SYNAPTIC PLASTICITY: UNDERSTANDING THE NEUROBIOLOGICAL MECHANISMS OF LEARNING AND MEMORY. PART II

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SUMMARY

Plasticity of the nervous system has been related to learning and memory processing as early as the beginning of the century; although, remotely, brain plasticity in relation to behavior has been connoted over the past two centuries. However, four decades ago, several evidences have shown that experience and training induce neural changes, showing that major neuroanatomical, neurochemical as well as molecular changes are required for the establishment of a long-term memory process. Early experimental procedures showed that differential experience, training and/or informal experience could produce altered quantified changes in the brain of mammals. Moreover, neuropsychologists have emphasized that different memories could be localized in separate cortical areas of the brain, but updated evidences assert that memory systems are specifically distributed in exclusive neural networks in the cortex. For instance, the same cortical systems that lead us to perceive and move in our environment, are used as neural substrates for memory retrieval. Such memories are the result of the repeated activity of millions of neurons assembled into distinct neural networks, where plastic changes in synaptic function leads to the strengthening of the same synaptic connections with the result of reconstructed permanent traces that lead to remembrance (Hebb Postulate). Elementary forms of learning and memory have been studied in simple neural systems of invertebrates, and as such have led the way for understanding much of the electrophysiological and neurochemical events occurring during LTP. Long-term potentiation (LTP) is the result of the increase in the strength of synaptic transmission, lasting as long as can be measured from hours to days. LTP has been detected in several areas of the brain, particularly, in the hippocampus, amygdala, and cortex, including several related limbic structures in the mammalian brain. LTP represents up to date the best model available for understanding the cellular basis of learning and memory in the central nervous system of mammals including humans.

Key words: Brain plasticity, synapses, learning, memory, long-term potentiation, experience, training.

RESUMEN

Uno de los fenómenos más nteresantes dentro del campo de la neurobiología, es el fenómeno de la plasticidad cerebral relacionada con los eventos de aprendizaje y el procesamiento del fenómeno de memoria. De hecho, estos fenómenos neurobiológicos empezaron a ser estudiados desde principios de siglo. Remotamente, el fenómeno de plasticidad cerebral en relación con el desarrollo y aprendizaje de las conductas fue ya concebido y cuestionado desde hace más de dos centurias. Sin embargo, desde hace cuatro décadas, múltiples evidencias experimentales han demostrado que tanto la experiencia o el entrenamiento en la ejecución de tareas operantes aprendidas, inducen cambios plásticos en la fisiología neuronal, incluyendo los cambios neuroquímicos y moleculares que se requieren para consolidar una memoria a largo plazo. Asimismo, diversos procedimientos experimentales han demostrado que la experiencia diferencial, el entrenamiento y el aprendizaje de conductas o la experiencia informal, producen cambios mensurables en el cerebro de los mamíferos. Más aún, la neuropsicología ha considerado desde hace varias décadas que diferentes tipos de memoria pueden ser localizados en diferentes circuitos neuronales en distintas áreas de la corteza cerebral. Sin embargo, los estudios recientes han demostrado que los sistemas de memoria están distribuidos en circuitos neuronales corticales específicos. Por ejemplo, los mismos sistemas corticales que procesan la percepción sensorial y las función motora, son los mismos sustratos neurales que se emplean para procesar los fenómenos de memorización. El fenómeno de la memoria y el aprendizaje es resultado de la actividad fisiológica repetitiva de millones de neuronas que, ensambladas en circuitos neuronales específicos, conllevan al reforzamiento de las conexiones sinápticas involucradas y a los cambios de plasticidad sináptica que se requieren para establecer estos fenómenos neurobiológicos. El fenómeno de potenciación a largo plazo, o LTP, es un evento neurofisiológico que resulta del incremento en el reforzamiento de la transmisión sináptica, que puede perdurar en las regiones cerebrales estudiadas desde horas a días. El modelo de LTP quizá representa el modelo funcional experimental más viable para entender las bases celulares del aprendizaje y la memoria en el SNC de los mamíferos, incluyendo el cerebro de los humanos.

Palabras clave: Plasticidad cerebral, sinapsis, aprendizaje, memoria, potenciación a largo plazo, experiencia, entrenamiento.

EXPERIENCE IMPROVES LEARNING ON SOLVING PROBLEMS

It has been shown by several research groups that enriched laboratory environments improve learning as

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well as problem solving abilities when subject animals are exposed to a wide variety of tests (Rosenzweig, 1996). This set of observations were briefly reported in advance by Hebb (1949) where he initially detailed that when young animals were allowed to explore his home for some weeks and then returned back to the lab, they showed better problem-solving ability than rats that had remained permanently in the lab. Hebb concluded that "the richer experience of the pet group mad during development made them better able to profit by new experience at maturity"- which is one of the characteristic of the "intelligent" human being (Hebb, 1949). Thus, these results demonstrated the effect of early experience on problem solving at maturity (Rosenzweig, 1996). Similarly, animals exposed to a conditioned environment, such as social (SC) and/or enriched conditioning (EC), which give greater opportunity to animals for informal learning as compared to a single animal or groups of animals maintained in an impoverished or isolated condition (IC), improve their abilities for learning and solving-test problems, even complex tasks, that are better performed when compared to those groups maintained under SC or IC (Renner & Rosenzweig, 1987). This does not mean that IC animals will be deprived of learning capabilities during growth and development, as it has been demonstrated that IC rats tend to catch up EC animals over longer periods of trials when tested to solve spatial problems. Therefore, early deprivation of experience does not produce a permanent deficit in learning capabilities (Rosenzweig, 1971). Although IC environment induces a decrease in cortical mass (less weight) in IC animals when left as long as 300 days in impoverished conditions, as compared to EC animals, this decrement of cortex tissue can be overcomed after few weeks of training and spatial solve-problem testing at least in complex spatial mazes (Cummings, 1973). These findings, the effects of training on brain plasticity, have been not only reported to occur in several species of mammals (mice, gerbils, squirrels, cats and monkeys) but have also been found in avian species (Renner and Rosenzweig, 1987). Thus, experience seems to affect anatomical substrates of the brain of distinct phylogenetical species which are more sensitive to the acquisition of learning experiences as do natural environments to enhance their learning capabilities. More interestingly, similar learning tests, have been performed in non vertebrate species, such as Drosophila, Aplysia, and Hermissenda (Krasne & Glanzman, 1995), where important synaptic changes in the nervous system result after animals are exposed to either training or differential experience (Davis, 1993; Heisenberg et al., 1995). For instance, in Aplysia, long-term habituation (see elementary forms of learning) reduces number of synaptic connections, whereas long-term sensitization increases number of same synaptic sites (Bailey & Chen, 1983). Synaptic connections involved in learning and memory storage, are quite sensitive to plastic changes, either decreasing or increasing the number of synapses, depending on the nature of the experience. Therefore, although experience-dependent synaptic plasticity has been widely documented in terms of species (Greenough et al., 1990), such synaptic changes in specific structures in the brain imply that mechanisms of memory are crucial for the consolidation of learning experiences.

On a similar track of experimental work, several studies have been conducted to analyze if rich experience influence the full growth of species-specific brain characteristics as well as the expression of specific behaviors in avian species. For instance, species that cache food for future use have larger hippocampal formation than related species that do not show similar behavior (Sherry et al., 1989; Clayton & Krebs, 1994). Such difference in the size of the hippocampus appear to be influenced after food storing has started, just after birds leave the nest (Healy and Krebs, 1993) and this increase is more notorious at the early stages of life span. Thus, it appears that experience influences the growth of brain structures, such as the hippocampus in birds, similarly to what has been found in the increase of the occipital cortex size in mammals (Rosenzweig, 1996).

EXPERIENCE AND TRAINING INDUCE NEUROCHEMICAL AND PLASTIC CHANGES IN THE BRAIN

Neurochemical and conducted behavioral studies have shown that rich environments induce an increase of protein synthesis and specific proteins in the brain cortex of animals (Bennett, 1964a). Even more, non mammal species, when subjected to specific training tasks, showed an increased rate of incorporation of label precursors into mRNA and protein in the forebrain tissue (Haywood, 1970) and in a similar context, mammal species exposed to conditioned environments have an increased expression of total RNA in the brain, as well as an increased translation of RNA products (Ferchim et al., 1970; Bennett, 1976; Grouse et al., 1979). Compared ratios of brain RNA to DNA showed that training causes an increase of this ratio in the rat cortex (Bennett et al., 1979). Such findings argue in favor of one of the earliest hypothesis, which enunciated that protein synthesis is required for memory storage (Katz & Halstead, 1950).

This issue was later investigated by different experimental approaches. One experimental approach was

based on the use of protein synthesis inhibitors, such as puromicin or cycloheximide, and although these substances are highly toxic in animals, their employment was useful to clarify that protein synthesis inhibition prevents memory formation after training (Barondes, 1970). However, when alternative protein synthesis inhibitors were employed, such as anisomycin (ANI), which is less toxic than the other two, this one did not prevent electrophysiological correlates of short-term habituation and sensitization in isolated ganglion of Aplysia (Schwartz et al., 1971). Although long term effects were not investigated when using this drug, it is quite difficult to argue that memory could be blocked or prevented when no data sustained that protein synthesis inhibition blocks long-term memory. One crucial finding, is that this drug has amnestic effects in rodents (Bennett, 1972). When ANI was administered at different doses so as to vary the duration of the amnestic effect in rodents, it was found that the stronger the training of animals, much more protein synthesis has to be inhibited to prevent long-term memory (LTM) (Flood et al., 1973, 1975). Moreover, for LTM to be established, protein must be synthesized in the cortex just after training, so that prevention of LTM should paralleled protein synthesis inhibition right after training. Both short-term memory (STM) and intermediate-term memory (ITM) are insensitive to protein synthesis inhibition, because they do not require protein synthesis for their formation (Bennett et al., 1972; Mizumori et al., 1985, 1987). So, there seems that different neurochemical processes underlie the formation of STM, ITM and LTM, respectively. This would make feasible according to Lashley's hypothesis (1950) that some kinds of memory appeared to be formed faster to allow growth of neural connections (this would be the case for LTM) considering that at that time no differences were made between STM and LTM), even though William James (1890) noticed the existence of two brain stores (as he defined them with different names). Based on these extreme distinctions (between STM and LTM) Hebb distinguished two types of memory processes: labile memory traces, and stable, structural memory traces.

Under such context, most of the neurochemistry of STM and LTM have been studied in avians, due to the fact that these animals can be trained rapidly for studying different stages of memory formation; can be tested either in short periods of time just after training or long after; learning and memory can be studied in the intact animal; and neurochemical processes involve in learning and memory formation occur relatively more slowly in chicks than in rats, for example, those which facilitate their characterization at each step during its consolidation (Rosenzweig, 1990; Rosenzweig et al., 1992). Under such basis, using the brain structure of chicks, several research works documented a cascade of events that are needed for memory formation, such as new synthesis of protein molecules that are substrates of the fine synaptic and neural plasticity (Ng & Gibbs, 1991; Rose, 1992a,b; Rosenzweig et al., 1992).

Though the concept of brain plasticity in relation to behavior started to clear out just a few decades ago, many evidences point out that training and experience produce numerous neurochemical and neuroanatomical changes in the brain tissue that allow the major changes needs it for long-term memory (Rosenzweig, 1996). Most chemical and molecular events are initiated by activation of organ specific receptors in sensory stimulated neural pathways. Neurotransmitters, such as ACh or glutamate have been shown to stimulate synaptic activity of afferent neurons that participate in the establishment of STM. Inhibition of such synaptic activity through blockage of ACh receptor activation by specific antagonist prevents STM. In a similar fashion, inhibition of glutamate receptor activation, such as the NMDA and AMPA receptors, affects STM formation. This memory process is also affected by altering calcium channel activity, K+ and Na+ channels, or inhibiting the activity of the major intracellular signaling pathways (e.g., adenylate cyclase, diacylglycerol), that relates to the generation of second messengers, such as cAMP and insositol phosphate (IP_3). Such messengers regulate the activity of several protein kinases or phosphorilation enzymes that catalyze the addition of phosphate residues to numerous protein molecules. Two kinds of protein kinases have been shown to be implicated in the formation of ITM and LTM; one refers to the calcium—calmodulin protein kinases (CaM-kinases), where pharmacological agents have been shown to inhibit ITM formation and others that do not inhibit CaM kinases, but potentially inhibit protein kinase A or protein kinase C affect LTM formation (Serrano et al., 1994; Rosenzweig et al., 1992). Using drugs that specifically inhibit PKC, an amnestic effect and decline of memory were seen in animals previously exposed to learning tasks and known to retain such learning for days and weeks (Serrano et al., 1995). Further studies demonstrated that training induces the activation and expression of immediate early genes and respective mRNA in the chick forebrain (Anokhin & Rose, 1991), and increases of dendritic spine density (Lowndes & Rose, 1994). Most of these effects were more conspicuously detected in the left hemisphere of chicks than in the right hemisphere. The neurochemical and molecular events involved in the memory formation in the animal model of the chick

have been shown to be extremely similar to the molecular cascade implicated in long-term potentiation in the mammalian brain (Colley & Routtenberg, 1993) as well as in the nervous system of invertebrates (Krasne & Glanzman, 1995). Interestingly enough, the endogenous opioid neurotransmission systems in avians, mammals and invertebrates including both synthetic and non-synthetic ligand agonists that activate such neural system impair memory formation. In a similar but opposite way, opioid antagonists enhance memory formation. As several experimental works have shown, several endogenous opioid substances seem to participate and regulate memory formation at different stages (Colombo et al., 1992, 1993; Patterson et al., 1989; Rosenzweig et al., 1992).

Thus, several experimental evidences have revealed that learning and experience induce chemical changes in the brain and that inhibition of specific chemical events around the time of learning blocks memory formation (Rosenzweig et al., 1996). Several parameters on the changes in the neural process involved in learning and memory have been considered as necessary and sufficient for formation of memory (Rose, 1992a), which briefly are described as follows:

A) As pointed out by several lines of research, localized region of the brain (as defined from the chick´s brain) during memory formation may show changes in the quantity of the system or substance, or demonstration of change of its rate of production or turnover.
B) The amount of change should be related to the strength or amount of training, up to a limit.
C) Related neural processes such as stress, motor activity, or others that accompany learning must not show structural or biochemical changes.
D) Those cellular and biochemical changes if inhibited during the normal period that memory formation occurs, then memory formation should be prevented and the animal should be amnesic (criteria paraphrased by Rose, 1992a).

Other criteria established and previously taken as important for memory establishment, as based for LTM formation is:

E) Removal of the anatomical site at which any biochemical, cellular and physiological change occurs should interfere with memory formation, and depending upon when, in relation to training this region is removed (Flood et al., 1973). This last criterion, has been recently criticized due to the fact that in some cases it has been shown that after removal of the primary area for memory formation, memory can consolidate in a secondary region, as shown from electrophysiological evidences and established in the next criteria:
F) Neurophysiological recording from the sites of cellular change should detect altered electrical responses from neurons during memory formation or a consequence from its result; and the time course of such cellular change must be compatible with the time course of memory formation (Entingh et al., 1975). In a similar context, several authors have pointed out that the brain regions involved in learning and memory storage should be identified by several converging parameters, such as neurochemical changes and inhibition of neurochemical events, corroborated by electrophysiological recordings and supported by lesion studies mentioned in the criteria previously listed. Thus, long-term potentiation (LTP) as pointed out, is the best single candidate to support that synaptic plasticity as part of a whole cellular process are crucial for memory formation, including those neurochemical, electrophysiological and neuroanatomical changes associated to LTM formation (Martinez & Derrick, 1996).

If learning induces anatomical changes in the nervous system, the induced plastic changes should parallel long-term memory storage. As described previously, neuroanatomical experiments have revealed that changes in the number of synapses and the degree of dendritic branching relate to the amount and sites of learning or experience (Greenough et al., 1990; Morris, 1989; Martinez & Derrick, 1996). In such context, several statements have been established supporting that the plastic changes induced in the brain equal LTM. For instance, one statement defines that the amount of dendrite density per neuron in the occipital cortex of the rat relates to the amount of stimulation or learning about its environment (after animals have been subjected to an enriched environment). Similar statements argue that changes in dendritic branching parallel changes in the number of synapses per neuron as they seem to be induced rapidly by training of a learning task, or similarly, by experience in an enriched environment. Most of the effects describing synaptic and dendritic changes in rodents have been also found in other mammals such as the cat and macaccus. Moreover, these morphological changes induced in the brain by training or learned-experience are quite specific, their occurrence is greater and different from that produced by mere activity. Furthermore, these changes are restricted to those brain areas involved specifically in learning processing and must parallel neurochemical events in the same specific brain regions (e.g., if learning of a detailed task is confined to one side of the brain, plastic changes occurring at synapses and dendrites must be localized exclusively in that side

of the brain). Several conductive experiments demonstrated that training and experience increased spacing of neurons in specific cortical regions (Witelson et al., 1994).

NEURAL NETWORKS AND PLASTIC CHANGES

During the past decades neuroscientists have been concerned with the elucidation of how synaptic changes can store information more than how neural networks can compute information. Since Hebb´s initial proposition that synaptic changes support the formation neural networks, investigators were interested in studying different complexities of neural circuits in order to better comprehend how information is stored and memory responses computed (Rosenzweig, 1996). For instance, using simplest experimental models of neural circuits, such as the monosynaptic reflex arc, it was possible to document the mechanisms of most simple forms of learning, as is habituation (Kandel, 2000; Kandel et al., 1987; Kupferman & Kandel, 1969) and sensitization (Kandel, 2000). As synaptic changes have been reported in monosynaptic reflex arc, similar changes necessary for learning and memory consolidation must occur in simple neural systems present in non-vertebrate species, as elementary forms of learning and memory (see box 1-3). For instance, in Aplysia, gill-withdrawal response persists and can be altered by training after surgical removal of the abdominal ganglion (Mpitsos & Lukowiak, 1985). Moreover, when this marine specie engages in sexual activity or has eaten, the CNS enters into a suppressed state, but even when central neurons are inactivated, the animal still responds to gill-withdrawal, a reflex mechanism, mediated by the peripheral nervous system of the organism (Leonard et al., 1989).

Current theories have proposed that same neural circuits can encode different types of memories, whether one neuron in each neural circuit could participate from a greater to a lesser extent in a particular form of memory (McNaughton & Morris, 1987). This is supported by several experimental observations that have shown that modification of the gill-withdrawal response in Aplysia depends not on a few ensemble of neurons in a simple monosynaptic reflex arc, but on a parallel distributed processing of large number of neurons, in which synaptic changes and plasticity occurs. For instance, from the 1000 neurons present in the abdominal ganglia of Aplysia, just 200 seem to respond to the touching of the siphon (Zecevic et al., 1989). These same numbers of neurons are responsible for the gill-withdrawal reflex as for breathing function (Wu et al., 1994). The fact that single neurons mediate different responses is because these neurons operating in a large neural network are capable in generating different activities, as opposed to the activity of neurons anatomically integrated into separate individual small neural systems mediating each response. Moreover, studies of learning and memory processes in birds and mammals demonstrate that these neural functions are spread all over the brain, as Hebb previously believed that memory occurs in cell assemblies.

Studies on aversive conditioning, it has been shown that several brain structures participate in the eyelid reflex conditioning, including simple neural substrates mediating the reflex response as well as complex structures such as the hippocampus and cortex, which are involved in complex forms of learning, thus, they seem to be influenced by aversive classical conditioning (Lavond et al., 1993). Furthermore, non invasive imaging techniques have shown that several brain areas in human subjects are specifically activated during eyelid conditioning, such as the cerebellum, pontine tegmentum, ipsilateral inferior thalamus/red nucleus, ipsilateral hippocampal formation, lateral temporal cortex and bilateral ventral striatum (Logan & Graffton, 1995).

Similar neuroimaging studies coupled to the research of several types of working memory, demonstrated that the performance of delayed response-tasks induce preferentially the activation of prefrontal cortex, including other brain regions that depend on the specific stimuli and detailed task performed by the subject (McCarthy, 1995). Although brain regions seem to cooperate in task performance, it should be more important to evaluate the neural circuits that facilitate such learning and memory processes in the human brain.

BRAIN PLASTICITY AND APPLICATIONS

Several works using induced brain plasticity have been adapted to clinical applications. For instance, the importance of the early experience to child´s intellectual development has been previously demonstrated and reported as well (Hunt, 1979). Despite of the various demonstrations showing that the lack of adequate intellectual stimulation can cause mental retardation, and that enriched stimulation or enriched conditioning can foster normal development, several attempts have been proposed to explore how enriched environments could improve the cognitive status of children raised in poor environments (Zigler & Muenchow, 1992). Several researchers have claimed that many cases of mild retardation are preventable and/or treatable by

appropriate early training and experience (Satcher, 1995). Also, several findings have shown that early-enriched experience, beginning early in life, helps to ensure the maintenance of several abilities in old age. For instance, infantile handling or late enriched experience helps to prevent hippocampal damage causes by stress (Meaney et al., 1988, 1991). Moreover, in experiments where neonatal rat pups are handled along their first 21 days, cognitive function such as performance of spatial memory are improved in such animals when tested at 3-24 months of age as compared to non handled neonatal rat pups. Similarly, old age rats expressed a large number of hippocampal corticosteriod receptors and an upgrade metabolic turnover of corticosterone to basal levels after stressful stimulations (Sapolsky, 1992). In addition, old handled age rats have low levels of corticosterone and lesser loss of hippocampal neurons after gluco-corticoid administration (whose chronic adminis-tration results to be toxic for hippocampal neurons particularly in aged rats) than unhandled adult rats. Furthermore, young rats exposed to 30 days of enriched conditioning experience showed, as infantile handled animals, higher expression of gene encoding glucocorticoid receptors in the hippocampus. These animals have increase expression of the nerve growth factor as a result of increase

BOX 1. SIMPLE FORMS OF LEARNING AND MEMORY

Cellular studies on memory storage show that most elementary forms of learning or implicit learning can be examined as simple behaviors in several invertebrate species, which are expressed as a variety of vertebrate reflexes. Such implicit forms of learning that mediate extensive kind of behaviors result from changes in the effectiveness of synaptic connections that make up the required network to express from simple to complex behaviors.

Habituation. The simplest form of implicit learning is when an animal learns about the properties of a non-noxious novel stimulus that is neither beneficial nor harmful. The animal first responds to the stimulus with a series of orienting responses, and subsequently learns after repeated exposure to ignore it. Similar responses occur with certain types of postural reflexes, in which repeated stimulation causes a decreased response to subsequent stimulus. Thus an habituation process of the stimulus has resulted due to the diminished synaptic effectiveness in the motor neuron pathways after consecutive repeated activation.

Intracellular recordings of spinal motor neurons that participate in spinal flexion reflexes in mammals have shown that habituation leads to a decrease in the strength of synaptic connections between excitatory interneurons and motor neurons. Since the neuronal organization in spinal cord of vertebrates is quite complex, much of the understanding of the cellular mechanism that drive the most simple forms of learning behaviors, such as habituation, sensitization and classical conditioning has been routinely analyzed in the simple nervous system of invertebrates, such as the marine sea slug, Aplysia Californica. Such animal models of learning and memory respond to stimuli with simple defensive reflexes by withdrawing superficial structures such as the gill and siphon. For instance, a mild tactile stimulus delivered at the siphon elicits a reflex withdrawal on both siphon and gill. These reflexes are mediated through the activation of sensory neurons that innervate the siphon, generating excitatory synaptic potentials in both interneurons and motor neurons. Thus, the monosynaptic excitatory synaptic potentials generated by sensory neurons impinging both interneurons and motor neurons, cause these cells to discharge repeatedly, leading to the initial response of a strong reflexive withdrawal of the gill. Upon repeated stimulation, these excitatory postsynaptic potentials decrease in both motor neurons and interneurons that parallely innervate motor neurons as well. Thus, the net result of such learning process is a decrement of the reflex response. Furthermore, such decrease of the synaptic strength results from a decrement in the number of transmitter vesicles in presynaptic terminals of sensory neurons and, therefore, in the release of glutamate transmitters employed as the chemical signal in this neural network. Both glutamate receptors expressed in motor neurons even show no changes in sensitivity once habituation is established, though this response reduction, as a result from habituation, lasts several minutes.

Physiologically, 90% of the sensory neurons in the Aplysia makes detectable synaptic connections into the gill motor neurons. However in animals trained to long-term habituation the same synaptic connections are reduced to 30%. This plastic changes last for a week and do not recover even for up to three weeks after the training. Moreover, at the structural level, this long-term inactivation of synaptic transmission is due to morphological changes in the sensory neurons. Not all synapses are reinforced by repeated stimulation, and moreover, the strength of those activated synapse are not easily changed with repeated activation. However, only those synaptic connections that mediate the withdrawal reflex, that have learned about the properties of the stimulus, and therefore, are involved in learning and memory storage, produce dramatic changes in synaptic strength, even when a small amount of training and applied stimuli are spaced out for minutes or hours. When repeated habituation stimuli are applied, with no resting periods between training session, a short-term memory is produced, but not a long term memory. This illustrates that for effective learning to be produced, spatial training is required to induce a long-term memory process (Kandel, 2000; Bayley & Chen, 1983; Castelluci et al., 1978; Thompson & Spencer, 1966).

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induction of the coding genes (Mohammed et al., 1993; Olsson et al., 1994). Therefore, enriched experience in adulthood, as suggested by several authors (similar to infantile handling) protects the aging of the hippocampus from glucocorticoid neurotoxicity.

Although, is it known that certain kinds of learning and performance decline with age after middle adulthood, other kinds of memory remain. For instance, people who are under continued learning activities obtain high levels of performance (Shimamura, 1995). More interestingly, several reports have shown that the severity of symptoms of Alzheimer's disease correlates strongly with loss of synaptic connections. Thus, as previuosly shown, enriched experience produces abundant neural networks, synaptic density and dendritic branching in several species so far studied. Thus, if similar conditions occur in the human brain, it could be postulated, that enriched experience and intellectual function of the brain should protect the reserve synaptic connections in adulthood

BOX 2. SIMPLE FORMS OF LEARNING AND MEMORY

Sensitization. Similarly to what occurs when a harmless stimulus is presented to an animal, and it learns to habituate to it, a harmful stimulus produces an intense response, so that the animal learns to respond equally to other non related stimuli, even to harmless ones. These withdrawal defensive reflexes are actually heightened when animals are exposed to harmful stimuli, and as such, allows the animal to escape from danger, helping him to react to nature itself and adapt him to his own environment. This natural behavioral response, the enhancement of reflex responses, is more complex than the habituation process. Thus, any stimulus applied to a neural pathway activates another reflex pathway, causing a synaptic strength in its neural connectivity, enhancing a reflex strength. For instance, a noxious stimulus applied to the tail, enhances synaptic transmission at several neural connections in the circuit network that mediates the gill withdrawal reflexes. A single shock to the animal's tail induces a short-term sensitization that lasts for minutes; prolonged stimulation induces a long lasting sensitization process which lasts from days to weeks. More interesting is the finding that similar connections that enhance the habituation learning process are involved in producing both short and long-term sensitization. This means that a single synapse participating in a learning processes and memory storage, may participate in other learning process and store more than one memory process as well. As such, a sensitizing stimulus can override the effects of habituation, an effect named by electrophysiologists as dishabituation. For instance, once a startle response to a noise is reduced by habituation, the initial intensive response to such stimuli can be restored through the application of a strong pinch. Although synaptic connectivity between both learning processes might be the same, the cellular mechanisms involved in each process are totally different when producing synaptic changes. While short-term habituation in this simple neural systems (Aplysia) is based in a homosynaptic process in order to provoke a decrease in synaptic strength, sensitization requires a heterosynaptic process that enhances the synaptic strength in modulatory or facilitating interneurons, that highlights the withdrawal reflex response a result of the enhanced synaptic connectivity in motor neurons. These facilitating interneurons release serotonin as neurotransmitter, therefore, serotoninergic transmission as the one that seems to be implicated in this learning-memory process, is made up by axo-axonic synapsis made between interneurons and presynaptic terminals of the sensory neurons. Molecular studies on this aminergic transmission have shown that serotonin and other neuromodulatory transmitters released from interneurons activate membrane-spanning protein receptors that are coupled to the heterotrimeric GTP binding proteins, activating proteins such as the $G\alpha s$. Similarly to the activation of GTP-coupled membrane protein receptors by natural endogenous ligand agonists in the nervous systems of mammals (Schulman & Hyman, 1998; Cooper et al., 1996; Gilman, 1995; Hill, 1994; Strader et al., 1994); Go/Gs proteins stimulate a intracellular signaling mechanism which includes the activation adenylyl cyclase by Go/Gs proteins that enhance the production of cAMP, and in turn, through the sequential activation of cAMP protein kinases, such as the protein kinase A (PKA) and protein kinase C, phosphorilate several intracellular protein substrates, that finally facilitate the release of neurotransmitters from presynaptic terminals of sensory neurons. As will be discussed later such molecular mechanisms, are involved in strengthening synaptic connections induced by repeated sensitizing stimuli, facilitating the establishment and consolidation of both short and long term memory storage. Repeated experience produces changes in the neural system, so that repeated stimulation converts short-term memory process into a long term memory form (Kandel, 2000; Chain et al., 1999; Hawkins et al., 1993).

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from the effects of the Alzheimer's disease (Terry et al., 1995). Therefore, enriched experience and used of cognitive faculties early in life, will set the functional maintenance of the brain and the mental capabilities in late adulthood and old age (Rosenzweig, 1996).

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BOX 3. SIMPLE FORMS OF LEARNING AND MEMORY

Classical conditioning. In essence, associative learning involves the formation of associations among stimuli and/or responses. It is generally subdivided into classical versus instrumental conditioning or learning. Classical or Pavlovian conditioning is the procedure in which a neutral stimulus, termed a conditioned stimulus (CS), is paired with a stimulus that elicits a response, termed an unconditioned stimulus (US), for example, food that elicits salivation or a shock to the foot that elicits limb withdrawal.

According to the traditional view, classical or Pavlovian conditioning is an operation that pairs one stimulus, the conditioned stimulus or CS, with a second stimulus, the unconditioned stimulus or US. The US reliably elicits a response termed the unconditioned response or UR. Repeated pairings of the CS and US result in the CS eliciting a response, defined as the conditioned response of CR. Critically important variables are:

1. Order: the CS precedes the US.
2. Timing: the interval between CS and US is critical for most examples of conditioning.
3. Contiguity: the pairing of contiguity of the CS and US is necessary for conditioning.

The traditional view of Pavlovian conditioning emphasized the contiguity of the CS and US. A more general and contemporary view of Pavlovian conditioning emphasizes the relationship between the CS and the US. That is, the information that the CS provides about the occurrence of the US is the critical feature for learning. This perspective on Pavlovian conditioning is consistent with current cognitive views of learning and memory. Indeed, in some situations the CR is quite different from the UR: foot shock causes an increase in activity (UR) in the rat; fear learned to a tone paired with this same foot shock is expressed as freezing (CR). Note, however, that both these responses are adaptive.

Conditioning involves learning about the relations between events in the organism's environment. In this view, contingency is a key factor in organizing the organism's environment. Consider the following experiment. A group of rats is given a series of paired stimuli in which tone (CS) and foot shock (US) are paired. The animals learn very well to freeze (CR) when the CS occurs. Another group of rats is given the same number of paired CS-US trials but is also given a number of presentations with the US alone. Animals in this group do not learn to freeze to the CS at all. Both groups had the same number of contiguous pairings of CS and US, but the contingency, the probability that the US is predicted by the CS, was very much lower in the group that was also given trials with the US alone (Rescorla, 1988).

Delivering a US, such as an electric shock to the tail or a peripheral nerve, releases a modulatory neurotransmitter, such as 5-HT, that nonspecifically enhances transmitter release from the sensory neurons. This nonspecific enhancement contributes to short-term sensitization. The associative learning results from the pairing of a CS (e.g., spike activity in one sensory neuron) with the US, an interaction that causes a selective amplification of the modulatory effects of the US in that specific sensory neuron. Unpaired activity does not amplify the effects of the US. The amplification of the modulatory effects in the paired sensory neuron leads to a pairing-specific enhancement of transmitter release from the sensory neuron.

In this proposed mechanism, increased Ca^{2+} levels resulting from spike activity in the sensory neuron alter adenylate cyclase levels via calmodulin and increase the cAMP level produced by 5-HT. Thus, Ca^{2+} and CaM appear to play a role in the activity-dependent neuromodulation underlying associative conditioning of the tail and gill withdrawal reflexes. In addition, activity and changes in the intracellular levels of Ca^{2+} in the postsynaptic neuron (i.e., motor neuron) may also contribute to associative changes in synaptic strength at the sensory-motor neuron synapse (Lin and Glanzman, 1994; Lechner and Byrne, 1998).

An important conclusion is that short-term associative learning operates via a mechanism that is an elaboration of the cAMP-dependent mechanisms contributing to a simpler form of learning-sensitization. This finding raises the interesting possibility that even more complex forms of learning may be achieved by using these simpler forms as building blocks. Indeed, theoretical studies have shown that a mathematical model of the learning rule (activity-dependent neuromodulation) for simple classical conditioning, when incorporated into simple neural circuits, has the capability to simulate higher-order features of classical conditioning such as second-order conditioning and blocking, as well as features of operant conditioning (Byrne, et al., 1990).

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