavioral task to see changes during encoding and retrieval of memories.

Recording studies have identified neurons with exquisitely tuned activity responses to external stimuli. For example, in human studies, neurons in the hippocampus have been identified that seem to respond only to a specific individual. This specific activity strongly implies that these neurons somehow participate in the brain's encoding of the individual to which the neuron responds, but this has been difficult to test directly. Mayford and Reijmers discuss a technique that allows this question to be addressed. By using regulatory elements from genes whose expression is induced by neural activity, essentially any exogenous gene can be expressed in the ensemble of neurons that are active in response to any behaviorally relevant sensory stimulus. This approach has been used in mice to show that the ensemble of neurons naturally activated in the hippocampus and cortex during learning of a specific place could, when artificially stimulated, substitute for the actual sensory experience of the place. This approach allows cellular, molecular, and electrophysiological studies to focus specifically on the sparse and distributed group of neurons that are naturally active during learning and should be useful in identifying the circuit modifications that occur to produce memory.

Recent advances in human noninvasive functional brain imaging have enhanced the ability to interrelate three major facets of systems neurosciences: brain activity, behavior, and computational modeling. Brown, Staresina, and Wagner describe how novel acquisition and analyses methods in fMRI advance mechanistic accounts of learning and memory in the human brain. These include hardware and protocol developments that now permit spatial resolution approaching 1 mm and in some cases even at the submillimeter range. Combination of high-spatial-resolution MRI with multivariate analyses techniques has markedly improved the capacity to tap into the representational content of brain activity signatures and hence contribute to better understanding the role of distinct brain areas in representing discrete stimuli and responses and the transformation of representational content of perceived events from their encoding to their retrieval.

An illustrative approach is representational similarity analysis (RSA), which examines the correlation between stimulus-specific patterns of brain activation, which can be used to query, for example, how the similarity of multivoxel patterns measured during encoding relate to subsequent memory. Another, related approach is multivoxel pattern analysis (MVPA), in which multivoxel activation patterns for two or more classes of stimuli are used to train a classifier to identify characteristic activity patterns that maximally discriminate between these stimulus classes. Pattern classifiers can also be used to generate probabilistic predictions about the new event's likely class, which provides a trial-specific quantitative measure of the strength of neural signatures. For example, it has been reported that the strength of content-specific (i.e., face vs. scene) neural signatures in visual cortex correlates with the magnitude of hippocampal fMRI activity at encoding and predicts whether that information will later be remembered or forgotten. This suggests that the success of hippocampally mediated encoding of event details covaries with the strength or fidelity of the corresponding cortical representations.

Multivariate pattern analyses hence open new possibilities in the study of representational content of types of stimuli in specific brain regions. It would be of interest to observe how this

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class of methods is harnessed to discern activity signatures of tokens within each stimulus type (e.g., individual face vs. generic face) or whether this would require human imaging methods with improved spatiotemporal resolution. In parallel, functional connectivity measures using currently available fMRI also provide new information on how different brain regions and subregions interact in support of encoding and retrieval of memory.

SUMMARY

As these chapters illustrate, a great deal of progress has been made in recent years in tapping into the biological mechanisms of learning and memory. In simple circuits that control behavior, the tools of cellular and molecular biology have revealed how individual neurons and molecular signaling pathways are modified by learning. Changes in synaptic strength produced by specific patterns of electrical activity or the action of modulatory transmitters can alter the processing of information to control behavioral performance. Both memory storage and synaptic plasticity have varying temporal phases, with the switch from short- to long-lasting synaptic and behavioral memory requiring new gene expression. The long-term phase seems to be using a number of synaptic and cell-wide mechanisms, such as synaptic tagging, changes in protein synthesis at the synapse, protein kinase-based cascades, and functional self-perpetuating prion-like mechanisms and epigenetic alterations for maintenance.

We are also beginning to uncover the structure of neural circuits in more complex forms of memory, among them those that involve the hippocampus and neocortex. Recent techniques for the genetic manipulation of neurons based on their natural activity during learning and recall are enabling investigators to investigate the function of distributed neural ensembles and their role in generating representations in complex memory. Finally, advances in functional brain imaging, combined with new electrophysiological and computational techniques for assessing neural activity in populations of neurons, are helping us to determine what regions of the human brain are involved in complex memory and to explore the coding properties and the transformation of the experience-dependent representation over time in consolidation, maintenance, and reactivation or reconstruction in retrieval. The application of these approaches over the next decade offer an exciting opportunity to uncover the computational rules for coding complex information in the mammalian brain and the nature of its modification with learning and memory.

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