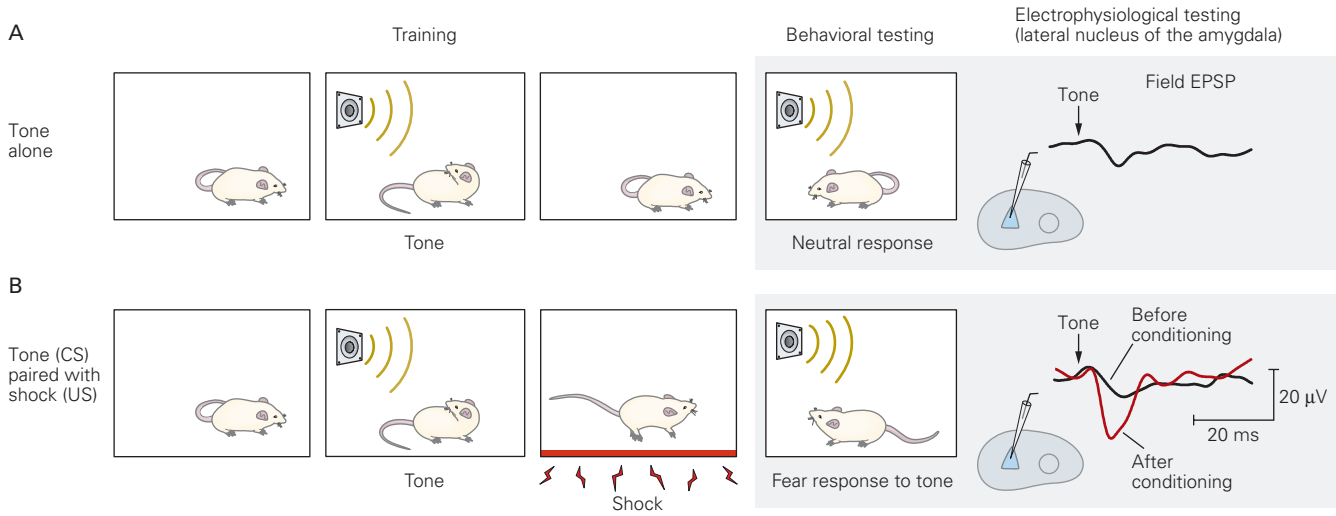

Figure 53–14 Threat learning produces correlated behavioral and electrophysiological changes.

A. An animal ordinarily ignores a neutral tone. The tone produces a small synaptic response in the amygdala recorded by an extracellular field electrode. This field excitatory postsynaptic potential (**field EPSP**) is generated by the small voltage drop between the recording electrode in the amygdala and a second electrode on the exterior of the brain as excitatory synaptic current enters the dendrites of a large population of amygdala neurons.

B. When the tone is presented immediately before a foot shock, the animal learns to associate the tone with the shock. As a result, the tone alone will elicit what the shock previously elicited: It causes the mouse to freeze, an instinctive defense response. After threat conditioning, the electrophysiological response in the lateral nucleus of the amygdala to the tone is greater than the response prior to conditioning. (Abbreviations: **CS**, conditioned stimulus; **US**, unconditioned stimulus.) (Reproduced, with permission, from Rogan et al. 2005.)

convergence of the tone (conditioned stimulus) and the shock (unconditioned stimulus) onto single neurons in the lateral amygdala (Figure 53–14).

It is generally thought that behavioral learning depends on synaptic plasticity. In an effort to understand how such plasticity might occur during learning in the lateral amygdala, researchers have studied *long-term potentiation* (LTP), a cellular model of plasticity. We initially discussed LTP in connection with excitatory synapse function in Chapter 13 and will examine it in detail in Chapter 54 in connection with explicit memory and the hippocampus. In brain slices that include the lateral amygdala, LTP can be induced by high-frequency tetanic stimulation of either the direct or indirect sensory pathways, which produces a long-lasting increase in the excitatory postsynaptic response to these inputs. This change results from a form of homosynaptic plasticity (Figure 53–15).

Long-term potentiation in the lateral nucleus of the amygdala is triggered by Ca^{2+} influx into the postsynaptic neurons in response to strong synaptic activity. The Ca^{2+} entry is mediated by the opening of both *N*-methyl-D-aspartate (NMDA)-type glutamate receptors and L-type voltage-gated Ca^{2+} channels in the postsynaptic cell. Because NMDA receptors are normally blocked by extracellular Mg^{2+}, they require a large synaptic input to generate enough postsynaptic depolarization to relieve this blockade (Chapter 13). L-type channels also require a strong depolarization to open. Thus, LTP is only generated in response to coincident synaptic activity. Calcium influx triggers a biochemical cascade that enhances synaptic transmission through both the insertion of additional α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptors in the postsynaptic membrane and an increase in transmitter release from the presynaptic terminals. As in *Aplysia*, monoamine neurotransmitters, such as norepinephrine and dopamine, released during tetanic stimulation provide a heterosynaptic modulatory signal that contributes to the induction of LTP.

Studies in awake behaving rodents indicate that similar mechanisms contribute to the acquisition of Pavlovian threat conditioning. This form of learning requires postsynaptic NMDA receptors and voltage-gated calcium channels in the lateral amygdala, and it is enhanced by norepinephrine released in lateral amygdala from the locus ceruleus.

In addition, the size of the LTP elicited by electrical stimulation in slices of the amygdala from animals previously trained is less than that found in slices from untrained animals. Because there is an upper limit to the amount by which synapses can be

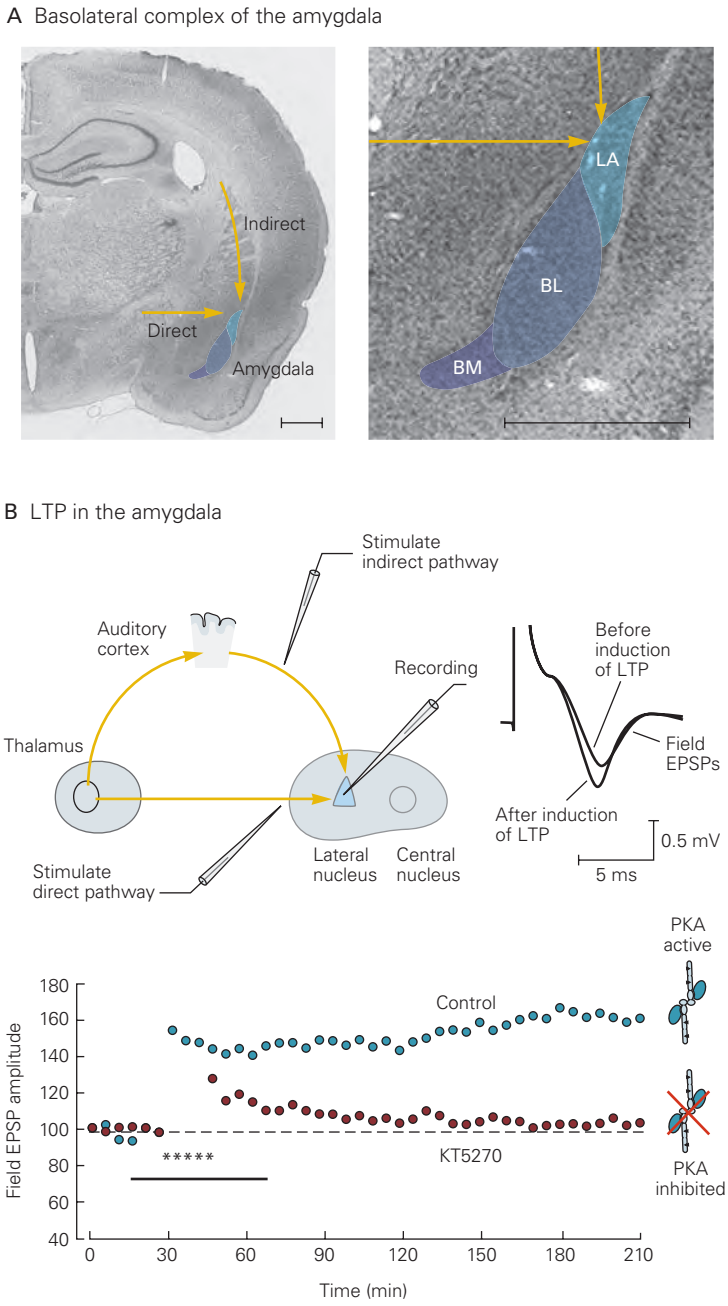

Figure 53–15 Long-term potentiation at synapses in the amygdala may mediate threat conditioning.

A. A coronal brain slice from a mouse shows the position of the amygdala. The enlargement shows three key input nuclei of the amygdala—lateral (**LA**), basolateral (**BL**), and basomedial (**BM**)—which together form the basolateral complex. These nuclei project to the central nucleus, which projects to the hypothalamus and brain stem. (Adapted, with permission, from Maren 1999. Copyright © 1999 Elsevier.)

B. High-frequency tetanic stimulation of the direct or indirect pathway from the thalamus to the lateral nucleus initiates long-term potentiation (**LTP**). The drawing shows the position of the extracellular voltage recording electrode in the lateral nucleus, and the positions of two stimulating electrodes used to activate either the direct pathway or indirect pathway. The plot shows the amplitude of the extracellular field excitatory postsynaptic potential (**EPSP**) in response to stimulation of the indirect cortical pathway during the time course of the experiment. When a pathway is stimulated at a low frequency (once every 30 seconds), the field EPSP is stable. However, when five trains of high-frequency tetanic stimulation are applied (**asterisks**), the response is enhanced for a period of hours. The facilitation depends on protein kinase A (**PKA**) and is compromised when the PKA inhibitor KT5720 is applied (the **bar**). Field EPSPs before and after induction of LTP are also shown. (Adapted, with permission, from Huang and Kandel 1998; Huang, Martin, and Kandel 2000.)

potentiated, this result is taken as evidence that threat conditioning recruits LTP, which precludes further LTP in response to electrical stimulation. Thus, artificially induced LTP and behaviorally induced LTP are closely related.

Two types of genetic experiments also strongly support the idea that an LTP-like phenomenon contributes to the cellular mechanism for storing the memory of a learned threat. First, genetic disruption of the GluN2B (NR2B) subunit of the NMDA receptor interferes both with threat conditioning and the induction of LTP in pathways that transmit the conditioned stimulus signal to the lateral amygdala. Moreover, this mutation affects only learned threats; it does not affect responses to unconditioned threats or routine synaptic transmission. Conversely, overexpression of the GluN2B subunit facilitates learning. Similarly, disruption of CREB signaling, a step downstream from Ca^{2+} influx, interferes with conditioning, whereas enhancement of CREB activity facilitates learning.

Does the LTP important for threat learning involve insertion of new AMPA receptors, as observed in brain slices? To address this question, researchers infected pyramidal neurons in the lateral nucleus with a genetically engineered virus that did not damage the neurons but caused them to express AMPA receptors tagged with a fluorescent label. Threat conditioning led to an increase in insertion of the tagged AMPA receptors into the cell membrane, similar to what is seen during experimentally induced LTP in brain slices. When a different virus was used to express a C-terminal portion of the AMPA receptor that competes with and prevents the insertion of endogenous AMPA receptors, memory for learned threat was substantially reduced, even though the virus infected only 10% to 20% of the neurons in the lateral nucleus. This surprising result suggests that LTP needs to be induced at nearly all activated synapses to effectively support threat learning.

One of the virtues of the Pavlovian paradigm is its amenability to experimental study due to the fact that specific stimuli are transmitted to the amygdala by known pathways. This has allowed experimenters to directly activate conditioned stimulus or unconditioned stimulus pathways, bypassing the normal sensory input. Such studies have provided convincing evidence implicating these pathways to the amygdala in threat learning.

Based on these findings, researchers explored whether threat learning could be induced when they paired an auditory conditioned stimulus (tone) with direct depolarization of lateral amygdala neurons, instead of using an external, pain-eliciting unconditioned shock stimulus to produce the depolarization via the unconditioned stimulus pathway to the lateral amygdala. To accomplish this, they used an optogenetic approach (Chapter 5). They injected a virus into the amygdala to express channelrhodopsin-2, a light-activated excitatory cation channel, in lateral amygdala neurons. Following pairing of the auditory stimulus with a light pulse that depolarized lateral amygdala cells, presentation of the tone alone elicited conditioned freezing. The amount of freezing was greater when norepinephrine was present, which is further evidence that modulatory pathways also have a role in synaptic facilitation in this circuit. Thus, an aversive shock itself is not necessary to induce threat learning. Rather, it is the association of a stimulus with activation of the lateral amygdala that is key.

Other studies demonstrated the possibility of artificially manipulating the amygdala to impair as well as to instantiate, threat memory. They first trained animals to associate a foot shock with optogenetic stimulation of auditory inputs to the amygdala. They then delivered a pattern of optogenetic stimulation that generated *long-term depression* (LTD) of the auditory input to the amygdala, a form of synaptic plasticity in which weak, repetitive stimulation decreases the strength of synaptic transmission. Induction of LTD was able to inactivate the memory of the shock. Then, using a pattern of optical stimulation that produced LTP of the same auditory input, they found that the memory of the shock could be reinstated. The findings that inactivation and reactivation of a memory could be engineered using LTD and LTP strengthened the possible causal link between synaptic strength and behavioral memory storage.

The persistence of the synaptic changes underlying the memory for a threat depend on gene expression and protein synthesis in the amygdala, much like long-term memory in *Aplysia* and *Drosophila*. Thus, cAMP-dependent protein kinase and MAPK activate the transcription factor CREB to initiate gene expression. The importance of CREB is underscored by the finding that different neurons in the lateral amygdala have varying levels of CREB expression prior to threat conditioning. Neurons that express a larger than average amount of CREB are selectively recruited during learning. Conversely, if neurons with a large resting level of CREB are selectively ablated after learning, memory is impaired.

While most of the work on the neural mechanisms of threat conditioning has involved the lateral nucleus of the amygdala, in recent years, evidence has accumulated that plasticity in the central nucleus is also important. The central nucleus receives direct and indirect inputs from the lateral nucleus and forms synaptic connections with neurons in the periaqueductal gray region in the midbrain, which projects to the brain stem to control a number of defensive reactions, including freezing behavior. Within a lateral cell group of the central nucleus, inhibitory cells called PKC delta neurons control the activity of the output neurons in the medial cell group that project to the periaqueductal gray.

Memory for threat conditioning in humans also involves the amygdala. Thus, in humans, damage to the amygdala impairs the implicit memory of threat conditioning but not the explicit memory of having been conditioned. Functional imaging studies have found that the amygdala is activated by threats even when the person is not aware of the presence of the threat because the stimulus was subliminal. Although human studies are limited in their ability to reveal neurobiological details, they demonstrate the relevance of the animal work for human psychopathology.

In summary, Pavlovian threat conditioning has emerged as one of the most useful experimental

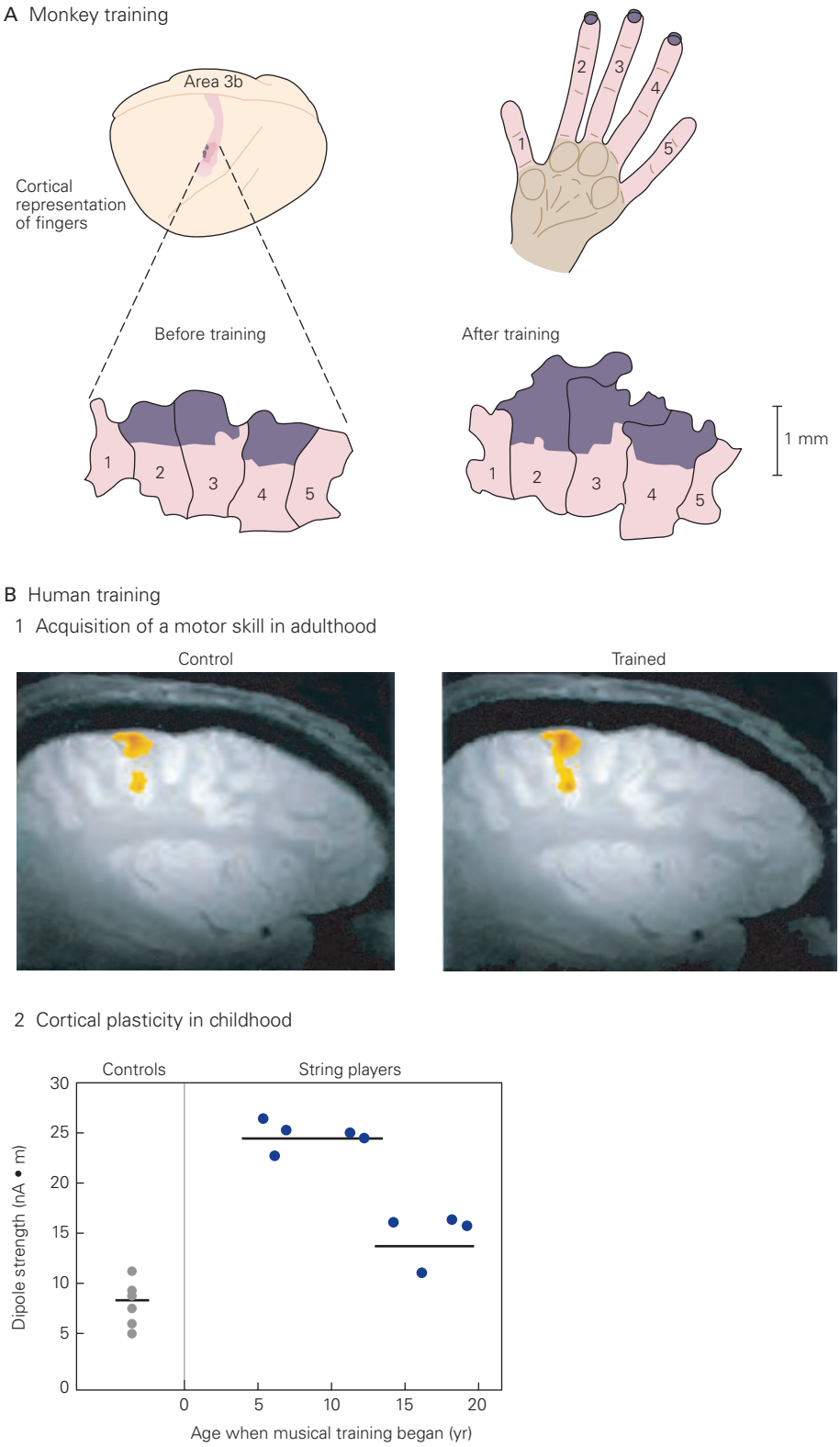

Figure 53–16 Training expands representation of inputs from the fingers in the cortex.

A. A monkey was trained for 1 hour per day to perform a task that required repeated use of the tips of fingers 2, 3, and occasionally 4. After training, the portion of area 3b of the somatosensory cortex representing the tips of the stimulated fingers (**dark color**) is substantially greater than normal (measured 3 months prior to training). (Adapted, with permission, from Jenkins et al. 1990.)

B. 1. A human subject trained to do a rapid sequence of finger movements will improve in accuracy and speed after 3 weeks of daily training (10–20 minutes each day). Functional magnetic resonance imaging scans of the primary motor cortex (based on local blood oxygenation level–dependent signals) after training show that the region activated in trained subjects (**orange region**) is larger than the region activated in untrained (controls). The control subjects received no training and performed unlearned finger movements using the same hand as control subjects. The change in cortical representation in trained subjects persisted for several months. (Reproduced, with permission, from Karni et al. 1998. Copyright © 1998 National Academy of Sciences.)

2. The size of the cortical representation of the fifth finger of the left hand is greater in string players than in nonmusicians. The graph plots the dipole strength obtained from magnetoencephalography, a measure of neural activity. The increase is most pronounced in musicians that began musical training before age 13. (Reproduced, with permission, from Elbert et al. 1995. Copyright © 1995 AAAS.)

models for studying associative learning and memory in the mammalian brain. In part, this is due to the fact that the behavioral paradigm has been applied successfully across diverse species, from flies to humans, and thus builds upon the earlier progress that invertebrate models have made.

Learning-Induced Changes in the Structure of the Brain Contribute to the Biological Basis of Individuality

To what extent do the anatomical alterations in synapses required for long-term memory storage alter the large-scale functional architecture of the mature brain? The answer is well illustrated by the fact that the maps of the body surface in the primary somatic sensory cortex differ among individuals in a manner that reflects their use of specific sensory pathways. This remarkable finding results from the expansion or retraction of the connections of sensory pathways in the cortex according to the specific experience of the individual (Chapter 49).

The reorganization of afferent inputs as a result of behavior is also evident at lower levels in the brain, specifically at the level of the dorsal column nuclei, which contain the first synapses of the somatic sensory system. Therefore, organizational changes probably occur throughout the somatic afferent pathway.

The process by which experience alters the maps of somatosensory inputs in the cortex is illustrated in an experiment in which adult monkeys were trained to use their middle three fingers at the expense of other fingers to obtain food. After several thousand trials of this behavior, the area of cortex devoted to the middle fingers expanded greatly (Figure 53–16A). Thus, practice may expand synaptic connections by strengthening the effectiveness of existing connections.

The normal development of somatosensory input to cortical neurons may depend on the level of activity in neighboring afferent axons. In one experiment using monkeys, the skin surfaces of two adjacent fingers were surgically connected so that the connected fingers were always used together, thus ensuring that their afferent somatosensory axons were normally coactivated. As a result, the normally sharp discontinuity between the zones in the somatosensory cortex that receive inputs from these digits was abolished. Thus, normal development of the boundaries of representation of adjacent fingers in the cortex may be guided not only genetically but also through experience. Fine tuning of cortical connections may depend on associative mechanisms such as LTP, similar to the role of cooperative activity in shaping the development of ocular dominance columns in the visual system (Chapter 49).

This plasticity is evident in humans as well. People trained to perform a task with their fingers show an expansion in the fMRI signal in the primary motor cortex during performance of the task (Figure 53–16B). Thomas Elbert explored the hand representation in the motor cortex of string instrument players. These musicians use their left hand for fingering the strings, manipulating the fingers in a highly individuated way. By contrast, the right hand, used for bowing, is used almost like a fist. The representation of the right hand in the cortex of string instrument players is the same as that of nonmusicians. But the representation of the left hand is greater than in nonmusicians and substantially more prominent in players who started to play their instrument prior to age 13 years (Figure 53–16B).

Because each of us is brought up in a somewhat different environment, experiencing different combinations of stimuli and developing motor skills in different ways, each individual's brain is uniquely modified. This distinctive modification of brain architecture, along with a unique genetic makeup, constitutes a biological basis for individuality.

Highlights

1. Many aspects of personality are guided by implicit memory. A great deal of what we experience—what we perceive, think, fantasize—is not directly controlled by conscious thought.
2. In mammals, both innate and learned defensive responses involve the amygdala. The amygdala-based defense system quickly learns about new dangers. It can associate a new neutral (conditioned) stimulus with a known threatening (unconditioned) stimulus on a single paired exposure, and this learned association is often retained throughout life.
3. During Pavlovian conditioning, the strength of synaptic transmission is modified in the lateral amygdala by pairing the conditioned and unconditioned stimuli. As a result, electrophysiological responses of neurons in the lateral amygdala are enhanced and behavioral learning occurs.
4. Many of the molecular mechanisms underlying threat conditioning in invertebrates also contribute to conditioning in mammals.
5. Damage to the human amygdala impairs implicit threat conditioning but does not affect the explicit memory of having been conditioned.

6. Habits are routines that are acquired gradually by repetition and are the result of a distinct form of implicit learning. As with all forms of implicit learning, habits are expressed in action alone, without conscious control, and independent of verbal reports.
7. As these arguments make clear, the empirical study of unconscious psychic processes was severely limited for many years by the lack of suitable experimental methods. Today, however, biology has a wide range of empirical methods that are providing cellular and molecular insights that are expanding our understanding of a wide range of mental activities.

Eric R. Kandel
Joseph LeDoux

Selected Reading

Alberini CM, Kandel ER. 2016. The regulation of transcription in memory consolidation. In: ER Kandel, Y Dudai, MR Mayford (eds). *Learning and Memory*, pp. 157–174. New York: Cold Spring Harbor Laboratory Press.

Bailey CH, Kandel ER, Harris KM. 2015. Structural components of synaptic plasticity and memory consolidation. Cold Spring Harb Perspect Biol 7:a021758.

Busto GU, Cervantes-Sandoval I, Davis RL. 2010. Olfactory learning in *Drosophila*. Physiology (Bethesda) 25:338–346.

Duvarci S, Pare D. 2014. Amygdala microcircuits controlling learned fear. Neuron 82:966–980.

Fanselow MS, Zelikowsky M, Perusini J, Barrera VR, Hersman S. 2014. Isomorphisms between psychological processes and neural mechanisms: from stimulus elements to genetic markers of activity. Neurobiol Learn Mem 108:5–13.

Hawkins RD, Kandel ER, Bailey CH. 2006. Molecular mechanisms of memory storage in *Aplysia*. Biol Bull 210: 174–191.

LeDoux JE. 2014. Coming to terms with fear. Proc Natl Acad Sci U S A 111:2871–2878.

LeDoux J. 2015. *Anxious: Using the Brain to Understand and Treat Fear and Anxiety*. New York: Viking.

LeDoux JE. 2019. *The Deep History of Ourselves: The Four-Billion Year History of How We Got Conscious Brains*. New York: Viking.

Nader K. 2016. Reconsolidation and the dynamic nature of memory. In: ER Kandel, Y Dudai, MR Mayford (eds). *Learning and Memory*, pp. 245–260. New York: Cold Spring Harbor Laboratory Press.

Phelps EA. 2006. Emotion and cognition: insights from studies of the human amygdala. Annu Rev Psychol 57: 27–53.

Tubon CT Jr, Yin JCP. 2008. CREB responsive transcription and memory formation. In: SM Dudek (ed). *Transcriptional Regulation by Neuronal Activity, Part III*, pp. 377–397. New York: Springer.

References

Bailey CH, Chen MC. 1983. Morphological basis of long-term habituation and sensitization in *Aplysia*. Science 220:91–93.

Bailey CH, Kandel ER, Si K. 2004. The persistence of long-term memory: a molecular approach to self-sustaining changes in learning-induced synaptic growth. Neuron 44:49–57.

Bear MF, Connors BW, Paradiso MA. 2001. *Neuroscience: Exploring the Brain,* 2nd ed. Chicago: Lippincott Williams & Wilkins.

Casadio A, Martin KC, Giustetto M, et al. 1999. A transient, neuron-wide form of CREB-mediated long-term facilitation can be stabilized at specific synapses by local protein synthesis. Cell 99:221–237.

Castellucci VF, Carew TJ, Kandel ER. 1978. Cellular analysis of long-term habituation of the gill-withdrawal reflex in *Aplysia californica*. Science 202:1306–1308.

Castellucci VF, Kandel ER. 1974. A quantal analysis of the synaptic depression underlying habituation of the gill-withdrawal reflex in *Aplysia*. Proc Natl Acad Sci U S A 71:5004–5008.

Claridge-Chang A, Roorda RD, Vrontou E, et al. 2009. Writing memories with light-addressable reinforcement circuitry. Cell 139:405–415.

Ehrlich DE, Josselyn SA. 2016. Plasticity-related genes in brain development and amygdala-dependent learning. Genes Brain Behav 15:125–143.

Eichenbaum H, Cohen NJ. 2001. *From Conditioning to Conscious Recollection: Memory Systems of the Brain.* Oxford: Oxford Univ. Press.

Elbert T, Pantev C, Wienbruch C, Rockstroh B, Taub E. 1995. Increased cortical representation of the fingers of the left hand in string players. Science 270:305–307.

Glanzman DL, Kandel ER, Schacher S. 1990. Target-dependent structural changes accompanying long-term synaptic facilitation in *Aplysia* neurons. Science 249:799–802.

Greco JA, Liberzon I. 2016. Neuroimaging of fear-associated learning. Neuropsychopharmacology 41:320–334.

Gründemann J, Lüthi A. 2015. Ensemble coding in amygdala circuits for associative learning. Curr Opin Neurobiol 35:200–206.

Guan Z, Giustetto M, Lomvardas S, et al. 2002. Integration of long-term–memory-related synaptic plasticity involves bidirectional regulation of gene expression and chromatin structure. Cell 111:483–493.

Hawkins RD, Abrams TW, Carew TJ, Kandel ER. 1983. A cellular mechanism of classical conditioning in *Aplysia:* activity-dependent amplification of presynaptic facilitation. Science 219:400–405.

Hegde AN, Inokuchi K, Pei W, et al. 1997. Ubiquitin C-terminal hydrolase is an immediate-early gene essential for long-term facilitation in *Aplysia*. Cell 89:115–126.

Herry C, Johansen JP. 2014. Encoding of fear learning and memory in distributed neuronal circuits. Nat Neurosci 17:1644–1654.

Huang YY, Kandel ER. 1998. Postsynaptic induction and PKA-dependent expression of LTP in the lateral amygdala. Neuron 21:169–178.

Huang YY, Martin KC, Kandel ER. 2000. Both protein kinase A and mitogen-activated protein kinase are required in the amygdala for the macromolecular synthesis-dependent late phase of long-term potentiation. J Neurosci 20:6317–6325.

Janak PH, Tye KM. 2015. From circuits to behaviour in the amygdala. Nature 517:284–292.

Jenkins WM, Merzenich MM, Ochs MT, Allard T, Guic-Robles E. 1990. Functional reorganization of primary somatosensory cortex in adult owl monkeys after behaviorally controlled tactile stimulation. J Neurophysiol 63:82–104.

Johansen JP, Diaz-Mataix L, Hamanaka H, et al. 2014. Hebbian and neuromodulatory mechanisms interact to trigger associative memory formation. Proc Natl Acad Sci U S A 111:E5584–E5592.

Kandel ER. 2001. The molecular biology of memory storage: a dialogue between genes and synapses. Science 294:1030–1038.

Kandel ER. 2006. *In Search of Memory: The Emergence of a New Science of Mind.* New York: Norton.

Karni A, Meyer G, Rey-Hipolito C, et al. 1998. The acquisition of skilled motor performance: fast and slow experience-driven changes in primary motor cortex. Proc Natl Acad Sci U S A 95:861–868.

Keleman K, Krüttner S, Alenius M, Dickson BJ. 2007. Function of the *Drosophila* CPEB protein Orb2 in long-term courtship memory. Nat Neurosci 10:1587–1593.

Klein M, Kandel ER. 1980. Mechanism of calcium current modulation underlying presynaptic facilitation and behavioral sensitization in *Aplysia*. Proc Natl Acad Sci U S A 77:6912–6916.

Krabbe S, Gründemann J, Lüthi A. 2018. Amygdala inhibitory circuits regulate associative fear conditioning. Biol Psychiatry 83:800–809.

Mahan AL, Ressler KJ. 2011. Fear conditioning, synaptic plasticity and the amygdala: implications for posttraumatic stress disorder. Trends Neurosci 35:24–35.

Maren S. 2017. Synapse-specific encoding of fear memory in the amygdala. Neuron 95:988–990.

Maren S. 1999. Long-term potentiation in the amygdala: a mechanism for emotional learning and memory. Trends Neurosci 22:561–567.

Martin KC, Casadio A, Zhu H, et al. 1997. Synapse-specific, long-term facilitation of *Aplysia* sensory to motor synapses: a function for local protein synthesis in memory storage. Cell 91:927–938.

Nabavi S, Fox R, Proulx CD, Lin JY, Tsien RY, Malinow R. 2014. Engineering a memory with LTD and LTP. Nature 511:348–352.

Pape HC, Pare D. 2010. Plastic synaptic networks of the amygdala for the acquisition, expression, and extinction of conditioned fear. Physiol Rev 90:419–463.

Pavlov IP. 1927. *Conditioned Reflexes: An Investigation of the Physiological Activity of the Cerebral Cortex*. GV Anrep (transl). Oxford: Oxford Univ. Press.

Pinsker H, Kupferman I, Castelucci V, Kandel ER. 1970. Habituation and dishabituation of the gill-withdrawal reflex in *Aplysia*. Science 167:1740–1742.

Rajasethupathy P, Antonov I, Sheridan R, et al. 2012. A role for neuronal piRNAs in the epigenetic control of memory-related synaptic plasticity. Cell 149:693–707.

Rogan MT, Leon KS, Perez DL, Kandel ER. 2005. Distinct neural signatures for safety and danger in the amygdala and striatum of the mouse. Neuron 46:309–320.

Sears RM, Fink AE, Wigestrand MB, Farb CR, de Lecea L, LeDoux JE. 2013. Orexin/hypocretin system modulates amygdala-dependent treat learning trough the locus coeruleus. Proc Natl Acad Sci U S A 110:20260–20265.

Sears RM, Schiff HC, LeDoux JE. 2014. Molecular mechanisms of threat learning in the lateral nucleus of the amygdala. Prog Mol Biol Transl Sci 122:263–304.

Si K, Giustetto M, Etkin A, et al. 2003. A neuronal isoform of CPEB regulates local protein synthesis and stabilizes synapse-specific long-term facilitation in *Aplysia*. Cell 115:893–904.

Si K, Lindquist S, Kandel ER. 2003. A neuronal isoform of the *Aplysia* CPEB has prion-like properties. Cell 115:879–891.

Spencer AW, Thompson RF, Nielson DR Jr. 1966. Response decrement of the flexion reflex in the acute spinal cat and transient restoration by strong stimuli. J Neurophysiol 29:240–252.

Squire LR, Kandel ER. 2008. *Memory: From Mind to Molecules*, 2nd ed. Greenwood Village: Roberts.

Yin JCP, Wallach JS, Del Vecchio M, et al. 1994. Induction of a dominant negative CREB transgene specifically blocks long-term memory in *Drosophila*. Cell 79:49–58.

54

The Hippocampus and the Neural Basis of Explicit Memory Storage

Explicit memory—the conscious recall of information about people, places, objects, and events—is what people commonly think of as memory. Sometimes called *declarative memory*, it binds our mental life together by allowing us to recall at will what we ate for breakfast, where we ate it, and with whom. It allows us to join what we did today with what we did yesterday or the week or month before that.

Two structures in the mammalian brain are particularly critical for encoding and storing explicit memory: the prefrontal cortex and the hippocampus (Chapter 52). The prefrontal cortex mediates working memory, which can be actively maintained for only very short periods and is then rapidly forgotten, such as a password that is remembered only until it is entered. Information in working memory can be stored elsewhere in the brain as long-term memory for periods ranging from days to weeks to years, and throughout a lifetime. Although long-term storage of explicit memory requires the hippocampus, the ultimate storage site for most declarative memory is thought to be the cerebral cortex.

In this chapter, we focus on the cellular, molecular, and network mechanisms of the hippocampus that underlie the long-term storage of explicit memory. Because the hippocampus receives its major input from a region of the cerebral cortex called the entorhinal cortex, an area that processes many forms of sensory input, we also consider how information from the entorhinal cortex is transformed by the hippocampus. In particular, we examine how neural activity in the entorhinal cortex and hippocampus contributes to

spatial memory by encoding a representation of an animal's location in its environment.

Explicit Memory in Mammals Involves Synaptic Plasticity in the Hippocampus

Unlike working memory, which is thought to be maintained by ongoing neural activity in the prefrontal cortex (Chapter 52), the long-term storage of information is thought to depend on long-lasting changes in the strength of connections among specific ensembles of neurons (neural assemblies) in the hippocampus that encode particular elements of memory.

The idea that memory storage involves long-lasting structural changes in the brain, first referred to as an "engram" by the German biologist Richard Semon in the early 20th century, dates back to the French philosopher Rene Descartes. In an attempt to locate an engram, the American psychologist Karl Lashley examined the effects of lesions in different regions of the neocortex on the ability of a rat to learn to navigate a maze. Since the performance in the maze seemed to be directly proportional to the size of the lesion, rather than its precise location, Lashley concluded that any memory trace must be distributed throughout the brain. Although it is now generally accepted that storage of an explicit memory is distributed throughout the neocortex, it is also clear that the process of storing memory requires the hippocampus, as demonstrated by the pioneering studies of Brenda Milner on patient H.M. (Chapter 52) and subsequent studies in animals with targeted lesions of the hippocampus. Thus, understanding how the brain stores explicit memory depends on an understanding of how the cortico-hippocampal circuit processes and stores information.

The nature of the basic mechanisms for memory storage was and remains the subject of much speculation and debate among psychologists and neuroscientists. One influential theory was proposed by the Canadian psychologist Donald Hebb, who suggested in 1949 that memory-encoding neural assemblies may be generated when synaptic connections are strengthened based on experience. According to *Hebb's rule*: "When an axon of cell A . . . excites cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells so that A's efficiency as one of the cells firing B is increased." The key element of Hebb's rule is the requirement for coincidence of pre- and postsynaptic firing, and so the rule has sometimes been rephrased as "Cells that fire together, wire together." A similar Hebbian coincidence principle is thought to be involved in fine-tuning synaptic connections during the late stages of development (Chapter 49). Hebb's ideas were later refined by the theoretical neuroscientist David Marr, based on a consideration of the hippocampal circuit.

The hippocampus comprises a loop of connections that process multimodal sensory and spatial information from the superficial layers of the nearby entorhinal cortex. This information passes through multiple synapses before arriving at the hippocampal CA1 region, the major output area of the hippocampus. The critical importance of CA1 neurons in learning and memory is seen in the profound memory loss exhibited by patients with lesions in this region alone, an observation supported by numerous animal studies. Information from the entorhinal cortex reaches CA1 neurons along two excitatory pathways, one direct and one indirect.

In the indirect pathway, the axons of neurons in layer II of the entorhinal cortex project through the *perforant pathway* to excite the granule cells of the dentate gyrus (an area considered part of the hippocampus). Next, the axons of the granule cells project in the *mossy fiber pathway* to excite the pyramidal cells in the CA3 region of the hippocampus. Finally, axons of the CA3 neurons project through the *Schaffer collateral pathway* to make excitatory synapses on more proximal regions of the dendrites of the CA1 pyramidal cells (Figure 54–1). (Because of its three successive excitatory synaptic connections, the indirect pathway is often referred to as the *trisynaptic pathway*). Finally, CA1 pyramidal cells project back to the deep layers of entorhinal cortex and forward to the subiculum, another medial temporal lobe structure that connects the hippocampus with a wide diversity of brain regions.

In parallel with the indirect pathway, the entorhinal cortex also projects directly to CA3 and CA1 hippocampal regions. In the direct pathway to CA1, neurons in layer III of the entorhinal cortex send their axons through the *perforant pathway* to form excitatory synapses on the very distal regions of the apical dendrites of CA1 neurons (such projections are also called the *temporoammonic pathway*). Interactions between direct and indirect inputs at each stage of the hippocampal circuit are likely important for memory storage or recall, although the precise nature of these interactions remains to be determined.

In addition to the above pathways that link different stages of the hippocampal circuit, CA3 pyramidal neurons also make strong excitatory connections with one another. This self-excitation through recurrent collaterals is thought to contribute to associative aspects of memory storage and recall. Under pathological conditions, such self-excitation can lead to seizures.