Associative Learning and Long-Term Potentiation in Rodents: Effects of Nutrition

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Long-Term Potentiation (LTP) and Learning-Dependent Changes in Synaptic Strength

Following the seminal proposal of Hebb[1] and many others, acquired learning abilities are assumed to be stored in the form of functional and/or structural changes in synaptic efficiency. Although there are many excellent studies in vitro of the electrophysiological processes and molecular events supporting activity-dependent synaptic changes, not much information is available on synaptic changes in strength (ie, synaptic plasticity) during actual learning in behaving animals. Functional changes evoked by learning should be susceptible to being detected at synapses relevant to the acquisition process. In 2006, our research group convincingly demonstrated that classical conditioning of eyelid responses in behaving mice was able to increase the synaptic strength of the hippocampal CA3-CA1 synapse (Fig 1).[2,3.]

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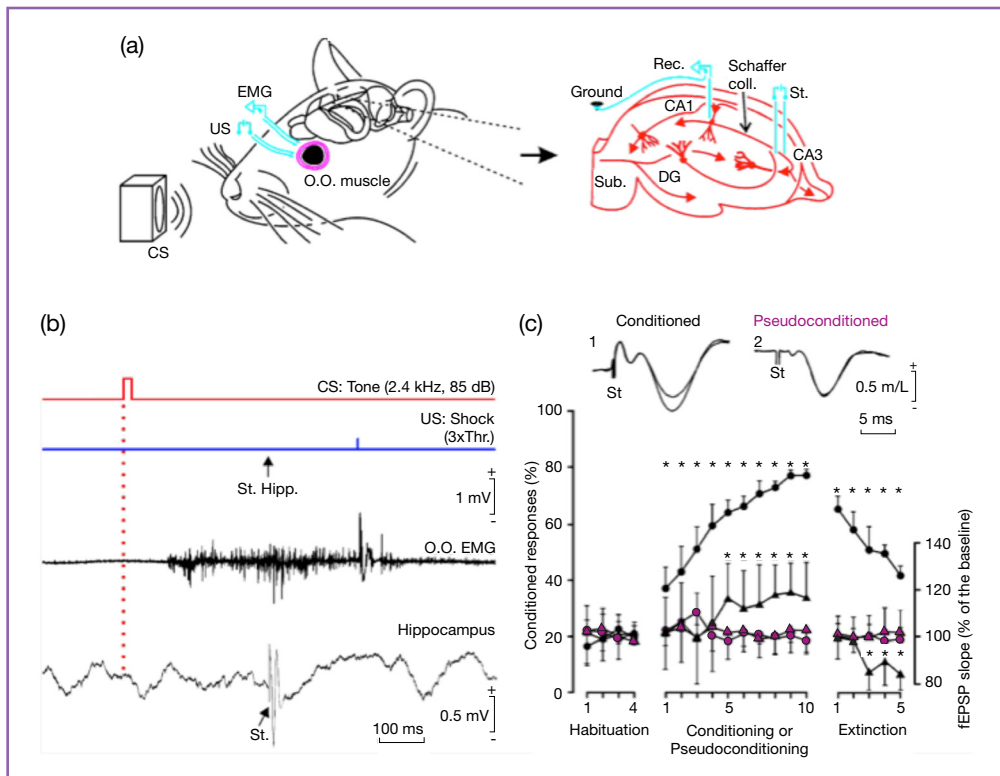

Fig 1. Learning-dependent changes in the slope (volt/s) of field excitatory post-synaptic potentials (fEPSPs) evoked in the hippocampal CA3-CA1 synapse across a classical conditioning of eyelid responses in behaving mice.[2,3] *A*, Experimental design. Stimulating electrodes (St.) were implanted on Schaffer's collaterals and recording electrodes were implanted in the hippocampal CA1 area. (DG=dentate gyrus, Sub=subiculum) ***B*,** Schematic representation of the conditioning paradigm. From top to bottom are shown the conditioned (CS, red) and unconditioned (US, blue) stimuli, the moment at which a single electrical pulse (100 µs, square, biphasic) was presented to the CA3-CA1 synapse (St. Hipp.), the electromyographic (EMG) activity of the orbicularis oculi (O.O.) muscle, and the extracellular record of hippocampal activity. The illustrated records correspond to the 7th conditioning session. Note the fEPSP evoked by the single pulse presented to the CA3-CA1 synapse. ***C*,** At the top (i) are illustrated fEPSPs evoked in the CA3-CA1 synapse 300 ms after CS presentation. Records were collected from conditioned and pseudoconditioned animals during the 1st and 10th conditioning sessions. (ii) Evolution of the percentage (%) of conditioned responses (CRs) during the successive sessions for conditioned (control, dots) and pseudoconditioned (circles) groups. Mean % values are followed by ±SD. Evolution of the fEPSP slope is also indicated for conditioned (black triangles) and pseudoconditioned

(open triangles) groups, expressed as the % change with respect to mean values collected during the habituation sessions. *P<0.001.

Another basic tenet of current neuroscience is that LTP could be the mechanism underlying certain forms of learning. LTP is evoked by high-frequency stimulation of selected afferent pathways, resulting in a long-lasting enhancement of synaptic efficacy. It was generally assumed that the experimental induction of LTP would disturb the synaptic changes taking place during associative learning in selected neural sites. Indeed, our research group and others have shown that LTP saturation of hippocampal circuits disrupts spatial and associative learning tasks.[2-5] It is important to point out that although LTP is evoked experimentally and activity-dependent synaptic plasticity takes place during actual learning, both plastic phenomena share some neural and synaptic properties, including the need for the proper activation of glutamatergic N-methyl-D-aspartate (NMDA) receptors at selected synaptic sites.[2,3] It should be kept in mind that LTP is evoked experimentally by high-frequency stimulation of selected synapses and that actual learning never evokes the huge increases in synaptic activity evoked by LTP. In this regard, LTP should not be used synonymously with synaptic changes evoked by actual learning and should not be confused with long-term memory (defined as lasting storage of acquired information). Nevertheless, the amount of LTP evoked, for example, in the hippocampus of an experimental group as compared with a control group of animals can give an idea of their relative learning capabilities.[5-9]

Environmental, Social, Neuronal, and Nutritional Factors Involved in the Proper Acquisition of New Motor and Cognitive Abilities

During the last few years, research has shown that many different neuronal postsynaptic and presynaptic receptors, as well as neurotrophic factors and molecular intraneuronal cascades, are involved in the proper acquisition of new cognitive and motor abilities. For example, we have recently shown that metabotropic glutamate (mGluR), dopaminergic (D1), and cannabinoid (CB1) receptors are also involved in the acquisition and/or retrieval of associative learning tasks.[6,7,9] In addition, many other environmental, emotional, and social factors can modify learning capabilities.[8]

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It also is feasible that selected dietary ingredients and nutritional factors can modify (at the least in the long run) learning and memory abilities of experimental animals and, by extension, of human beings. For example, we showed a few years ago that mangiferin and morin hydrate (two antioxidant polyphenols obtained from mango and mulberry fruits) prevent excitotoxic death and oxidative stress in cultures of rat cortical neurons and facilitate the recovery of associative learning capabilities in an in vivo model of stroke carried out in behaving rats (Fig 2).[10]
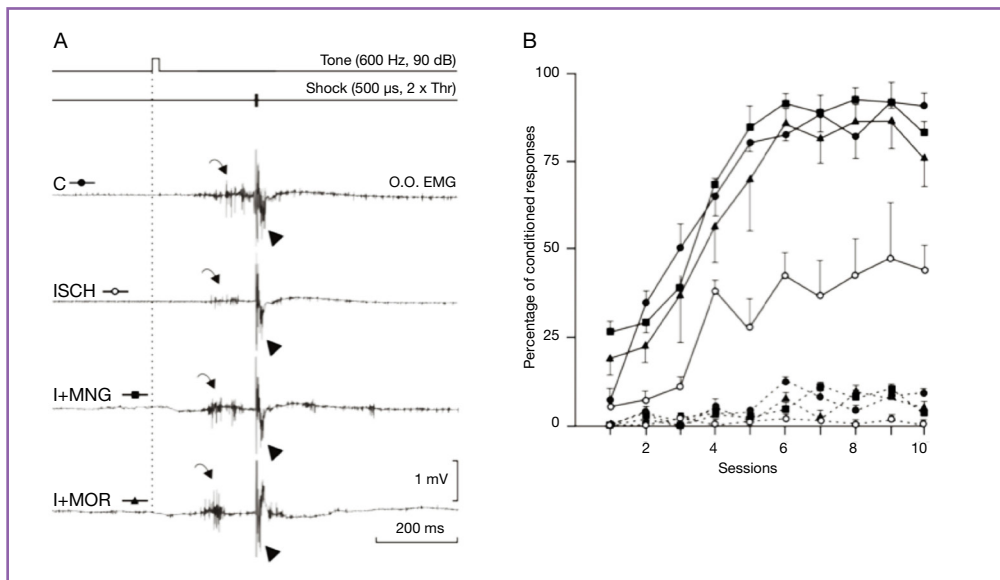

Fig 2. A quantitative analysis of classically conditioned eyelid responses from control (C, and dots) and ischemic (ISCH, and circles) rats and from ischemic rats treated with mangiferin (I + MNG, and squares) or morin (I + MOR, and triangles).[10] *A*, Electromyographic (EMG, in mV) recordings from representative animals of each of the indicated experimental groups collected during the 9th conditioning session. For trace conditioning, a tone (600 Hz, 90 dB) was presented for 20 ms as a conditioned stimulus (CS). The tone was followed 270 ms later by an electrical shock (500 µs, 2 × threshold) presented to the supraorbital nerve as an unconditioned stimulus (US). Bent arrows indicate the presence of conditioned responses (CRs). Arrowheads indicate the appearance of unconditioned eyelid responses. ***B*,** Graphs of mean percent conditioned responses across the 10 conditioning sessions for the four experimental groups. Results collected from conditioned groups are indicated by continuous lines, whereas results corresponding to pseudoconditioned groups are indicated by discontinuous lines. Note the low learning curve corresponding to ischemic animals.

In addition, preliminary studies carried out in our laboratory indicate that the chronic administration of a human-milk derivate has a positive effect on LTP evoked in behaving rats as well as on their learning capabilities to solve Skinner box tasks.

Cortical and Subcortical Circuits Involved in Learning and Memory Processes

The hippocampus has been traditionally implicated in a variety of learning paradigms, including spatial learning, object recognition, and classical conditioning of eyelid responses.[2,3] As a particular case, the cerebellum also has been related to the acquisition of the classical conditioning of eyelid responses using a delay paradigm. Nevertheless, our in vivo studies carried out in different experimental animal models (mice, rats, and rabbits) have shown convincingly that many other cortical (sensory, premotor, and motor) and prefrontal cortices seem to be necessary for the acquisition of complex cognitive and behavioral tasks.[11-13] For example, more than the hippocampus, prefrontal and striatal circuits seem to be involved in the acquisition of instrumental learning tasks.[11]

Interestingly, two recent reports from our laboratory[12,13] have opened a new experimental approach to the study of food as a natural reward and its putative effects in selected neuronal circuits. We have shown that appetitive behaviors (ie, to press a lever to obtain a food pellet) activate specific (CA3-CA1 synapse) hippocampal circuits, while consumatory behaviors (collect the pellet) depress it.[12] To our surprise, internal rewards as electrical self-stimulation of the medial septum by behaving mice placed in a Skinner box produce identical effects on hippocampal circuits.[13] These original experimental approaches open new possibilities for determining the rewarding effects of selected nutrients.

Summary and Conclusions

Neural mechanisms underlying learning and memory processes have to be studied in alert behaving animals. Our group has developed different technical procedures for the study of the firing and synaptic activities of selected brain sites during the acquisition and recall of different types of associative (classical and instrumental) learning tasks. We also have shown that LTP evoked experimentally in laboratory animals shares some synaptic properties and molecular mechanisms with learning-dependent changes in synaptic strength. Synaptic changes evoked by LTP and/or

by actual learning can be modified by environmental, social, and emotional factors, as well as by many different drugs and putative dietary ingredients.

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