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SYNAPTIC PLASTICITY STUDIES AND THEIR APPLICABILITY IN MOUSE MODELS OF NEURODEGENERATIVE DISEASES

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Abstract

During the past few years, there have been many important contributions to the better understanding of the different types of memory and the putative neural structures that generate them. Moreover, various studies on neurodegenerative diseases in human beings have added useful information about learning and memory formation, and about their loss in patients with these disabling diseases. The development of sophisticated pharmacogenetic tools applied to mouse models, reproducing different types of neurodegenerative disease or, at least, some of their main symptoms, has turned out to be extremely useful for the further development of contemporary neuroscience. In addition, ingenious behavioral and electrophysiological approaches have been developed to study the activity-dependent changes in synaptic strength during learning and memory processes in the best possible way – that is, in the alert behaving animal. Collected data from these numerous studies have enabled us to know more about the role of many different molecular components integrating the synaptic cleft, and to draw some conclusions about the concordance between *in vitro* and *in vivo* recorded data, and the generalization of the results to other types of learning and/or brain-related structures. As described here, changes in synaptic strength studied in key neural synapses during learning and memory processes in genetically manipulated mice can represent an interesting and powerful approach to the better understanding of neural processes underlying the acquisition of new motor and cognitive abilities and how they are affected by different human diseases involving the neural tissue.

Keywords

• Synaptic strength • Long-term potentiation • Learning and memory

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Learning and memory deficits in neurodegenerative diseases

Both acute and chronic brain damages are caused by a wide range of different disorders such as stroke, trauma, or neurodegenerative processes, most of them including the progressive loss of structure and/or function of the neurons involved [1]. Many neurodegenerative diseases, including Parkinson's disease, Alzheimer's disease, and Huntington's disease, occur as a result of a variety of devastating processes, which can affect numerous different functions of neuronal circuitries, ranging from the molecular level to the behavioral and physiological ones. With advancing research, many similarities have appeared these disorders, mainly at the subcellular level [2].

One of the greatest risks for neurodegenerative diseases is aging. Indeed, aging can be accelerated by mitochondrial DNA mutations, as well as by oxidative stress. The behavioral and neural alterations associated with aging are usually accompanied by altered medial temporal and frontostriatal systems —i.e., those that make possible episodic memories and executive functions. Moreover, aggregated b-amyloid protein plaques, among others causes, have substantial effects on neural circuits involving memory functions. However, it has been observed that the same neural systems can be differently affected in individuals of the same age [2].

Memory impairment is widely accepted as being one of the most-common complaints affecting patients with neurodegenerative diseases [3,4]. Memory includes three neural information processes (encoding, storage, and retrieval) and it is the basic mechanism for learning, by which the nervous system adapts to environmental constraints by generating new adaptive behaviors. The investigation of human memory in neurodegenerative disorders suggests that the interaction of networks subserving episodic, semantic, and working memories contributes to the fundamental homeostatic processes of retrieval and learning. Among other neural structures, the hippocampus has been widely used as a model structure for the study of different cortical functions (learning, memory, emotion, motivation, etc.) and, in general, of many different types of plastic neural mechanism. Therefore, the hippocampal formation is identified as an excellent experimental subject for the study of the changes in strength that take place at the synaptic level during a wide variety of learning and memory tasks, as well as in specific clinical disorders [5].

Some of the above-mentioned aspects have been deeply studied in rodent models. For example, naturally aging mice show clear impairments in both associative learning and synaptic plasticity. Indeed, it has been reported that 18-month-old mice are unable to acquire conditioned eyeblinks in a hippocampal-dependent trace paradigm, whereas 3-month-old animals acquire this associative learning normally [6]. Interestingly, identical results

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are found in different mouse models of Alzheimer's disease [single-transgenic (APP, PS1) and double-transgenic (APP+PS1) mice] of the same age. Collected results from mice of intermediate age (12 months old) indicate that double-transgenic (APP+PS1) and single-transgenic (PS1) animals present an earlier impairment for the acquisition of associative learning than their wild-type and transgenic littermates, suggesting that b-amyloid deposits accelerated in some unknown way the decline of learning capabilities in mice. The intermediate-aged mice show more variability in the behavioral and physiological tests, compared with the categorically young or old mice, mainly in Alzheimer's disease models. These results are similar to those collected from humans [2]. Thus, it seems evident that factors besides plaque deposits are involved in the functional deficits observed in aged wild-type and transgenic (APP and/or PS1) mice [6,7].

Aging studies can also provide some unexpected data. A recently published study [8] demonstrated that mice lacking DNA polymerase m (Polm$^{-/-}$) maintain their learning and hippocampal synaptic facilitation capabilities at ages when wild-type mice do not. DNA polymerase m is a novel accessory partner for the non-homologous end-joining DNA repair pathway for double-strand breaks, and its deficiency causes reduced DNA repair. The absence of Polm could produce a less efficient but more conservative non-homologous end-joining repair, affecting mitochondrial biological efficiency and maintaining a lower chronic rate of reactive oxygen species (ROS) generation. Thus, the global physiological cell status can delay the typical organism evolution that accompanies aging.

Synaptic plasticity

The term synaptic plasticity was introduced by Jerzy Kornoski in 1948 to describe changes in the synaptic strength of preexisting synapses evoked by the acquisition of new motor or cognitive abilities. Many neuroscientists (included the neurohistologist Santiago Ramon y Cajal) had already proposed these changes in synaptic efficacy as the mechanism underlying information storage in the brain [5]. This proposal was well accepted, and one of the most-basic assumptions of contemporary neuroscience is that newly acquired learning capabilities are registered and stored in the brain in the form of functional (and/or structural) changes in synaptic efficiency [9-12]. Given the number of functions ascribed to synaptic plasticity, it can be assumed that more than one mechanism will be needed to explain it. A further proposal is that all excitatory synapses in the mammalian brain simultaneously express different forms of synaptic plasticity [13].

There are many excellent studies on the subcellular and molecular events underlying learning-dependent synaptic changes in animals, as well as on the electrophysiological (*in vitro*) processes feasibly related to learning and memory phenomena generated *in vivo* [10,14-16]. For many years, however, not much information was available regarding synaptic functional events taking place during the learning process in alert behaving animals. This experimental limitation was an important drawback for the proper understanding of functional neural states supporting the acquisition of new motor and/or cognitive abilities in humans [12,17]. A significant change began with the availability of new data on synaptic plasticity mechanisms collected during learning and memory tasks in alert behaving rodents in three different laboratories [18-20]. These three important contributions to the better understanding of neuronal mechanisms taking place during actual learning were mentioned in Science Journal's list of the top ten breakthroughs of the year 2006.

Synaptic plasticity induced experimentally

There is broad agreement that long-term potentiation (LTP) is the leading candidate to be the neural mechanism underlying memory processes [11,13,14,16,21]. LTP is usually evoked (both *in vitro* and *in vivo*) by high-frequency stimulation (HFS) of selected afferent pathways, resulting in a long-lasting enhancement of synaptic efficacy or strength. The hippocampus has been widely used as a model structure for the study of different high functions (such as learning, memory, emotion, motivation, etc.) and, in general, of many different types of plastic neural process. Indeed, the hippocampal formation is identified as a suitable structure for the study of the changes in strength that take place at the synaptic level during a wide variety of learning and memory tasks, as well as in more-or-less specific neurodegenerative disorders [5]. For many years, the experimental analysis of the properties and mechanisms of LTP has concentrated on the Hebbian form of synaptic plasticity exhibited by the perforant path projection to granule cells of the dentate gyrus and by Schaffer-commissural afferents to the pyramidal CA1 area (CA3-CA1 synapse).

In a series of experiments carried out in our laboratory in alert behaving mice, we have studied LTP processes at the hippocampal CA3-CA1 synapse and their relationship with associative learning [12,17]. Prior to any experimental procedure, mice are implanted under anesthesia with tungsten electrodes for the proper recording (CA1 area) and stimulation (CA3 area) of the CA3-CA1 synapse (Figure 1A). The LTP protocol starts with single stimuli (3/min) presented at Schaffer collaterals to obtain a baseline [18]. Schaffer-collateral stimulation evokes a field excitatory postsynaptic potential (fEPSP) in the CA1 area (Figure 1C) that is the result of the multiple excitatory postsynaptic potentials. The shape of the evoked fEPSP depends on the final placement of the extracellular recording electrodes: it is small and positive when recordings are collected from the somas, and large and negative when recordings are collected from apical dendrites (Figure 1D). After HFS, the previous single stimulus is presented for 2 h at the same rate (3/min). In successful experimental protocols, the fEPSP of wild-type mice increases to 150-200% of baseline values, and this potentiation is sustained for several days (for methodological details, see [18,22,23]).

Synaptic plasticity induced by learning

When LTP was proposed as the most plausible mechanism for memory storage, the

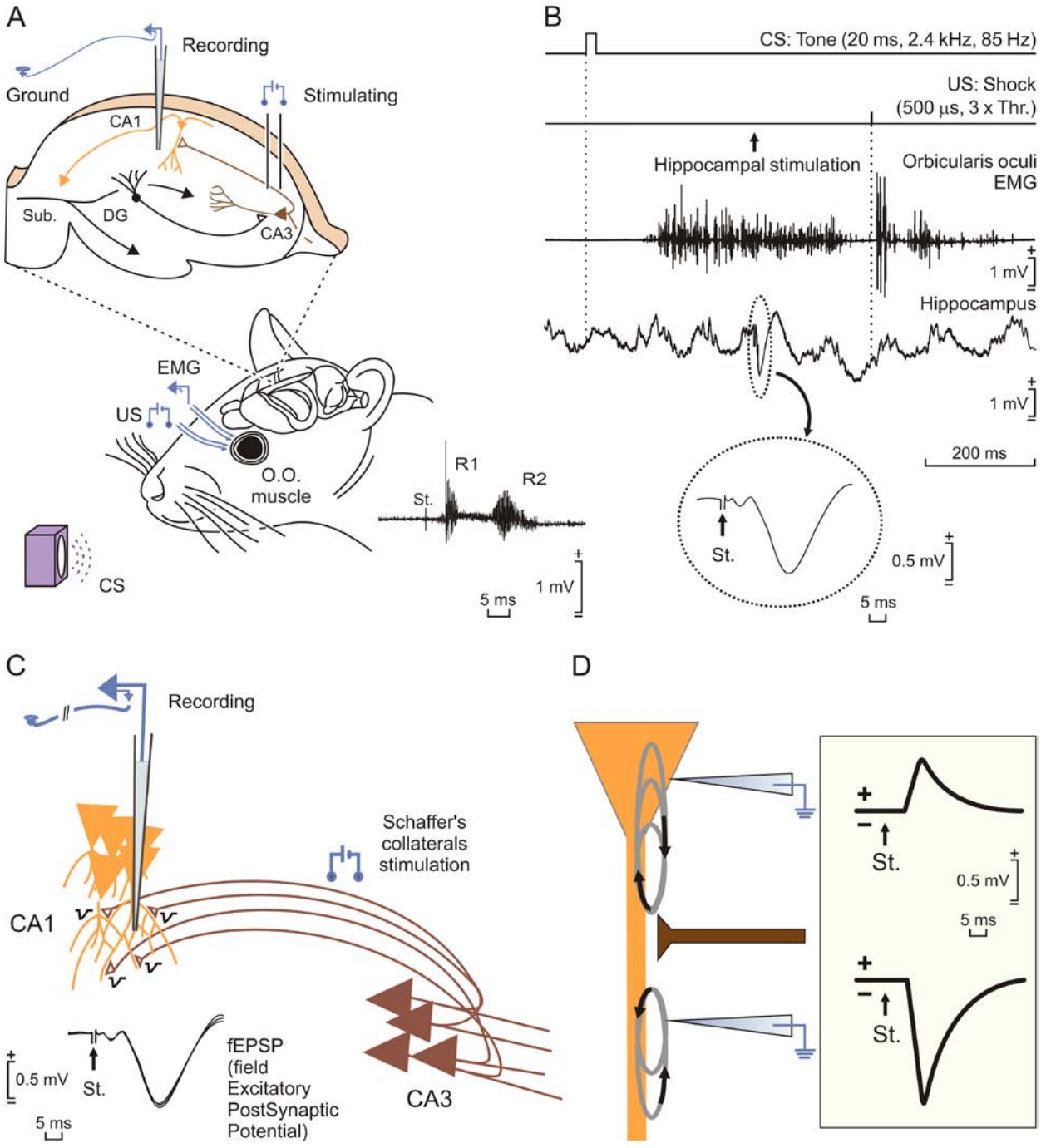

Figure 1. Experimental design for studying long-term potentiation and classical conditioning of eyelid responses in alert behaving mice. A) Location of chronically implanted electrodes for stimulation and recording of cortical neural sites. The loudspeaker for the conditioned stimulus is placed in front of the animal. At the bottom right is illustrated an example of blink reflex evoked in the *orbicularis oculi* (o.o.) muscle by the electrical stimulation (St.) of the ipsilateral trigeminal nerve. The short- (R1) and long- (R2) latency components are characteristic of the blink reflex in mammals. B) Schematic representation of the conditioning paradigm, illustrating the conditioned (CS) and unconditioned (US) stimuli, an example of an electromyographic (EMG) recording from the *orbicularis oculi* muscle, as well as an extracellular recording of hippocampal local field activity. Short arrows indicate the moment at which a single pulse (100 ms; square, biphasic) is applied for hippocampal stimulation. The inset shows in detail the evoked field excitatory postsynaptic potential (fEPSP). C) Schematic drawing indicating that the fEPSP recorded in the hippocampal CA1 area is the result of excitatory postsynaptic potentials induced by Schaffer-collateral stimulation in multiple CA3-CA1 synapses. D) A diagram illustrating that the final placement of the extracellular recording electrodes defines fEPSP shape and sign: small and positive in the somatic area and large and negative in the apical dendrites. (Reproduced and modified with permission from Gruart *et al.* 2006 [18])

experimental data were obtained mainly from *in vitro* experiments. Thus, it became necessary to find out whether, during actual learning and memory processes in behaving animals, some facilitation could be recorded in key cortical synapses that would reproduce, in some ways at least, the LTP phenomenon [18,20]. One of the most-extended experimental models for studying neural processes underlying learning is the classical conditioning of eyelid responses, using a trace paradigm (Figure 1B). For evoking this classical conditioning task, a tone is presented to the experimental animal as a conditioned stimulus (CS) and an electrical stimulation in the supraorbitary branch of the trigeminal nerve, some milliseconds afterwards, as an unconditioned stimulus (US) [18]. At the same time, the fEPSP evoked in the hippocampal CA1 pyramidal cells by the electrical stimulation of the ipsilateral Schaffer collateral-commissural pathway can be recorded *in vivo* (see inset in Figure 1B). The changes in strength of fEPSPs evoked at the CA3-CA1 synapse during the CS-US interval can be followed during habituation, acquisition, extinction, retrieval, and reconditioning sessions. In order to prepare the experimental mice for classical eyeblink conditioning, during the surgery, a pair of electrodes is implanted for stimulation of the trigeminal nerve (subsequently used as unconditioned stimulus), whilst a second pair of electrodes is implanted in the *orbicularis oculi* muscle to record its electromyographic activity during eyelid responses (Figure 1A). Implanted electrodes do not disturb blink kinematics, and allow the normal generation of spontaneous, reflexively evoked, and classically conditioned eyelid responses [18]. Tungsten electrodes for proper recording (CA1 area) and stimulating (CA3 area) procedures are also chronically implanted (Figure 1A).

During classical eyeblink conditioning, mice present a typical learning curve that reaches the maximum percentage of conditioned responses by the 10th conditioning session (Figure 2A). An important question is whether this type of associative learning can modify the synaptic strength of the hippocampal CA3-CA1 synapse. Results collected in our laboratory [18] convincingly show that the

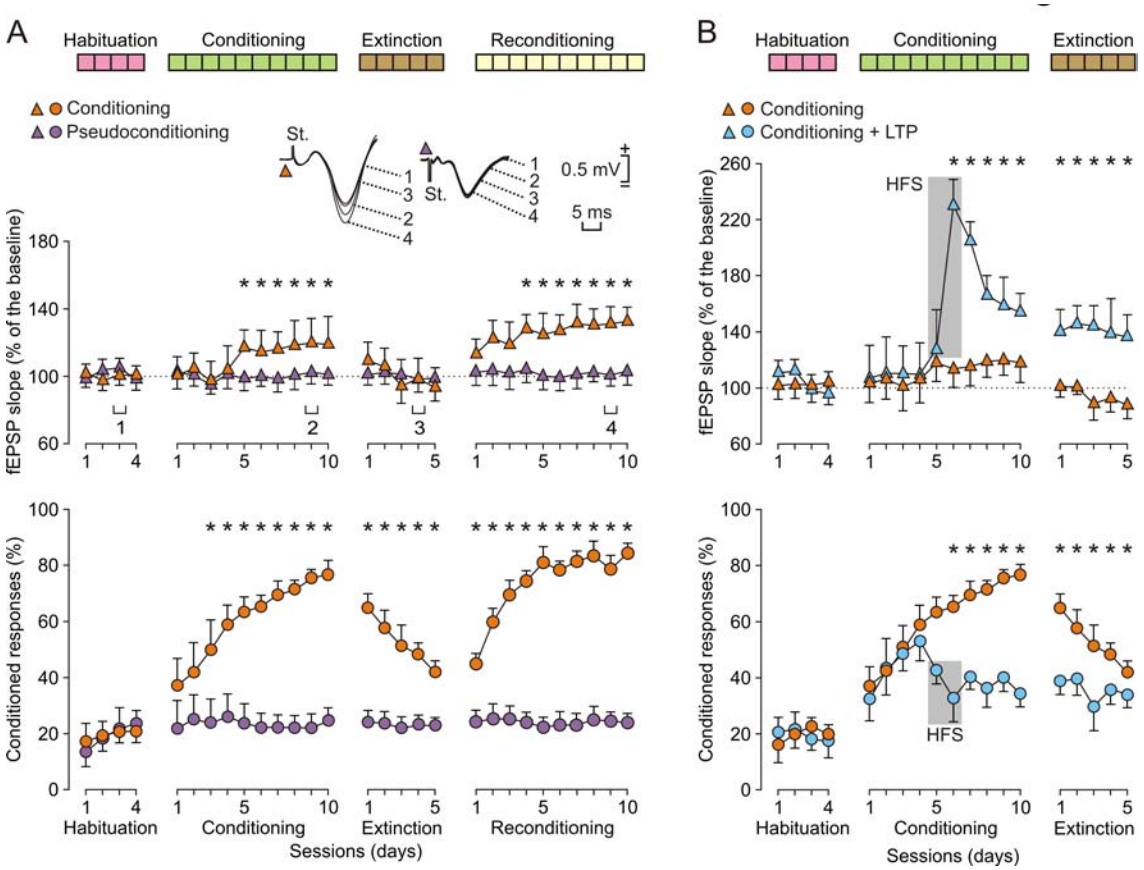

Figure 2. Evolution of changes in synaptic strength during classical eyeblink conditioning and following the occlusion effects produced by experimentally evoked long-term potentiation (LTP). A) Synaptic field potentials and learning curves for conditioned (orange symbols) and pseudoconditioned (violet symbols) mice during four consecutive learning phases: habituation, conditioning, extinction, and reconditioning. At the top are illustrated selected examples of field excitatory postsynaptic potentials (fEPSP) recorded in the CA1 area after a single pulse presented to the ipsilateral Schaffer collaterals 300 ms after conditioned stimulus presentation, in a conditioned (left) and in a pseudoconditioned (right) mouse. Recordings correspond to the penultimate session of each learning phase (1-4). B) Synaptic field potentials and learning curves for control (conditioning, orange symbols) mice and for mice that received two high-frequency stimulation (HFS) sessions during the 5th and 6th conditioning sessions (conditioning + LTP, blue symbols). Note that the experimentally evoked LTP evokes an occlusion effect in the learning process. (Adapted and reproduced with permission from Gruart *et al.* 2006 [18])

slope of evoked fEPSPs increases progressively across conditioning, reaching maximum values during the 8th and 9th conditioning sessions (Figure 2A). In contrast, the percentage of conditioned responses and fEPSP values collected during pseudoconditioning sessions (i.e., during the unpaired presentations of tones and eyelid shocks) do not show any significant change. Finally, the percentage of conditioned responses and the increase in fEPSP slopes are maintained during retrieval sessions (carried out a week after the last conditioning session) and they are still easily recovered, and even exceeded, during reconditioning sessions (Figure 2A).

The involvement of hippocampal unitary activity in classical conditioning of nictitating membrane/eyelid responses is very well known [12]. Using unitary *in vivo* recordings, it is found that hippocampal pyramidal cell firing to CS presentations increases several sessions in advance of behavioral conditioning [24,25]. Although the discharge rate of hippocampal CA1 pyramidal neurons seems not to encode the kinetic peculiarities of conditioned eyelid responses, it is linearly related to the progressive acquisition of the eyelid learned response [25]. The slow building up of hippocampal neuronal responses across conditioning is similar to the small increase in the slope of fEPSPs evoked at the apical dendrite of CA1 pyramidal cells by single pulses applied to Schaffer collaterals during trace conditioning in mice [18]. The reported modulation of CA3-CA1 synaptic strength during acquisition, extinction, retrieval, and reconditioning sessions seems to be a slow process originated by changes in the probability of releasing synaptic vesicles by CA3 terminals and/or by subtle modifications in the number of presynaptic active zones and/or postsynaptic receptor sites.

An interesting question is whether the convincing data found for eyeblink classical conditioning training can be extended to other types of learning, for example, for instrumental conditioning —another well-known type of associative learning. Although instrumental learning involves the use of sensorimotor and cognitive abilities typically associated to the hippocampus (spatial orientation, object recognition, temporal association of environmental cues, etc.), there is still some controversy with regard to the participation of this structure in operant conditioning tasks [26-28]. Instrumental conditioning protocols demonstrate that food-deprived mice are able to acquire operant training tasks of certain difficulty in a Skinner box, using small pellets of food as a reward [27,28]. During mouse training, the fEPSPs evoked at the CA3-CA1 synapse at the very moment of the performance of five different behaviors carried out during the task —i) resting in the Skinner box, ii) going to the lever, iii) pressing the lever, iv) going to the feeder, and v) eating the rewarded pellet— were also measured. Instead of a single electrical pulse, animals were presented with a double pulse, since the paired-pulse ratio (second/first x 100) is indicative of changes in synaptic strength taking place at presynaptic sites [23,29]. The slope of the fEPSP evoked during typical appetitive behaviors (going to the lever, pressing the lever) is significantly bigger than during consummatory ones (going to the feeder, eating a pellet). In contrast, fEPSPs evoked by the second pulse presented to Schaffer collaterals are smaller in slope when the animal goes to the lever than when on the way to the feeder. In addition, the paired-pulse ratio during eating behavior is significantly bigger than when going to the lever and during the resting situation. These results could explain the involvement of short-term plastic synaptic mechanisms in the changes of fEPSP slopes observed for appetitive versus consummatory behaviors during animal performance in a Skinner box [28]. However, in contrast to the results collected during classical eyeblink conditioning, there is an absence of long-lasting synaptic changes in strength at the CA3-CA1 synapse during the acquisition of an operant conditioning task, since recorded fEPSPs do not change across training sessions [28]. In any case, and although the hippocampus seems not to participate in the acquisition of operant conditioning tasks, it certainly plays an active role during the performance of involved behavioral situations, mainly when animals are over-trained and/or reach an appropriate understanding of the contextual circumstances involved. Thus, selective changes in CA3-CA1 synaptic strength are dependent on both/either the behavioral display and/or the learning stage.

Long-term potentiation can occlude associative learning and concomitant changes in synaptic strength

If we assume, from the previously explained data, that the hippocampal CA3-CA1 synapse is functionally related to associative learning using classical conditioning paradigms, it can be hypothesized that any experimental procedure capable of disturbing hippocampal patterns of synaptic activities should be enough to prevent such a cognitive process. The assumption is that the huge wave of plasticity generated experimentally by an LTP protocol would interfere with the activation of hippocampal memory networks during actual learning. Indeed, humans with hippocampal lesions present both anterograde and (immediate) retrograde amnesia, and it has been shown in rats that spatial learning is prevented when saturating LTP is evoked in the perforant pathway or when evoked by the repeated stimulation of a large number of hippocampal synaptic contacts [30]. Finally, LTP evoked at the hippocampus is more evident for the acquisition of new learning skills and in recently acquired knowledge than for remote memory retrieval [31].

Some experiments carried out in our laboratory have addressed the above issue [18]. To start with, after LTP induction, the amount of eyelid conditioned responses using a trace paradigm decreases to ~40% independently of the phase in which the HFS is applied, meaning that this type of associative learning is dependent on the functional state of hippocampal circuits (Figure 2B). The capability of remembering during retrieval sessions is lower in the group that received an HFS protocol on the testing day than in mice that received the same stimulation, but 7 days before two retrieval sessions. According to these results, it seems that recent memories become somewhat more labile when retrieved. Perhaps the hippocampus is not necessary after memory consolidation, or it is not required in the same way [18,21,31].

Importantly, LTP deleterious effects on learning and memory are present not only during the period in which fEPSP slopes are displaced from baseline physiological values. Trace eyeblink conditioning is severely disrupted even when fEPSP values have returned to their baseline level five days before the first conditioning session [22]. Interesting, when a longer period of time (more than 25 days) is allowed between the end of a detectable change in synaptic strength evoked by HFS and the beginning of conditioning sessions, LTP-evoked mice are able to acquire the conditioning test as controls do. Moreover, the intra-hippocampal injection of ZIP, a membrane-permanent peptide inhibitor of protein kinase Mzeta (PKMz), in the CA3-CA1 area, selectively reverses LTP and speeds the recovery of both the evoked fEPSPs in CA1 after Schaffer-collateral stimulation and the eyeblink conditioned responses [19,32]. Two very important conclusions can be drawn from these experiments: i) classical eyeblink conditioning can be occluded for a long time after LTP, even after fEPSPs evoked at the CA3-CA1 synapse reach baseline values; and ii) these occlusion effects can be totally reversed with time —namely, around 25 days. This time interval can be shortened by the administration of ZIP [32].

The clear results found when evoking LTP during classical conditioning experiments could not be extended in the same way to other types of associative learning, such as instrumental conditioning [27]. The reason why LTP fails to disrupt (or to occlude) instrumental conditioning is still unclear. Nevertheless, some facts related to the procedural and motivational characteristics of operant learning can give some clues. The appetitive tasks (for example, the use of food as a reward) used in instrumental learning require that mice are submitted to a severe food deprivation (until reaching 80% of their body weight). Therefore, animals are highly motivated and emotionally activated during training sessions. Any circuit related to this type of learning process could thus be over-excited by these strong motivational and emotional circumstances. Other neural circuits are also supporting similar behaviors, protecting the animal's survival. In contrast, classical eyeblink conditioning is an almost automatic activity, not relevant for mouse survival and much more constrained to the pairs of stimuli presented.

Taking all these data together, a pertinent question is whether LTP is the underlying neural mechanism for memory storage and learning formation or, to the contrary, just an experimental phenomenon that produces some neural effects resembling those processes. Some recent evidence would suggest the second hypothesis as the more correct one [22,28].

Synaptic plasticity in mouse models of neurodegenerative diseases

Recent efforts have been addressed to taking advantage of genetically manipulated mice, which are affected at different stages of the learning and memory process. Many of these mice are accurate models of human neurodegenerative diseases [6,33]. In this sense, classical eyeblink conditioning studies that were preferentially carried out in rabbits are now extended to transgenic and knockout mice.

It is known that hippocampal *N*-methyl-D-aspartate (NMDA) receptors are involved in the acquisition of conditioned eyeblink responses and in the induction of LTP, both studied in the CA3-CA1 synapse [18,34,35]. LTP seems to depend on both local dendritic protein synthesis and nuclear transcription; therefore, many different signaling pathways have been proposed as key responsible source of the postsynaptic changes that produce LTP and classical conditioning. Moreover, different types of presynaptic receptor (adenosine A_1 and A_{2A}, cannabinoid CB1, muscarinic and nicotinic cholinergic, $GABA_A$ and $GABA_B$ metabotropic glutamate, TrkB, etc.) are also able to exert specific excitatory or inhibitory effects on transmitter release [23,29,36,37]. This scenario opens the door to studying different receptors and molecular signals, mainly through genetically manipulated mice together with the use of selective pharmacological tools.

Firstly, the effect of selected neurotrophic factors on associative learning and LTP in behaving animals can be tested. For example, mice with a mutation at the TrkB PLCg-docking site are impaired in the acquisition of trace eyeblink conditioning, and also show impairments in learning-related changes in synaptic efficacy and LTP in the CA3-CA1 region [38]. In contrast, mice with a mutation at the TrkB SHC-docking site show normal acquisition of trace eyeblink conditioning, but to some extent augmented synaptic efficacy and LTP. These results indicate some specificity in the molecular pathways underlying both associative learning and LTP triggered at the CA3-CA1 synapse. Interestingly, mice overexpressing TrkC receptor present enhanced hippocampal synaptic activity and LTP, and reduced efficiency of classical conditioning, similarly to the occlusion effects evoked with the HFS protocols [18,39]. Overexpression of TrkC leads to significant changes in the level of hippocampal expression of NMDA receptor subunits, but not of AMPA receptors —an effect that can be considered to serve as the "set point" for the control of synaptic plasticity.

Many different studies have pointed to CREB as an essential component of the molecular switch that controls the conversion of short-term forms of plasticity into long-term forms, including those underlying LTP [40]. The study of the forebrain expression of a strong constitutively active CREB variant, VP16-CREB, shows an increase in the *in vivo* LTP and significant changes in the input/output curve and paired-pulse facilitation evoked at the CA3-CA1 synapse [41]. Electrophysiological experiments carried out in behaving VP16-CREB transgenic mice support a critical role of CREB-dependent gene expression in plasticity and memory, and demonstrate, as in previous studies in other genetically modified mouse strains [39], that enhanced hippocampal LTP in response to HFS is not necessarily associated with better performance in hippocampal-dependent tasks. It is important to note that changes in CREB activity occur during learning, but that the timing and duration of these changes are tightly regulated and that any deviation from this sequence can clearly disrupt the learning process [41]. Metabotropic glutamate receptor 1 (mGluR1) is another receptor necessary both for the acquisition

of trace eyeblink conditioning and for the proper enhancement in synaptic strength taking place in hippocampal circuits across conditioning [42]. Some synaptic components have been associated to modulation and smooth control of the synaptic activity, rather than to the neurotransmitter transmission itself. For example, adenosine is a prototypic neuromodulator present in the nervous system which tunes on-going synaptic transmission [43] through the activation of high-affinity G-protein inhibitory (A_1) and excitatory (A_{2A})-coupled receptors. Adenosine A_{2A} receptors co-localize and act synergistically with mGluR receptors to potentiate NMDA effects at the hippocampal CA3-CA1 synapse [44]. These A_{2A} receptors also interact with TrkB receptors, through a cyclic AMP-mediated process [45] or through transactivation of TrkB receptors [46]. Mice injected with a highly selective A_{2A} receptor antagonist, SCH58261, are completely incapable of acquiring a classical eyeblink conditioned task, and no evolution of the CA3-CA1 synapse strength is found during these training sessions. Moreover, the injected mice do not show experimentally evoked LTP after the HFS protocol presentation as the controls do [47]. It seems that A_{2A} receptors have a pivotal effect on associative learning and on relevant hippocampal processes, including activity-dependent changes at the CA3-CA1 synapse.

Associative learning depends on multiple cortical and subcortical structures, including striatum, hippocampus, and amygdala. In this regard, and besides glutamatergic receptors, both dopaminergic and cannabinoid neurotransmitter systems have been implicated in learning and memory consolidation. The role of dopamine was studied using two models of dopamine D1 receptor (D_1R, *Drd1a*) loss: D_1R knock-out mice (*Drd1a*$^{-/-}$) and mice with intrahippocampal injections of *Drd1a*-siRNA (i.e., small interfering RNA; see [48]). D1R loss clearly reduced spatial learning, fear learning, and classical conditioning of eyelid responses, as well as the related activity-dependent synaptic plasticity and experimental LTP evoked at the hippocampal CA3-CA1 synapse. In each learning task, the performance of *Drd1a*-siRNA mice was identical to that of *Drd1a*$^{-/-}$ animals, indicating that hippocampal knock-down is as effective as the global inactivation of D1 receptors, and that the observed effects are caused by loss of D_1R and not by any indirect developmental effects of *Drd1a*$^{-/-}$.

We have also studied the effects of the activation of cannabinoid CB1 receptors, located on presynaptic terminals of hippocampal (among other structures) principal neurons [49]. The administration of a CB1 agonist decreases the acquisition of an associative learning task and the concomitant increase in strength of the CA3-CA1 synapse (Figure 3A), as well as the LTP evoked at the same hippocampal synapse. Similar results were collected from CB1R-siRNA-injected animals (Figure 3B). Unexpectedly, CB1$^{-/-}$ mice showed a decrease in the percentage of conditioned eyelid responses, a diminished potentiation of the CA3-CA1 synapse across training, and a lower LTP (Figure 3C), suggesting the presence of delayed compensatory mechanisms evoked in this genetically manipulated animal. Importantly, part of the learning impairment produced by cannabinoids is mediated also by non-hippocampal CB1 receptors [49]. That study confirms the involvement of hippocampal CB1 receptors in learning and memory processes, but it also reinforces the putative role of presynaptic mechanisms [23] in activity-dependent changes in synaptic strength.

Similar electrophysiological *in vivo* approaches can also be used for the study of mouse models of other types of human disease —for example, prionopathies or Down syndrome. Prionopathies are characterized by spongiform brain degeneration, myoclonus, dementia, and periodic electroencephalographic disturbances. The hallmark of prionopathies is the presence of an abnormal conformational isoform (PrP^{SC}) of the natural cellular prion protein (PrP^{C}) encoded by the *Prnp* gene. Studies of the putative functions of PrP^{C} demonstrate that it is necessary for the proper homeostatic functioning of hippocampal circuits. We have shown that overexpression of the PrP^{C} protein increases susceptibility to the seizures after kainate administration and enhances synaptic facilitation in paired-pulse experiments and hippocampal LTP [50]. On the other hand, mental retardation in human Down syndrome is variable, and many human and mouse models identify some regions of chromosome 21 (Hsa21) as being linked to cognitive deficits. However, the trisomy of the 12 genes found in the 0.59 Mb (*Abcg1-U2af1*)Hsa21 sub-telomeric region in mice (Ts1Yah) produces defects in novel object recognition, open-field, and Y-maze tests, but induces an improvement of the hippocampal-dependent spatial memory in the Morris water maze [51]. Moreover, in the same study it was found that HFS applied to Ts1Yah mice evokes a larger increase in fEPSP slopes during the LTP test than in controls. Thus, trisomy of the *Abcg1-U2af1* interval impacts the hippocampal functions in a very sophisticated way, disturbing the short-term memory while facilitating the spatial learning and LTP. All these data suggest that the variable Down syndrome cognitive phenotypes likely result from the complex interactions of several genes or regions which can have negative or even positive contributions to the cognitive performance.

In conclusion, the classical conditioning of eyelid responses in combination with electrophysiological measurements of changes in synaptic strength taking place during the learning process is an excellent tool for evaluating mouse models of neurodegenerative diseases and the degree of recovery following putative treatments. All of the molecular and subcellular components described here, and many others, with their complex mechanisms, have a specific role in the physiological basis of learning and memory. Therefore, they are good experimental models for studying these processes and as well as certain neurological processes directly or indirectly related to some neurological diseases. The huge increase in the number and sophistication of methods and techniques at many different levels (molecular, physiological, behavioral, etc.) in recent years has shed light on some of the mechanisms that underlie the storage and use of relevant information by brain circuits. Present knowledge also allows us to predict that memory mechanisms are probably not kept in one single neural structure, but are the emergent result of many activated structures in the mnesic circuits, which means an extra level of complexity in the system. Interaction between basic experiments and clinical experience will also speed the understanding

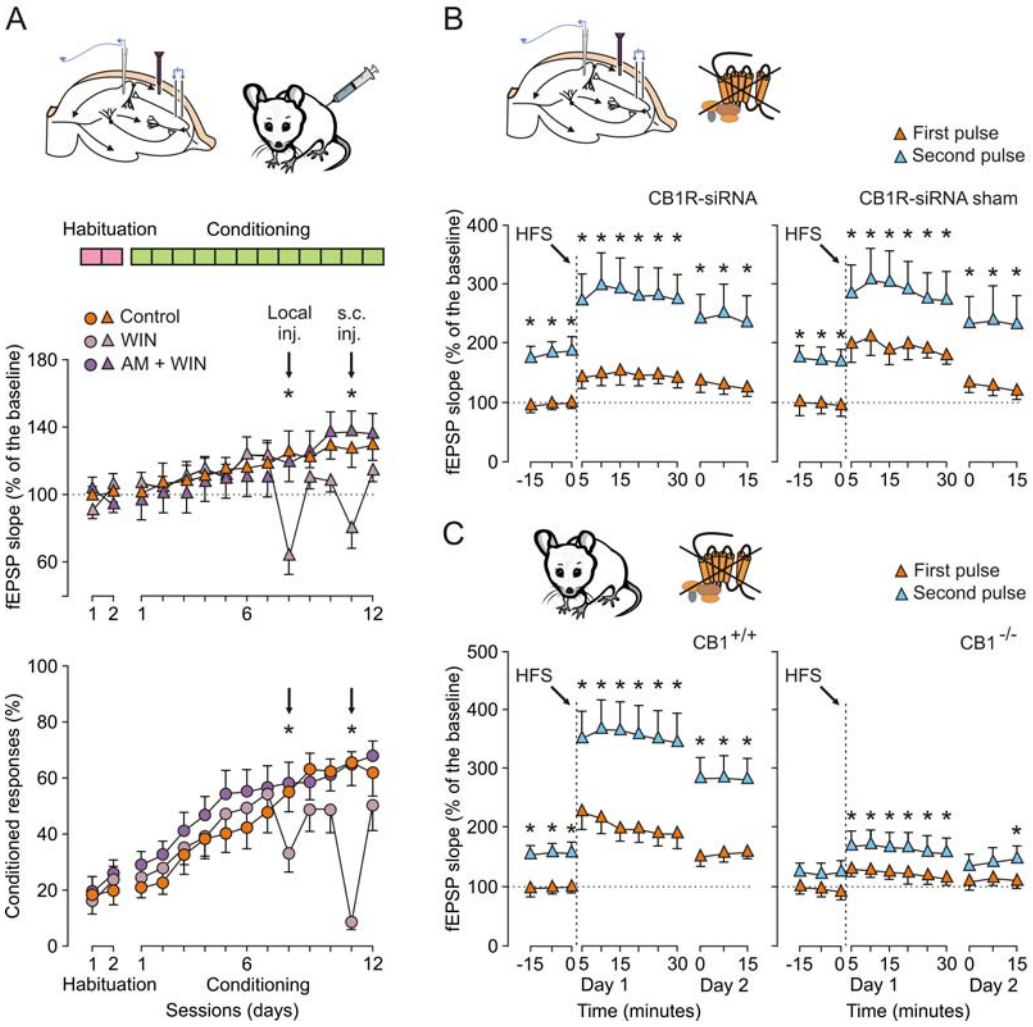

Figure 3. Role of cannabinoid CB1 receptor in classical eyeblink conditioning and in hippocampal CA3-CA1 synaptic plasticity. A) Comparison of the effects on field excitatory postsynaptic potentials (fEPSPs) evoked in CA1 area after Schaffer-collateral stimulation, and conditioned responses of local injections (Local inj.) of WIN55,212-2 (a CB1 receptor agonist) or a subcutaneous injection (s.c. inj.) of AM251 (a CB1 receptor antagonist). For the local injection, a cannula is implanted in the dorsal hippocampus. fEPSP slope evolution and percentage of conditioned responses along the successive learning sessions for control (orange symbols), and for WIN- (pale violet symbols) and WIN+AM-injected (dark violet) groups. B) Evolution of fEPSPs evoked by double-pulse stimulation in CB1R-siRNA- (left) and CB1R-siRNA sham- (right) injected groups after a high-frequency stimulation (HFS) session. The HFS evokes a small, but significant, long-term potentiation (LTP) to the first pulse (blue triangles) that is bigger in the sham group. The fEPSP slopes evoked by the second pulse (orange triangles) present similar values in both groups. C) Evolution of fEPSPs evoked by double-pulse stimulation in $CB1^{+/+}$ (left) and $CB1^{-/-}$ (right) mice after an HFS session. The LTP evoked by the HFS to the first pulse (orange triangles) is larger in the $CB1^{+/+}$ mice than in the $CB1^{-/-}$ mice. The differences between the two groups are more drastic for the LTP evoked by the HFS to the second pulse (blue triangles), since the values of the fEPSP slopes recorded in the $CB1^{-/-}$ mice are half those in the $CB1^{+/+}$ mice. (Adapted and reproduced with permission from Madroñal *et al.* 2012 [49])

of complex mechanisms, such as learning and memory processes, in the same way that *in vivo* experiments have enabled confirmation or rejection of the hypotheses stemming from *in vitro* studies.

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