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Visual discrimination learning and LTP-like changes in primary visual cortex

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Introduction

Visual discrimination learning is a visual process that refers to the ability to differentiate one visual target from another. This ability is fundamental for an individual to interact with the environment. For example, the ability to learn to visually discriminate letters and words becomes essential in learning to read and deficits in visual discrimination are a common cause of reading problems. One must be able to discriminate visually in terms of colour, foreground-background, form, size, and position in space.

Visual discrimination learning is a property of visual perception that is supposed to rely upon modifications of synaptic strength in neurons of neural structures concerning visual perception. The study of the physiological and cellular mechanisms underlying this plasticity contributes to increase our knowledge about how the brain makes use of visual information from the external world. This knowledge is fundamental to better understand how to intervene in diseases related to visual discrimination skills.

The principal mechanisms involved in visual discrimination learning is probably visual perceptual learning, which is defined as the increase in visual abilities after training. Numerous studies tried to explain the link between visual perceptual learning and the sensorial plasticity underlying it. Results from these studies showed that cortical areas operate in this process as low as primary visual cortex (V1) at the first levels of perception (Vogels & Orban 1985, Shiu & Pashler 1992 and Schoups *et al.* 1995). In particular, V1 is actually known as the cortical field in which visual perceptual learning is more likely to take place involving simple visual stimuli such as gratings (Schoups *et al.* 2001, Furmansky *et al.* 2004, Maertens & Pollmann 2005, Frenkel *et al.* 2006, Pourtois *et al.* 2007 and Yotsumoto *et al.* 2008).

The aim of this study is to verify the relation between visual discrimination learning and Long Term Potentiation (LTP), which is one of the best characterized forms of synaptic plasticity underlying various kinds of learning (Rogan *et al.* 1997, Moser *et al.* 1998, Rioult-Pedotti *et al.* 2000 and Whitlock *et al.* 2006). However, before analysing this topic

in details, it is useful to spend some words about the general concepts of learning and memory.

The concept of learning

Learning is the acquisition of new knowledge or skills from the external world. The expression of learning is the emergence of new behavioural patterns or simply the modification of pre-existing ones. Since the concept of learning implies that information has to be conserved to be subsequently recalled or re-used, investigating learning implyes the study of memory. Memory is the faculty that allows information from the environment to be stored. A basic and generally accepted classification of memory is based on the duration of memory retention, and identifies two distinct types of memory, short term memory and long term memory (Mc Gaugh 1966). When sensorial information is transferred to short-term memory, this allows one to recall it from several seconds up to minutes. Short-term memory is supported by transient patterns of neuronal communication (Bauer & Fuster 1976 and Jonides *et al.* 1998). Storage of short-term memory generally has a strictly limited capacity and duration. Information is available for a certain period of time, but is not retained indefinitely. To be long lasting a memory trace has to be consolidated through processes involving long term memory storage (Kesner & Connor 1972). In biological terms, long-term memories are maintained by stable and permanent changes in neural connections widely spread throughout the brain (Ordy & Schjeide 1973 and Markowitsch 1985).

Another kind of classification divides memory in two distinct, independent and parallel systems. This idea about memory organization became a topic of experimental interest when evidence from normal subjects, amnesic patients, and experimental animals converged on the same view (Scoville & Milner 1957 and Squire 1992). A fundamental distinction can be made between declarative or explicit memory that is accessible to awareness and non-declarative or implicit memory that is not (Leritz *et al.* 2006 and Speekenbrink *et al.* 2008).

Declarative memory

Declarative or explicit memory refers to the capacity of acquiring or modifying knowledge about facts and events. It is the kind of memory that is impaired in amnesia and it can be divided into semantic memory concerning facts about the world and episodic memory concerning the capacity to re-experience an event in the context in which it originally occurred (Tulving 1983).

Focusing on visual declarative memory, it has been shown that when information is acquired through the visual pathways, visual stimuli are coded by declarative memory systems as explicit knowledge (Desimone 1996 and Wolfe 1998). Initially, information processing occurs in a group of anatomically linked cortical fields, the so-called object-analyzer system, often called the "ventral visual stream" (Murray *et al.* 2007). It comprises several visual areas including V1 and the inferior temporal cortex (Stotnick 2004). Subsequently, to persist as memories, visual features are to be consolidated by the temporary intervention of the medial temporal lobe (MTL).

Determination of specific systems involved in memory consolidation began with the finding that damage to the MTL produced severe amnesia (see Warrington & Weiskrantz 1969). MTL is a term of convenience for referring collectively to the hippocampus, dentate gyrus, subicular complex, amygdala, and perirhinal, entorhinal, and parahippocampal cortex. These structures make selective contributions to declarative memory. Hippocampus is involved in processing information about places and paths, while perirhinal cortex seems to be more involved in processing information about objects. During amnesia, while remote memories usually remain intact, recently acquired declarative memories do not. This happens because amnesic patients with MTL damage have great difficulty in forming new long term memories (Scoville & Milner 1957, Penfield & Milner 1958 and Corkin 1984). Another important observation is that when brain pathology includes damage to the neocortex, remote memory is often impaired (Graham & Hodges 1997, Squire *et al.* 2001 and Bayley *et al.* 2003).

These findings suggest that initial acquisition and retrieval of declarative memories require MTL, while subsequent storage of information in various neocortical areas occurs without a further significant MTL contribution (Squire 1992, McClelland *et al*. 1995,

Squire *et al.* 2004 and Nadel & Moscovitch 1997). Memories gradually become independent from MTL as they are consolidated in neocortical circuits that serve as remote memory storage (Alvarez & Squire 1994 and Squire & Alvarez 1995). Studies of hippocampus-dependent memory in animals have largely confirmed this idea (Zola-Morgan & Squire 1990, Kim & Fanselow 1992, Kim *et al.* 1995, Anagnostaras *et al.* 1999, Frankland *et al.* 2001, Sutherland *et al.* 2001 and Clark *et al.* 2002). Moreover, there is strong evidence suggesting that synaptic structural changes take place in the neocortex during consolidation (Maviel *et al.* 2004 and Frankland *et al.* 2004), probably in order to stabilize remote memories. Consolidation processes are likely to be distribuited in different regions of the neocortex, including visual areas (Roland & Gulyàs 1995 and Mc Gaugh 2000).

But why does declarative memory need two complementary systems? Gradual interleaving of memories into the neocortex is essential for discovery of generalities and the eventual formation of knowledge structures. Using connectionist models, it can be shown that the rapid incorporation of new information into an existing knowledge system would cause catastrophic interference (Marr 1970, Marr 1971 and McClelland *et al.* 1995).
Essentially, new information would dominate and erase previously acquired information. Probably this explains why cortical consolidation is a slow, extended process, and why the hippocampus is needed as a temporary link between distributed cortical memories. New memories need to be incorporated into existing knowledge structures in the cortex through a gradual, interleaving process to avoid the loss of old information. This might happen during periods of inactivity and sleep, when bursts of activity, called sharp-waves (SPWs), are generated in the hippocampus (Buzsaki 1989 and Hasselmo 1999). SPWs could provide the activation required to drive intercortical plasticity and to promote cortical consolidation. This periodic activity seems to operate by a mechanism called synaptic re-entry reinforcement (Shimizu *et al.* 2000, Cui *et al.* 2004 and Wittenberg & Tsien 2002). Recent observations show that experiences are replayed during sleep synchronously in the hippocampus and in the visual cortex (Mehta 2007). During slow-wave sleep in rats, multicell spiking patterns in the visual cortex and in the hippocampus are organized into frames, defined as periods of stepwise increase in neuronal population activity. The multicell firing sequences evoked by awake experience are replayed during these frames in

both regions and coordinated to reflect the same experience (Ji & Wilson 2007). This probably implies simultaneous reactivation of coherent memory traces in the visual cortex and hippocampus during sleep. This reactivation may contribute to or reflect the result of the memory consolidation process.

Non-declarative memory

Non-declarative or implicit memory refers to processes known to be dispositional, expressed through performance, that have the ability to gradually extract common elements from a series of separate events. Memories occur as modifications within specialized performance systems. They are categorized in groups based loosely on functional properties and sometimes more strongly on functional or anatomical dissociations. They are revealed through reactivation of the systems within which learning originally occurred (Schacter 1992, Ashby & Waldron 1999 and Smith 2008). Typical examples are non-associative learning (habituation, sensitization and dishabituation), associative learning, skill learning, priming, perceptual learning and emotional learning (Roediger 3rd 1990).

Habituation, sensitization and dishabituation are the simplest forms of learning giving rise to non-declarative memory (Carew 1989). During habituation, repetition of a non relevant stimulus leads to a decrease in reflexive response, while during sensitization a strong aversive stimulus leads to an increase in sensitivity of other aversive sensory/motor reflexes. Dishabituation is the case in which sensitization is formed to override the previous habituation. These forms of implicit learning are non-associative because habituation involves the modification of a single sensory channel and not the association of two different ones, while sensitization is not specific to any sensory channel.

Associative learning occurs when two or more sensory streams, motor rules or cognitive rules are associated. The best described forms are classical conditioning and operant conditioning. In classical conditioning a non relevant conditioned stimulus (CS) is coupled with a relevant unconditioned stimulus (US), so that the CS becomes subsequently relevant (Pavlov 1927). In operant conditioning the positive or the negative reinforcement

of a behavioural pattern leads to the modification of the subsequent use of the same specific behaviour (Skinner 1935).

Skill learning is the acquisition of new behavioural abilities with practice and is defined as facilitation on a range of abilities in a particular task (Squire 1992 and Squire & Zola 1996). It relies upon basal ganglia and cerebellum activity. The initial cognitive stage requires working memory capacity. This stage is the categorization of skills used to guide behavior. In the subsequent associative stage behavior becomes tuned and errors are eliminated, while in the subsequent autonomous stage there is a gradual continued improvement of skill with little reliance upon working memory.

Perceptual learning is the specific and relatively permanent modification of perception and behavior following sensory experience (Schacter 1990). It involves structural and functional changes in primary sensory cortices.

Priming is an improvement in a perceptual or conceptual task from a one trial learning perceptual exposure to the stimulus being used in the task (Squire 1992). Priming is thought to happen in primary sensory areas and results from an improvement in processing efficiency. Much of priming results in a decrease in response time or in an increase in probability of correct response.

Emotional learning concerns the unconscious learning by storage of information about the emotional significance of events (LeDoux 1993). The neural system underlying emotional learning critically involves the amygdala and structures with which it is connected. It includes all the emotional reactions that are built over time by simple exposure.

Visual discrimination learning

During visual discrimination learning, the process of perception becomes adapted to the environment. Experience increases the attention paid to features that are important, and decreases the attention to irrelevant ones. Attention can be selectively directed toward important stimulus aspects at several different stages in information processing. Researchers in animal learning and human categorization have described shifts toward the use of dimensions that are useful for tasks (Nosofsky 1986) or have previously been useful (Lawrence 1949). Thus, experience can lead to the separation of perceptual dimensions comprised in a single stimulus. Dimensions that are originally treated as fused often become segregated with development or training. The subject shifts from perceiving stimuli in terms of holistic, overall aspects to analytically decomposing objects into separate dimensions. This trend has received substantial support from developmental psychology.

As mentioned before, two memory systems can be distinguished in terms of the different kinds of information they process and the principles by which they operate. In visual discrimination learning both systems may be potentially utilized. The critical aspect is the strategy implemented during the discrimination learning, which reflects which memory system is principally engaged. Categorizing the objects that are to be discriminated requires attending to the object-based spatial frequency information collected by different spatial frequency channels of the visual system. This drives a visual perceptual learning process of the spatial frequencies that facilitate the particular categorization of the object (Sowden & Schyns 2006). However, sinse retinal information about object spatial frequencies varies in size with distance, the critical bands of diagnostic spatial frequencies are seen by different channels. Support is provided by knowledge whenever the ability to abstract and generalize is needed to optimize visual discrimination performance (Sowden & Schyns 2006). Thus, in a visual discrimination task, recognition may also be useful and an interaction may happen between the top-down conscious object-based indications and the bottom-up information coming from the spatial frequency channels organization of the visual system. The top-down and bottom-up contribution in discrimination may vary according to the complexity of the object features and to the categorizing ability of the discriminating organism (Sowden & Schyns 2006).

In visual pattern discrimination tasks, monkeys with large MTL lesions show no deficit concerning learning and retention of pattern discriminations (Squire & Zola-Morgan, 1983). Amnesic patients learn such tasks in a few trials, like normal individuals, but they later loose awareness of what they previously learned (Squire *et al.* 1988). The difference appears to lie in the fact that monkeys learn the pattern discrimination task gradually, during several hundred of trials in a manner reminiscent of visual perceptual learning (Iversen, 1976) while humans approach the task as a simple problem of conscious memorization. These findings show that it is possible to observe an experimental situation in which only one of the two systems is substantially working, but more generally, almost anytime visual discrimination is occurring, both systems might be utilized together with different respective contributions, according to different strategies usable to learn a task. It is often problematic to completely isolate the single contribution of one of the two different complexes engaged, especially working with animals which cannot suggest a verbal check of the conscious aspects of the information acquired.

Visual perceptual learning

Visual perceptual learning is maybe the principal mechanism operating in visual discrimination learning concerning simple visual stimuli such as gratings (Schoups *et al.* 2001, Furmansky *et al.* 2004, Maertens & Pollmann 2005, Frenkel *et al.* 2006, Pourtois *et al.* 2007 and Yotsumoto *et al.* 2008). It involves relatively long-lasting changes to an organism's visual system that improves its ability to respond to the environment. A major consequence of visual perceptual learning is that perceptions become increasingly differentiated from each other. By differentiation, stimuli that were initially perceptually indistinguishable become separated. Laboratory studies have extensively studied training effects involving simple discriminations, noting that improvement comes after several training sessions (Magnussen & Greenlee 1999) or, in some cases, as the effect of a mere exposition to a stimulus (Magnussen 2000). In order to focus the investigation exclusively on the non-declarative aspects of learning coinciding with visual perceptual learning, the impact of verbal or categorical coding has to be minimized. In current research this impact

is reduced by studying the retention of single dimensions or attributes of the visual stimulus. The decay of memory is tracked in delayed discrimination tasks with variable time intervals interposed between the stimuli that are to be compared. Memory performance is indexed by the resultant discrimination thresholds or some equivalent measures (Kinchla & Smyzer 1967, Laming & Scheiwiller 1985, Regan 1985, Magnussen *et al.* 1990 and Magnussen & Greenlee 1999).

Visual perception is known to concern various proprieties of visual stimuli including orientation, direction of motion, texture, deepness, spatial position and spatial frequency (Shapley & Lennie 1985, Baker Jr & Mareschal 2001 and Derrington *et al.* 2004). In order to make visual perceptual learning strictly specific, during the training discrimination improvement is directed to one particular property of the stimuli (Gilbert 1994). This specificity has deep implications for the understanding of neural mechanisms underlying visual perceptual learning. For example, some features such as orientation, contrast and colour exhibit a slight decay in the short-term memory range of visual perceptual learning, whereas others, such as spatial frequency and motion, are stored with precision (Nilsson & Nelson 1981, Vogels & Orban 1986, Lee & Harris 1996, Blake *et al.* 1997 and Reinvang *et al.* 1998). Moreover, trained performance on a horizontal discrimination task frequently does not transfer to a vertical version of the same task (Fahle & Edelman 1993 and Poggio *et al.* 1992), nor does it transfer to new retinal locations (Fahle *et al.* 1995 and Shiu & Pashler 1992), and it does not completely transfer from the trained eye to the untrained eye (Fahle *et al.* 1995).

During the task of discriminating changes along a single property (for example spatial frequency) in a multiple property test, human observers are able to extract the relevant information from concurrent changes along other properties, for example contrast or orientation, as precisely as when the stimuli to be compared vary along a single property (Burbeck 1987 and Heeley *et al.* 1993). These observations suggest an interesting model for visual discrimination. A set of second-order neural representations might combine information from neural representations tuned to different properties of the visual stimulus (Magnussen *et al.* 1998 and Olzak & Thomas 1999). These second-order mechanisms might be organized in a modular way. Parallel mechanisms that are dedicated to the processing of one property (for example, spatial frequency) would abstract information

across other properties simultaneously (for example, orientation and contrast). Each property-dedicated mechanism would be organized in terms of an array of memory stores that would be linked in a lateral inhibitory network and each store would code a restricted range of values along the property. According to this model the operating system should be a neural structure where representation of the basilar proprieties of the stimulus is strictly organized.

Learning tasks concerning simple stimuli, with specificity for properties like spatial frequency or stimulus orientation, are likely to be mediated by mechanisms involving the first steps of cortical elaboration (Vogels & Orban, 1985; Shiu & Pashler, 1992 and Schoups *et al.* 1995), where receptive fields are smaller, visual topography is finely organized and there is a fine selectivity for orientation and spatial frequency. V1 is known to have neurons, called simple cells, with high selectivity for stimulus orientation (Hubel & Wiesel 1959), which is an important feature of the organization of V1 columnar architecture (Hubel & Wiesel 1977). Recent investigations utilizing Magnetic Functional Resonance (fMRI) and Transcranical Magnetic Stimulation (TMS) found direct evidence that visual discrimination improvements show changes at the first states of visual information cortical elaboration (Furmansky *et al.* 2004 and Maertens & Pollmann 2005). For example, one of these studies directly showed that improved visual perceptive performance was linked to increased V1 neural activity (Furmansky *et al.* 2004). Subjects were trained to recognize a low contrast grating, while fMRI recordings occurred before and after the training. Primary visual cortex response was increased after learning and this effect was specific for location and orientation of the training stimulus.

Important findings about V1 involvement in visual perceptual learning were also obtained by electroencephalogram (EEG) recording experiments. One study examined the period in which visual perceptual learning took place in subjects trained in a texture discrimination task (Pourtois *et al.* 2007). This approach had a temporal resolution which was able to define the latency of the effects observed after training. The target produced a change in the visual evoked potential in V1, which was the earliest component of the whole cortical response. This effect only occurred when target was present in a previously trained location and in corrispondence of the upper part of the visual field. Thus, this study showed that plasticity in V1 can underlie the consolidation of a recent perceptive ability. This

ability is acquired by modeling the initial charge of sensorial input which occurs at the first visual cortical area.

All these ideas deriving from imaging experiments, were also supported by findings of correlations between electrophysiological recordings and orientation learning in monkeys V1 (Schoups *et al.* 2001). Behavioral improvement in this type of learning has been linked to an improved neuronal performance of trained compared to naive neurons. Improved long-term neuronal performance resulted from changes in the characteristics of orientation tuning of individual neurons. More particularly, the slope of the orientation tuning curve, that was measured at the trained orientation, increased only for the subgroup of trained neurons, most likely to code the orientation identified by the monkey. No modifications of the tuning curve were observed in orientations for which the monkey had not been trained.

However, in another study (Ghose *at al.* 2002) the authors showed that in V1 perceptual learning consisted only in a little reduction of the response amplitude of single neurons tuned on the training orientation. There were no modifications in the receptive field proprieties. They argued the psychophysical change was more probably obtained by a decoding strategy specifically optimized for training, than by a better neural representation of orientation in the primary visual areas. However, differences in the specificity of training experimental design may influence the contribution of the brain areas involved. In particular, learning observed by Schoups and colleagues was eye and location specific (Schoups *et al.* 2001), which is consistent with neural changes in V1, while Ghose et al. found a transfer of learning improvement from one eye to another and between different retinotopic locations (Ghose *at al.* 2002).

This discrepancy between these two studies might be explained by a recent interesting fMRI study (Yotsumoto *et al.* 2008). Authors examined V1 changing activity during the occurrence of visual perceptual learning, in a texture discrimination task. During every training session subjects were asked to point to a letter while a target stimulus was constantly presented for a brief time in a peripheral location of their visual field. Subjects were asked to identify the letter and to define the orientation of the target stimulus. In separated imaging session, fMRI activity of subjects was measured while the level of the task performance was evaluated. Relation between the level of performance and V1 activity

deferred along the whole period of learning task. Visual plasticity could be distinguished in two phases. In the first one, an increasing of performance level corresponded to an increasing fMRI signal recorded on the visual cortex. Authors suggested that an increase in the number and strength of synapses might have occurred during this phase. These changes probably underlined both the fMRI signal increase, and the higher level of performance. In the second phase, instead, a stable saturation of improved performance occurred together with a decrease of the cortical fMRI signal. After saturation of performance level, the number and the strength of synapses involved, might have been reduced and only the ones that were essential to continue the task might have been kept activated. This experiment suggested a model in which the local network of visual cortex can be reorganized to acquire and consolidate information during learning, but once the task has been completed the level of performance can be kept without further reorganizations.

While in primates the neural substrate involved in perceptual learning may have a deep dependence on training specificity, in rodents the relation between learning and neural changes might be more simple. Repeated exposition to a stimulus of defined orientation, leads to a specific potentiation of the response in primary visual cortex of awake mice (Frenkel *et al.* 2006). This was recently demonstrated by the evidence in V1 of changes in amplitude of visual evocated potentials (VEPs), recorded during visual exposure tests. Repeated exposure to a specific oriented stimulus leads to an increase to its evoked response. Modifications underlying potentiation were resistant even after the subsequent exposure to an orthogonally oriented stimulus. The animals were in a condition of passive exposure to stimuli, so the cortical modification required in case of an active training might have a more consistent effect. It is anyway interesting to note that cortical changes observed in mice are very similar to fMRI signal increase caused by training in human V1 (Furmansky *et al.* 2004) and are also similar to the results obtained by recordings in V1 of monkeys (Schoups *et al.* 2001).

Perceptual learning may also be associated to changes in the contextual modulation of neurons response in V1 (Crist *et al.* 2001 and Li *et al.* 2004). After a training of bisection of three lines or after a Vernier training (little discrepancy of orientation discrimination) monkeys showed an improvement with no change in the receptive fields basal proprieties in V1. However, there was a change in the contextual modulation, a high order property of V1

cells: the modulation of the response due to the introduction, out of one cell receptive field, of further stimuli having spatial relation with the cell preferred stimulus.

When improvement requires a mechanism that takes count of the contest, top-down interactions between multiple brain areas control physiological changes by a combination of local circuitry and feed back connections from higher cortical levels (Grossberg 1999, Gilbert *et al.* 2000 and Gilbert & Sigman 2007). Indeed, the function of primary visual cortex is known to result from an interaction between feed forward and top-down information. Internal representations of the world, behavioral requirements, attention and expectation affect the brain strategy for analyzing the visual field. Complex information that is represented at higher stages may control perceptual learning by influencing simpler processes occurring at antecedent stages by top-down corticocortical connections.

In search of a physiological model for visual discrimination learning

I discussed how visual discrimination learning requires processes of visual perceptual learning, which are principally related to changes occurring specifically in the primary visual cortex (V1). This issue makes V1 the visual area most likely to show plastic changes and the most reasonable to choose for an in-depth examination aimed at the investigation of physiological and cellular mechanisms operating in the network of neurons involved.

However, before analysing in details a suited model for visual discrimination learning, it is useful to review the most used approaches for the study of biological aspects of memory and learning.

Cellular mechanisms underlying memory and learning

The concept of neuronal networks is dated as early as 1884 (Exner 1884) and was further defined by describing algorithms that developed its fundamental principles (Hodges 1983). Since the birth of this concept, ensembles of neurons are thought to participate in maintaining a representation that serves as a memory trace. Such ensembles require dynamic interactions among neurons and the ability to modify these interactions. This implies use-dependent changes in the activity of the network, which is most easily altered by changes in synaptic function. Hebb formalized this idea in his postulate: "When an axon of cell A is near enough to excite cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased (Hebb 1949) ". The use of the Hebb rule in a distributed memory system can lead to an efficient storage of a number of representations within the same neural network.

Since the first attempts on the research of the physiological and molecular modifications underlying memory and learning, neural networks resulted very complex. Thus, this pioneer approach to neuroscience began with the study of the simpler nervous system of invertebrates, in order to define the synaptic plasticity underlying simple forms of learning, like habituation, sensitization and classical conditioning. The experiments on *Aplysia californica* are the most famous example. Results showed that molecular mechanisms underlying classical conditioning in *Aplysia Californica*, are a modification of the simpler processes known to involve sensitization (Castellucci *et al.* 1970, Carew *et al.* 1971, Walters *et al.* 1979 and Kandell 2001). All results converged suggesting that complex kinds of learning might be constituted by an ensemble of mechanisms similar to those underlying simpler kinds (Kosower 1972). This observations moved a lot of researchers to pass to an appropriate model suitable for the more complex mammalian nervous systems.

Among mammals, rodents are ideally suited for such a research in neurobiology. Understanding their neural structures might be crucial for elucidating the fundamental structure and function of the mammalian brain, because rodents are the largest order of mammals, representing over 40% of mammalian species. They are among the smallest

mammals known with adult weights in the range of few hundreds grams and they have a short generation time, on the order of ten weeks from being born to giving birth. This make rodents relatively easy to house. Females breed prolifically in the lab with an average of 5-10 pups per litter, this number is big enough to allow statistically robust sample sizes. Moreover, most laboratory-bred strains are relatively docile and easy to handle.

As soon as the brain of rodents was recognized as a suited mammalian model for neurobiology, investigation about physiological mechanisms of learning and memory started principally with the study of hippocampal circuitry. In fact, since the occurrence of amnesia after hippocampal lesion was well known, hippocampus has always been acknowledged as one of the functional structure principally involved in declarative memory. Electrophysiological studies of hippocampal circuitry soon revealed a phenomenon of increase in synaptic transmission whose mechanisms might also underlie the occurrence of learning and memory. This phenomenon is called Long Term Potentiation (LTP) and is probably the most powerfull model to investigate physiological and cellular mechanisms operating in the network of neurons involved in synaptic plasticity.

The discovery of LTP

Bliss & Lømo first found the proof that hippocampal neurons show plastic modifications that might be the ones necessary for declarative memory (Bliss & Lømo 1973). They observed that tetanic stimulation of the perforant path in anesthetized rabbits increased the slope of the population excitatory post-synaptic potential (field EPSP) recorded extracellularly in the dentate gyrus of the hippocampus. They labelled that phenomenon Long-Term Potentiation (LTP).

LTP is the long-lasting improvement in synaptic transmission between two neurons, occurring after a high-frequency stimulus to the presynaptic fiber. LTP improves the ability of neurons to communicate with one another across synapses and it is recognized as one of the best known form of synaptic plasticity. LTP is ruled by multiple mechanisms that vary

according to different brain region, animal age and species. In the best known form of LTP, enhanced communication is predominantly reached by improving the postsynaptic neuron sensitivity to neurotransmitters.

Most types of LTP are known to be *N*-methyl-D-aspartate (NMDA) receptor dependents (Collingridge *et al.* 1983) and this dependence is directly correlated to two important proprieties: associativity and cooperativity. Indeed, in order for LTP to occur, depolarization of postsynaptic cells and contemporaneous presynaptic activity (as predicted by Hebb's postulated) is necessary and the cooperation of more than one activated fibre is needed. Associativity and cooperativity depend on NMDA receptor which is well suited to be involved in hebbian plasticity mechanisms (Tsien 2000, Brown *et al.* 1988 and Collingridge & Bliss 1995).

NMDA is a voltage-dependent glutamate receptor subtype. For LTP induction, the NMDA receptor must be activated by the neurotransmitter glutamate while simultaneously there must be sufficient depolarization of the postsynaptic membrane to relieve a Mg^{2+} block in the NMDA-associated ion channel, which allows the entry of Ca^{2+} into the postsynaptic neuron. Ca^{2+} activate a number of Ca^{2+}-sensitive second messenger pathways. Because NMDA receptors are sensitive to both presynaptic transmitter release and postsynaptic depolarization, they act as hebbian coincidence detectors. This property can explain cooperativity and associativity through temporal and spatial summation. Thus, activated NMDA receptors at synapses that are proximal to active sites of depolarization, may be depolarized sufficiently to relieve the Mg^{2+} block and initiate the cascade of events that leads to LTP induction. This cascade may occur even if the activity of a particular synapse alone is not sufficient to induce LTP. NMDA receptors can account for the association of two separate afferent projections to the same cell, one strongly and the other weakly active (Kelso & Brown 1986 and Levy & Steward 1979), and for the cooperative requirement by which a threshold number of fibers are active.

LTP: a physiological substrate for memory and learning

Since neurons communicate by synapses and memories are believed to be stored within synapses, it is not surprising that LTP is probably the most popular accepted cellular mechanisms of how memory traces could be stored in the neuronal networks. The reason for such popularity is probably the fact that those changes in the synaptic strength fitt quite well with the theoretical predictions of Hebb's postulate. (Bliss & Lynch 1988, Morris 1989 and Montague & Sejnowski 1994). If memory is stored in networks of neurons and if network efficiency is mediated by persistent activity, then LTP induced by persistent stimulation of an afferent pathway appears as a likely mechanism by which the brain stores information.

Progress has been made in demonstrating that LTP possesses a number of features that would be expected for a computational device used to store information. Among these are the fact that LTP meets the durability requirement for longer lasting memories and the fact that repetition of LTP induction produces longer lasting LTP (Barnes 1979), just as practice improves behavioural retention. Moreover, there are three similarities between LTP and learning in support of the notion that LTP is a memory mechanism: LTP is specific to tetanized inputs, it is associative and it lasts for a long time.

Simple neural reflexes may be incorporated into conditioned reflexes that are known to involve specific neural pathways. Both pre- and postsynaptic specificity have been demonstrated under certain conditions also in LTP. LTP is specific in the way that only tetanized afferents show potentiation. However, the idea of specificity of tetanized afferents has become clouded by reports that LTP induction might involve molecules and retrograde messengers that diffuse into adjacent neurons (O'Dell *et al.* 1991, Schuman & Madison 1991, Bonhoeffer *et al.* 1989). This lack of specificity might however play a role, in the sense that diffuse alterations in different presynaptic elements may permit the storage of the temporal order of inputs (Montague & Sejnowski 1994).

Another interesting property of LTP, which led some researchers to suggest that it is a memory mechanism, is associativity. As already said, if weak non-LTP-inducing stimulation in one afferent is paired with strong LTP-inducing stimulation in another

afferent to the same cell population, then the weakly stimulated afferent will also exhibit LTP (Levy & Steward 1979, McNaughton *et al.* 1978). The property of associativity is reminiscent of classical conditioning, but the temporal constraints of associative LTP are dissimilar to those of classical conditioning. In addition, the necessary temporal ordering of CS and UCS are absent in associative LTP, and a mechanism as simple as associative LTP cannot account for the behavioral complexity observed in classical conditioning. Associative LTP does, instead, bear comparison to sensory preconditioning, another psychological example of learning (Mackintosh 1974).

Even if all these observations suggest that LTP is a substrate of memory, they are not sufficient to validate this hypothesis. In order to demonstrate such hypothesis, stronger evidence from more specific experimental approaches is needed.

Pharmacological approaches

Since the first evidence of LTP in hippocampal neurons, the pharmacological approach has been one of the most common attempts to verify the involvement of LTP-like mechanisms in learning. Unfortunately, administration of drugs is often far from being selective on networks directly involved in learning and memory. It might induce an effect on learning through a sensory, motor, motivational, attentional or other variable (Martinez *et al*. 1991). These concerns complicate the interpretation of studies using this strategy. Here the discussion is limited to pharmacological studies that used relatively localized, or at least intra-CNS administration of drugs, so that as far as possible the effects described can be interpreted of an action of the drug in a circumscribed area of the brain.

Subsequent to the demonstration of the important role of NMDA-type glutamate receptors in LTP induction, a number of behavioural researchers rushed to characterize the effects of NMDA-receptor antagonists on learning. One of the most comprehensive study examined intracerebroventricular (ICV) administration of AP5, the selective NMDA antagonist, on learning in a Morris water maze task (Morris *et al.* 1986). Prior research indicated that the hippocampus is important in the acquisition of this task, that is, when rats

have to learn the location of a hidden platform in a water pool, with respect to distal cues in the environment (Morris *et al.* 1982). Researchers first assured that NMDA antagonist caused no apparent sensorimotor impairments, then infused a group of animals with AP5 and showed a significant, but not striking, spatial learning impairment. Authors suggested that learning in the Morris water maze can involve non-spatial elements and that other hippocampus-independent strategies are employed in the initial stages of learning. In this view, spatial deficits should be most apparent at the point of asymptotic learning, and performance in the probe trials should be sensitive to spatial-learning deficits. Thus, for many researchers, the most convincing indication of memory deficits is observed in the probe trials. During this test the platform is removed, and the amount of time the animal spends in the quadrant where the platform was located is measured. Animals treated with AP5 showed no preference for the original location of the platform. By contrast, animals that received either saline or the inactive stereoisomer of AP5 showed a significant preference for the quadrant where the platform had been located, which indicates that the animals treated with NMDA antagonist had no spatial memory of the platform. The effect of AP5 on LTP induction was also assessed to compare the behaviour-impairing and LTP-induction-impairing action of AP5. LTP was induced by stimulation of the perforant-path dentate synapse. The drug had no effect on the low-frequency evoked responses, while it impaired acquisition of the water maze task and completely blocked LTP induction (Morris *et al.* 1982).

It is important to take consideration, however, that animals can also choose different strategies that do not include the expected learning strategy. Indeed Morris and his colleagues, using the Morris swim task (Bannerman *et al.* 1995), have provided evidence that, under some circumstances, LTP may not be necessary to learn the solution of a spatial problem. Normally, acquisition of this task is prevented by hippocampal NMDA receptor blockade; however, if rats are pretrained in the same apparatus in a different room, acquisition is essentially normal under NMDA receptor blockade but is nevertheless prevented by hippocampal lesions. It is assumed that rats solve the swim task by learning the relationships between the remote visual cues and the hidden platform. If this were true, one might conclude that hippocampal LTP is unnecessary for this form of learning. Two other possibilities are the uncertainty of whether NMDA receptor blockade is sufficiently

complete and the possibility that the rats do not always use a spatial strategy to solve the problem.

Metabotropic glutamate receptors seem also to be implicated in the induction of LTP. This evidence prompted the assessment of the role of these glutamate receptors in spatial learning. Perfusion of the metabotropic antagonist [RS]-α-methyl-4-carboxyphenylglycine (MCPG) did not produce deficits in animals during acquisition of a Morris water maze (Richter-Levin *et al.* 1994), although a significant deficit was observed in probe trials given 24 h after the last training trial. In these same animals, equivalent quantities of MCPG attenuated the magnitude but did not block the induction of perforant-path dentate LTP. Thus antagonism of metabotropic glutamate receptors produces some late deficits in LTP and spatial learning. In summary, localized receptor blockade does produce observable deficits. These deficits are similar to those observed with extensive hippocampal lesions.

More recent experiments of pharmacological intervention enlarged this kind of approach to different type of molecular interference. For example, both spatial memory and LTP were influenced in parallel, also by a GABA-B receptor antagonist (Stäubli *et al.* 1999), by an oxytocin antagonist (Tomizawa *et al.* 2003) and by an antisense oligodeoxynucleotide used to inhibit a protein known to be associated with cytoskeletal proteins in hippocampal neurons. Pharmacological approaches also seem to be easily effective in various brain structures and types of learning, like shown by blockade of NR2B subunit of the NMDA receptor, which impaired the induction of cingulate LTP and the formation of early contextual fear memory (Zhao *et al.* 2005).

These findings suggest not only that LTP may contribute to mechanisms underlying various forms of learning, but also that it may be a fundamental mechanism of information storage.

Genetic approaches

Long term changes of cell function occurring in long-term memory storage are known to be controlled by gene expression and resultant protein production. Many research groups investigated the chain of cellular events that underlie induction and maintenance of LTP (Grant *et al.* 1992, Silva *et al.* 1992a, Silva *et al.* 1992b) by recurring to genetic approaches. In these studies the mouse model was chosen, given that its genome is well characterized. Expression of specific genes was altered and the resultant effect was studied in whole transgenic mice for LTP and learning. When alteration leads to a complete blockage of gene expression the animals are called knock-out mice. The gene of interest, usually a well-characterized gene, is cloned and this altered DNA is introduced into embryonic stem cells derived from blastocysts. The gene combines with the DNA of the stem cells, and those cells in which the gene is inserted at appropriate regions of the DNA can be isolated and inserted into developing blastocysts. Subsequent cells arising from these altered cells have the modified gene. The resulting animal is a heterozygous chimera (combination of normal and mutant cells) that, with cross breeding, can generate progeny that are homozygous for the modified targeted gene.

In studies of genes related to LTP, an area of focus in the study of transgenes has been kinases. A group of researchers (Silva *et al.* 1992b) engineered knockout mice to be deficient in α-calcium-calmodulin-dependent kinase II (α-CaMKII). Although the probability of induction of LTP was greatly reduced in the mutants, LTP in some animals was virtually indistinguishable from LTP observed in wild-type controls. A subsequent study (Silva *et al.* 1992a) assessed the ability of α-CaMKII mutants to learn the Morris water maze. The α-CaMKII mutants were impaired in their ability to find the hidden platform on the first session of training. In the probe trial, the mutant mice took roughly twice as long to find the platform. An additional test employed a randomly located platform. Some trials were conducted with the hidden platform randomly located at other sites. Mutant mice took as long to find refuge at the random sites as to find refuge at the original location, whereas wild-type mice took less time to find the original location and longer times to find the random platforms, which indicates negative transfer. Thus, the evidence suggests that the α-CaMKII mutants had a deficit in the ability to learn the spatial

maze. What is not so clear is whether this spatial deficit is related to LTP. In the mutant mice only the probability of LTP induction was altered. LTP induction, however was not abolished.

Other groups targeted genes specific for subtypes of the glutamate receptor. One group (Sakimura *et al.* 1995) created mice with a mutation of the GluRe subunit of the NMDA-receptor channel. During training in the Morris water maze the mutants showed an initial latency deficit that disappeared by the end of training. The authors considered their findings positive evidence for the participation of the GluRe subunit of the NMDA receptor in both LTP and the acquisition of spatial learning. The gene mutation, however, did not abolished LTP nor spatial learning.

One group created a metabotropic glutamate receptor 1 (mGluR1) mutant to test involvement of mGluR1 in LTP and contextual-fear conditioning (Aiba *et al.* 1993). The mGluR1 mutants had a reduced LTP magnitude and were impaired in the hippocampus-dependent contextual-fear conditioning task. The authors concluded that the mGluR1 receptor modulates neural plasticity, apparently expressed as the magnitude of LTP. Another group (Conquet *et al.*1994) found that in Morris water maze, the mGluR1 mutants could not find the platform and evidenced no learning. They concluded that the observed deficit was due to an impairment of spatial ability mediated by mGluR1 receptors and probably in the mossy-fiber CA3 system, because LTP was greatly reduced only in the mossy fiber-CA3 system.

Knockout strategy provided a strong evidence that LTP is a substrate of learning. However despite of its specificity of elimination this strategy was weakened by the complexity of the mutant creature that had developed without a particular gene. Many questions concerned whether an animal's motor and sensorial systems were competent to perform what was required. Fortunately, these problems has been in part overcome by the use of inducible and reversible form of transgenic mutants (Mansuy *et al.* 1998 and Malleret *et al.* 2001). Subsequent studies succeeded in confirming that mutant forms of specific molecular factors interfering in the mouse forebrain, cause changes in both memory storage and LTP induction efficacy (Miller *et al.* 2002, Morozov *et al.* 2003, Kelleher 3rd *et al.* 2004, Seeger *et al.* 2004, Moosmang *et al.* 2005, Costa-Mattioli *et al.* 2005 and Moretti *et al.* 2006).

Does learning produce LTP-like changes?

In order to gain further evidence of the LTP pertinence as a model for memory and learning, a reasonable check is the verification that changes found after the induction of LTP match the same modifications noticed after the learning task, when analysing the memory structure principally involved. For example, a group of researchers recorded responses in the mossy-fiber projections of the hippocampus, as animals learned a radial arm maze (Mitsuno *et al.* 1994). Incremental increases were observed in mossy-fiber field EPSPs over the course of learning. Changes in evoked responsiveness were evident three days after learning. In another study specific learning task was substituted with enriched environment, as rearing animals in complex environments produces changes that are thought to be a result of increase in learning opportunity (Bennett *et al.* 1964, Greenough e*t al.* 1973, Rosenzweig *et al.* 1962). The field EPSP slopes of in *vitro* hippocampal slices taken from animals exposed to an enriched environment was larger in rats raised in a complex environment than in rats housed in standard laboratory conditions (Green & Greenough 1986).

LTP-like changes after learning are likely to happen also in other brain areas and structures beside hippocampus, suggesting that similar cellular mechanisms are involved wherever synaptic plasticity underlies formation of memory traces. For example, fear conditioning is known to induce associative LTP-like changes in the amygdala (Rogan *et al.* 1997). This has been seen by measuring CS evoked field potentials in lateral nucleus of amygdala (LA), before, during and after fear conditioning in freely behaving rats. The CS was an acoustic tone able to trigger the acquisition of an evoked waveform from the electrode in LA. Slope and amplitude of the waveform were unchanged by unpaired presentation of the CS and the aversive unconditioned stimulus US, but increased significantly when the CS was paired with the US.

There is also a study that shows evidence for LTP like modifications following learning involving the cerebral cortex: rats were trained to reach their food through a hole in a box with a single forpaw, in order to retrieve small food pellets using a grasping motion (Rioult-Pedotti *et al.* 1998). Field potentials evoked by stimulation of primary

motor area horizontal connections were increased after learning and practicing the skilled reaching task.

In summary, all these studies show changes in synaptic strength that may be due to LTP-like mechanisms. However, to reach such conclusion, there's still an important matter to point out. Why should changes in evoked-response amplitude following a single learning episode be detectable? According to the view of distributed memory systems, changes underlying learning should occur in a very small fraction of the available synapses, and there is no reason to expect that such sparse changes would be evident in synaptic activation evoked by the stimulation of thousands of afferent fibers activated by a stimulating electrode. The amygdala and hippocampal memory systems could have a small capacity and utilize most synapses when storing information. In such a system an evoked response might reveal the existence of a stored memory. The information in these low-capacity systems would have to be erased or have to decay rapidly in order to store new information. Some researchers suggest that mossy-fiber projections to CA3 constitute a low-capacity store (Lynch & Granger 1986) because LTP in mossy fibers can decay quite rapidly (within hours) in vitro (Mitsuno *et al.* 1994). However, learning-induced changes in evoked mossy-fiber responses are observed three days after the end of training, bringing evidence against the neural changes representing a transient, low-capacity store.

The troubles regarding non measurable changes can be overcome by a suited strategy. Synaptic changes in responses mediated by a large number of afferents do not need to be observed. The evoked response may be utilized as an integral part of the learning task. Stimulating randomly a large number of fibers is not necessary to detect specific changes induced by learning. Indeed, the artificial stimulation of these fibres can be substituted by behavioural (learning) task which is able to activate these same fibres. This strategy has been used in studies concerning a shuttle avoidance task with a foot shock as US (Matthies *et al.* 1986, Ott e*t al.* 1982, Reymann *et al.* 1982). High-frequency perforant-path stimulation was the CS. Low-frequency evoked responses were recorded in the dentate gyrus before, during, and after 10 daily training sessions. Changes of the field EPSP slope corresponded to changes in learned behavior. The increases in the field EPSP followed learning across days and asymptotic performance occurred on the days of asymptotic LTP.

Another way to overcome the trouble in detecting specific learning-induced changes is to use a multielectrode recording array that is able to cover a large area, in order to get a separate recording track of different locations. This has been done in a recent study in which synaptic transmission in CA1 was monitored by stimulating Shaffer collateral axons before and after the inhibitory avoidance paradigm (Whitlock *and al.* 2006). During the training, animals were allowed to walk through the apparatus without the shock or given the shock only. After that experience, animals returned to the recording box. When the strength of synaptic transmission was monitored, the majority of channels showed a slight decrease after behavioural conditioning, but two channels exhibited a substantial increase, which was apparent immediately, and persisted for the duration of the recording session.

Taken together, these studies still preserve the claim that learning may induce an increase in responsiveness of neurons involved, resembling the consequences observed following LTP induction.

Does the induction of LTP influence subsequent learning?

With repeated tetanic stimulation of an afferent pathway, the level of LTP does not increase infinitely, but approaches an asymptotic state (Bliss & Lømo 1973). Another way to test the LTP–learning hypothesis is the predicted blockade of memory formation following saturation of LTP. LTP induced prior to learning might impair it by saturating LTP processes that normally participate in learning. In order to find out if learning is blocked by saturation of synaptic strength, a sufficient proportion of synapses has to be enhanced. Behavioural impairment may be observed even before full saturation is reached, so the aim should be at least to minimize the number of synapses that can be further potentiated in subsequent behavioural tests. Indeed, if memories in the hippocampus are likely to be sparse and distributed according to a reliable model of neural code (Marr 1971 and Mc Naughton *et al*. 1987), effects of saturation of LTP on subsequent learning would follow a sigmoidal function. This implicates that impairment of memory formation would occur before the entire synaptic population has been saturated (Barnes *et al.* 1994).

The first attempt to run such a saturation experiment concerned the effects of LTP induction on the acquisition of classically conditioned nictitating membrane response (NMR). LTP induced unilaterally in the perforant path facilitated the subsequent acquisition of a classically conditioned NMR in rabbits (Berger 1984). Yet, the hippocampus is not essential for learning of simultaneous classical conditioning of the NMR, so this may be a modulatory effect, rather than a direct effect on a learning mechanism. Two years later, an opposite effect was observed in a circular platform task, which is a procedure known to concern spatial learning (McNaughton *et al.* 1986). During the training, animals were set in an illuminated open platform with various holes around the border, but only one of those was connected to a shelter box below the platform. The only way to escape from the light, was to remember the position of this safety hole. After the acquisition of this behavioural performance LTP was inducted by stimulating the angular bundle of the hippocampus. The induction did not interfere with subsequent retention and retrieval of the previous learned location. When induction was applyed before a new learning task, animals made more errors in learning the new goal location. These results suggested that instead of retention and retrieval, acquisition was more likely to be affected by LTP-induction.

Subsequently, another group of researchers elicited LTP saturation by stimulating the same locus of the hippocampus and observed a memory impairment in water maze learning task (Castro *et al.* 1989). Animals that received high frequency stimulation (HFS) sessions for 15 days, showed an impaired performance and learning capacity recovered in the same amount of time that it took LTP to decay. As a control, the ability to locate a visible platform was assessed, and no difference was observed between the stimulation groups, which indicates that the stimulation did not affect sensory capacity. Rats in which LTP was induced and then allowed to decay, did not show any learning deficits. Thus, in this case saturation was more likely to disrupt the retrieval of information instead of the acquisition. The discrepancy between these two experiments has remained without an explanation. Moreover subsequent attempts failed to replicate Castro's study (Korol *et al.* 1993) (Jeffery *et al.* 1993) (Sutherland *et al.* 1993) (Cain *et al.* 1993) and saturation of LTP did not appear to affect standard eight-arm radial maze task acquisition (Robinson 1992). Disparities between these different attempts, are probably due to problems in reaching

saturation. First, stimulating of the angular bundle with a single stimulation electrode may not increase synaptic weights sufficiently. Second, the number of potentiated synapses following HFS may be reduced by intrinsic inhibitory activity. Third, excitatory consequences of LTP (postsynaptic desensitization, new spine and new synapses formation) may reduce the amount of saturation. Fourth, LTP saturation does not prevent the induction of long term depression (LTD) (Linden & Conner 1995), which is also a potential memory mechanism (Sejnowski 1977, Stent 1973). Finally, learning impairment may differ for different learning tasks indicating different task susceptibility to LTP saturation. This seems the case of another study (Barnes *et al.* 1994) in which the same saturation procedure produced a deficit in the circular platform acquisition learning task, but not in the Morris water maze.

However, an ingenious study succeeded later in overcoming the problems of reaching saturation by improving sensitivity of the protocol (Moser *et al.* 1998). The volume of available hippocampal tissue was reduced by removing the hippocampus and dentate gyrus unilaterally, and a specially designed array of concentric bipolar stimulation electrodes was implanted contralaterally in order to increase the proportion of synapses undergoing saturation. Stimulation with cathode on one side and anode on the other side of the angular bundle (cross-bundle stimulation) was applied. Within a single day, LTP was induced by repeated cross-bundle tetanization. To check whether LTP was saturated, researchers tested whether more LTP could be induced through a ’naive’ central stimulation electrode. Only rats in which no further LTP was obtained were unable to learn the water maze task. The results with cross-bundle stimulation suggest that the amount of saturation is a critical factor. Learning was impaired only if the perforant path synapses had been potentiated maximally. These findings may explain why previous attempts to impair spatial learning by saturation of LTP had failed. With a single tetanization electrode, it may not be possible to recruit sufficient fibres to block further synaptic enhancement in the behaving rat.

Taken together, these data suggest that LTP itself, rather than non-specific effects of stimulation, is essential for learning because saturation-impaired acquisition of spatial learning tasks and the ability to learn are reinstated with the decay of LTP. In summary, there is convincing evidence that, at least in the hippocampus, a suit protocol of LTP

saturation is able to provoke impairment of learning by interfering with the same synaptic mechanisms probably required by learning itself.

Does learning influence the induction of LTP?

The LTP-learning hypothesis may further be verified by a reverse strategy of the previous approach: if LTP processes are a substrate of learning, full employment of these processing in learning activity should reduce the amount of potentiation after LTP induction. This strategy has an evident advantage: it raises the possibility to avoid the non specific effects of LTP induction on behavioural learning task. However, in spite of this advantage, researchers have to plane a learning task able to affect the studied neural structure as much as possible, in order to see an effect after subsequent LTP induction. This problem can be overcome also by recording simultaneously from different locations of the examined neural structure. In the already mentioned Whitlock's study, a group of animals was trained with the inhibitory avoidance paradigm and changes in field EPSP slope after training were compared with the subsequent enhancements induced by repeated application of HFS to saturate LTP (Whitlock *and al.* 2006). Electrodes where field EPSP were enhanced after training, showed less subsequent LTP in response to HFS.

The purpose to verify LTP induction after learning allows researchers to benefit from another consistent advantage. They have the possibility to use an *in vitro* electrophysiological technique, which can be used only when the animals have not to be employed in subsequent behavioural sessions. Indeed, an *in vitro* experiment requires slice preparations that are known to have a significantly reduced inhibition. Tetanic stimulation often results in a 100% increase of the slope of the field EPSP, either in the perforant-path synapses of the dentate gyrus (Hanse & Gustafsson 1992), the mossy-fiber synapses of CA3 (Zalutsky & Nicoll 1990) and the Schaffer-collateral synapses of CA1 (Kauer *et al.* 1988, O'Dell *et al.* 1991). Moreover, GABA antagonists can be added to the bath medium to facilitate potentiation. In the intact brain, instead, less potentiation is obtained. The field EPSP slope is seldom increased beyond 30%–40% in freely moving animals, at least as measured in the dentate granule cell layer during stimulation of the perforant path (Barnes

1979, Barnes *et al.* 1994, Cain *et al.*1993, Jeffery & Morris 1993 and McNaughton *et al.*1986). Most classes of dentate and hippocampal inhibitory interneurons have axon collaterals coursing extensively along the longitudinal axis of the hippocampal formation (Buckmaster & Schwartzkro 1995, Han *et al.*1993, Sik *et al.*1997, Sik *et al.*1995, Sik *et al.*1994 and Struble *et al.*1978). These collaterals are likely to be severed in a transverse slice preparation. Because of the massive inhibition present in the intact brain, physiological stimulation in anesthetized and behaving rats is more unlikely to induce saturation of all, or even most, synapses theoretically potentiable.

This discrepancy between the *in vitro* and *in vivo* approach is probably present also in other structures beside hippocampus. A learning-LTP relation was successfully demonstrated also in the primary motor cortex (M1) just by using an electrophysiological *in vitro* approach (Rioult-Pedotti *et al.* 2000). Rats were trained to reach and to retrieve small food pellets from a box, until success rate became asymptotic. After learning, evoked field potentials were recorded across layer II/III horizontal M1 connections in slice preparations. Repeated theta burst stimulations produced less LTP in the trained animals than in untrained ones. Thus, these results make the LTP-learning hypothesis suitable to be verified in neocortical circuitry.

LTP: a cellular point of view

Once recognized the validity of the LTP model, it’s important to spend some words defining its cellular and molecular mechanisms. Indeed, these same cellular processes are likely to match the ones involving the physiology underlying memory and learning. From its earlier phase, the major evidence for the LTP expression is an enhancement in the postsynaptic response. High frequency stimulation (HFS) induces LTP by a huge release of glutamate from the presynaptic terminals with a subsequent strong depolarization on the postsynaptic neurons. The effect of this residual post-tetanic potentiation ends within few seconds from the end of the strong presynaptic stimulation. However, postsynaptic response keeps a level that remains elevated for several hours and may even further

increase. This long lasting potentiation is the result of the activation of the intracellular paths following the previous induction. During HFS, strong postsynaptic depolarization is able to activate NMDA receptors and L-type voltage-gated calcium channels (VGCCs). The influx of calcium into postsynaptic neurons through NMDA receptors and VGCCs is the triggering event in hebbian plasticity mechanisms like LTP (Nicoll & Malenka 1995 and Magee & Johnston 1997). This influx of Ca^{2+} can engage signalling cascades that activate some kinases, the principally recruited factors during the early phase of the LTP. Kinases involved have their molecular pathways in the subsynaptic cytoskeleton or scaffold, the postsynaptic density (PSD), where they are embedded with glutamate receptors, channels, signalling molecules and various phosphatases that couple synaptic activity with postsynaptic biochemistry (Sheng & Kim 2002 and Kennedy 1997). In particular, Ca^{2+}/calmodulin protein kinase II (CaMKII), mitogen-activated protein kinase (MAPK), and adenosine 3',5'-cyclic monophosphate (cAMP)-dependent protein kinase (PKA) are some of the major components of the PSD and are all required for the induction of LTP. MAPK and CaMKII can promote the phosphorylation of each other, and MAPK is required for an increase in CaMKII levels produced by LTP-inducing stimulation. PKA activity promotes CaMKII phosphorylation by indirectly inhibiting the protein phosphatase PP1, which would otherwise limit the degree or persistence of CaMKII activation by dephosphorylating the kinase. This signalling cascade may be restricted to appropriately stimulated dendrites, but MAPK and PKA can also translocate to the nucleus, where they regulate gene transcription (Berardi *et al.* 2003 and Thomas & Richard 2004).

Synapses undergoing LTP seem to do so by moving from an active state to a potentiated state (Montgomery & Madison 2002). In the CA1 region of the hippocampus, this shift is known to be just NMDA-receptor-dependent. The effectiveness of LTP is amplified due to the presence of some called silent synapses, having normal NMDA receptors, but lacking AMPA receptors (Isaac *et al.* 1995, Montgomery *et al.* 2001, Faber *et al.* 1991, Kullmann 1994, Liao *et al.* 1995 and Durand *et al.* 1996). Potentiation leads silent synapses to a "recently silent" state in which they have both AMPA and NMDA receptors. Since silent synapses are active only after the induction of LTP, their presence brings to a huge increase of test stimulus response during the potentiation period. Thus, synaptic potentiation can be reached by increasing AMPA receptors activity and by changing their

localization (Benke *et al.* 1998 and Derkach *et al.* 1999). Indeed, CaMKII phosphorylates and stabilized GluR1 AMPA receptors subunit, in order to increase their channel conductance (Lee *et al.* 2000), inhibiting the internalization of newly inserted receptors (Lee *et al.* 2003) and increasing the insertion of those receptors into the postsynaptic membrane through an indirect mechanism (Hayashi *et al.* 2000).

The maintenance of long lasting LTP requires not only protein kinase activation and protein phosphorylation but also protein synthesis from existing mRNAs and gene expression (Bliss & Collingridge 1993). Behavioral approaches to learning suggested that these same cellular processes are involved in the establishment of long-term memory (Brinton 1991). In its late phase, LTP is known to trigger the transcription of many genes. These include immediate early genes (IEGs) such as cAMP-responsive element binding protein (CREB) and Zif268, that are both required for the consolidation of recognition memory (Bozon *et al.* 2003). Synthesis of proteins involved in synaptic plasticity probably occurs in the dendrites (Roberts *et al.* 1998). Local synaptic protein synthesis would then allow rapid, localized changes in synaptic strength. This mechanism requires mRNA to be transported from the soma to the dendrites and than to be translated specifically at stimulated synapses.

A clue to understand how mRNA release is regulated in dendrites comes from the phenomenon of synaptic tagging. In hippocampus, when synapses are weakly stimulated, they nonetheless develop long lasting LTP if neighbouring synapses are strongly stimulated within a brief interval. The strong stimulus needs to be able to induce protein synthesis, which may occur in the cell body or in the dendrites. The weakly stimulated synapses thus generate an identifying tag, which then allows them to capture proteins made in response to the strong stimulation of their neighbours (Barco *et al.* 2002; Frey & Morris 1997, Frey & Morris 1998a, Frey & Morris 1998b). Messenger RNAs are packaged in granules together with ribosomes and multiple proteins and synaptic tagging is likely to be related to the release of mRNA from granules at specific dendritic sites. The capture and mobilization of mRNA by stimulated synapses would logically precede synaptic protein synthesis because the mRNA in granules cannot be translated (Krichevsky & Kosik 2001), which fits with the observation that tagging in hippocampus does not require protein synthesis.

Synaptic protein synthesis provides the molecules necessary to increase synaptic transmission. These could be the same as the ones involved in the induction of LTP. Thus, synthesis of CaMKII, glutamate receptors, and scaffold proteins may help sustain the synaptic enhancement seen during the early phase of LTP. There is evidence that induction of LTP and memory formation also lead to changes in the number or shape of dendritic spines (Weiler *et al.* 1995, Nikonenko *et al.* 2002, Sorra & Harris 2000, Yuste & Bonhoeffer 2001 and Muller *et al.* 2002). Spines are specialized protrusions on dendrites that contain a PSD. Spines provide a closed compartment that allows rapid changes in the concentrations of signalling molecules, such as calcium, and therefore make it possible an efficient responses to inputs (Nimchinsky e*t al.* 2002). Modulation of the number of dendritic spines and/or their morphology has been proposed to contribute to alterations in excitatory synaptic transmission during learning (Bailey & Kandel 1993 and Nimchinsky e*t al.* 2002). The architecture of spines, and therefore their ability to change, depends on the specialized underlying structure of cytoskeletal filaments (Matus 2000). These microfilaments are composed of actin, which is present throughout the spine cytoplasm in close interaction with the PSD. Developmental studies have shown that changes in spine stability and motility depend on actin polymerization (Fischer *et al.* 1998 and Dunaevsky *et al.* 1999). Reorganization of actin could therefore contribute to the structural plasticity of spines after LTP induction.

LTP in the primary visual cortex

For many years it appeared that NMDA-receptor dependent LTP might be a phenomenon expressed primarily in the hippocampus due to this difficulties in reliably eliciting it in neocortex. Fortunately, the procedural difficulties have been soon overcome, and nowadays it is well known that neocortical synapses also support robust LTP (Tsumoto 1992 and Bear & Kirkwood 1993).

In rat visual cortex slices, it's possible to evoke field EPSP by applying stimulation to the white matter through vertical connections and recording from layer II/III. There is

evidence suggesting that active synapses in layer II/III are not modified if the level of postsynaptic activation during a high-frequency tetanus is low, depressed if the level of postsynaptic activation is moderate and potentiated if the level of postsynaptic activation is high (Artola *et al.* 1990). Activity-dependent synaptic plasticity in the superficial layers of adult rat visual cortex has been compared with that in CA1 hippocampal field (Kirkwood *et al.* 1993). The susceptibility to undergo white matter LTP (WM-LTP) is known to be age-dependent: at 2 weeks of age HFS brings to a high levels of potentiation, while at 4 weeks post synaptic response is no longer potentiated (Kato *et al.* 1991) unless GABA-A receptors are partially blocked (Kirkwood & Bear 1994). Interestingly, this period of susceptibility to WM-LTP nearly coincides with the critical period of sensibility to monocular deprivation. This suggests that WM-LTP is probably involved in the maturational processes of the visual cortex that occur during the critical period of plasticity in early life.

HFS of neocortical layer IV instead, induces LTP in layer III (IV-LTP) also during the adulthood and with precisely the same types of stimulation protocols that were effective in CA1 area of the hippocampus. As in the hippocampus, IV-LTP is specific to the conditioned pathway, input specific and dependent on the activation of NMDA receptors (Kirkwood *et al.* 1993). These observations provided strong evidence for the view that common principles may govern experience-dependent synaptic plasticity in CA1 and throughout the superficial layers of the visual cortex. IV-LTP is not observed in layer V neurons responses, suggesting a preferential involvement of synapses on layer III neurons. IV-LTP well satisfies the definition of a "Hebbian" modification as it could also be produced by pairing low-frequency synaptic stimulation (approximately 100 pulses at 1 Hz) with strong intracellular depolarization of layer III neurons (Kirkwood & Bear 1994).

The critical difference between IV-LTP and WM-LTP is not the magnitude of the responses to single stimuli delivered to the two different sites, but it probably lies in the postsynaptic depolarization during high-frequency stimulation. Consistent with this idea, in the adult visual cortex associative LTP could be elicited from white matter only when converging but independent inputs from the white matter and layer IV simultaneously receive tetanic conditioning stimulation (Kirkwood & Bear 1994). Inhibitory circuitry in layer IV normally seems to act as a sort of band-pass filter that constrains the types of

activity patterns that can gain access to the modifiable synapses in layer III. By stimulating in layer IV there is the possibility, instead, to bypass this filter and to overcome the threshold for LTP induction in layer III (Kirkwood & Bear 1994).

Current-source density (CSD) analysis has been performed to determine how the patterns of cortical activation differ in layer IV and white matter stimulation conditions. Superficial current sinks, at a depth of approximately 200 microns, are virtually eliminated by high concentrations of divalent cations after white matter stimulation, but not after layer IV stimulation, suggesting that stimulation at the two sites recruits different circuits (Aizenman *et al.* 1996). Moreover, while there is little evidence of a paired-pulse interaction after stimulation of layers IV, there is a marked suppression of superficial layer III current sinks after paired-pulse stimulation of the white matter. White matter stimulation seems to activate layer III neurons either by a monosynaptic route and by a disynaptic route. The disynaptic input originates in layer IV and it is controlled by inhibition. Thus, the recruitment of disynaptic layer IV inputs is required for the generation of LTP in layer III and layer IV input efficacy is strictly dependent on the inhibitory cortical tone. These observations agree with the evidence that age-dependent synaptic plasticity relies upon changes in the excitatory-inhibitory balance (Hensch 2005) and make layer IV stimulation the suited procedure to induce LTP in order to study synaptic plasticity in the adult visual cortex.

LTP can also arise by activity-dependent mechanisms within layer II/III horizontal projections (0.5mm-1mm) and persistent changes in the effectiveness of functional interactions of cortical neurons can be triggered. These changing interactions suggest a likely mechanism to recognize underlying cortical pattern representation. However, while field potential recordings were used in various rat cortical areas to investigate these modifications (Hess & Donoghue 1994, Bilkey 1996 and Yun *et al.* 2000), none is known about LTP inducted by horizontal projections stimulation in the visual cortex.

PKA, MAPK and CaMKII, the same three kinases involved in LTP induction in the hippocampus, are necessary for LTP induction also in the visual cortex (Kirkwood *et al.* 1997, Liu *et al.* 2003 and Di Cristo *et al.* 2001). The same three kinases are known to be involved in visually driven activation of synaptic plasticity and MAPK in particular is also powerful activated by patterned vision in neurons of the visual cortex (Cancedda *et al.*

2002). Each kinase is activated by a specific pattern of extracellular signals and the possible targets are at two different levels: the cytoplasm and the nucleus. In the first case, in a local and rapid action, these kinases phosphorilate substrates that are crucial for synaptic transmission, neuronal excitability and morphological stabilization. In the second case, their activity is involved in gene expression and protein synthesis, that are also necessary for long lasting changes in neuronal circuitry (Mower *et al.* 2002 and Taha & Stryker 2002). Thus, the pattern of kinase activation has to be translated into a pattern of gene expression, probably through the activation of transcription factors.

An important hint for the molecular identity of those transcription factors necessary for plasticity, is offered by the finding that the activation of CREB is necessary for ocular-dominance plasticity (Mower *et al.* 2002, Liao *et al.* 2002 and Pham *et al.* 1999) and may be involved also in the maintenance of LTP (Akaneya & Tsumoto 2006). To cause CREB phosphorylation, activated kinases must translocate to the nucleus, where they start the expression of genes under the cAMP-response-element (CRE) promoter, with the consequent production of gene transcripts essential for establishment and maintenance of plastic changes (Silva *et al.* 1998). Both PKA and MAPK are well characterized activators of CREB (Impey *et al.* 1996, Mayr & Montminy 2001), but MAPK in particular seems to be the final effector linking extracellular signals with gene expression in the visual system at least during the critical period (Cancedda *et al.* 2002).

LTP in primary visual cortex and visual discrimination learning

The previous discussion about visual discrimination learning argues that declarative and non-declarative processes seem to converge, at least in part, towards plastic modifications in the primary visual cortex. At the same time, I have highlighted the strong evidence that LTP is a valid model to study mechanisms of synaptic plasticity underlying memory and learning. This has been seen by different approaches: showing that both LTP and learning are impaired by the influence of the same drug, showing that specific transgenic mutants have alterations influencing both learning and LTP, showing that the effects of learning mimics the effects of LTP, showing that learning can be occluded by previous LTP saturation and showing that LTP can be occluded by previous intensive learning sessions. However, while this LTP-learning relation is well established in the hippocampus, little is known about the role of LTP in the neocortex, and in particular in the primary visual cortex.

In the present thesis study different behavioural and electrophysiological techniques were used to investigate the possibility that visual discrimination learning brings to changes in synaptic function coinciding, at least in part, with the same physiological processes triggered by the induction of LTP. To verify this hypothesis, three of the five approaches previously mentioned were used: drug administration, mimicry and LTP occlusion by learning. This thesis study used rats that had the possibility to improve their visual discrimination ability by a specific training in a behavioural test.

Theoretically, improvement can involve various proprieties of a visual stimulus like orientation or spatial frequency. However, improvement in terms of orientation seemed to be less effective. Indeed, neurons of rat primary visual cortex are strictly selective for few orientation values (horizontal, vertical, 60° and 30°) with very tight orientation tuning curves (Keller *et al.* 2000). Spatial frequency tuning curves instead are broader, although neurons show a best response to a preferred value. Moreover the entire spectrum of preferred spatial frequency values is larger if compared to the few values of preferred orientations (Keller *et al.* 2000). These observations suggest that neurons of rat primary

visual cortex seem more suitable to be subject of further modifications concerning their spatial frequency channels rather than their orientation selectivity.

Thus, in this behavioural test, animals were introduced to a discrimination task concerning two vertical oriented gratings of different spatial frequency values. During the test, a standard grating had a fixed spatial frequency value. The value of the other grating was changed in order to make it more similar to the standard one depending on the animal's performance.

After the visual discrimination task, animals were used to verify mimicry by recording f-EPSP from slices taken from the primary visual cortex. In order to verify the occlusion of IV-LTP and III-LTP, slices were also used to test the effect of HFS on vertical and horizontal connections.

At the end of their behavioural test, some animals had the orientation of their stimuli changed. This alteration was performed to confirm the selectivity of this visual discrimination task for the stimuli orientation, in order to assure that neural modifications were principally elicited in the primary visual cortex. Moreover, the parallel pharmacological effect on visual discrimination learning and LTP was assessed by treating a group of rats with U0129, a drug known to impair LTP effects.

Materials and methods

Animals

In this study Long Evans 31 rats 2–3 months old were used. Experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86.609.EEC) and were approved by the Italian Ministry of Health. Animals were housed in a room with a temperature of 21 C°, 12/12 light/dark cycle, and food and water available ad libitum.

Experimental settings

Visual water task

The method for training animals in perceptual learning derives from the task utilized for the behavioural assessment of visual acuity in mice and rats (Prusky *et al.* 2000). Mice and rats are instinctive swimmers and this task exploits their natural inclination to escape from water to reach a hidden platform, the position of which is predicted by a visual cue. Before the test of visual acuity begins, animals are conditioned to distinguish between a low spatial frequency square-wave grating and homogeneous gray. Subsequently small incremental changes in the spatial frequency of the stimulus are made between successive blocks of trials until the ability of animals to distinguish a grating from gray fails. The highest spatial frequency achieved consistently is recorded as the acuity threshold.

This kind of conditioning procedure can also be used to investigate various visual abilities besides visual acuity, like movement direction or functional recovery after

deprivation and to evaluate the visual contribution to the execution of a cognitive task (Prusky *et al.* 2000).

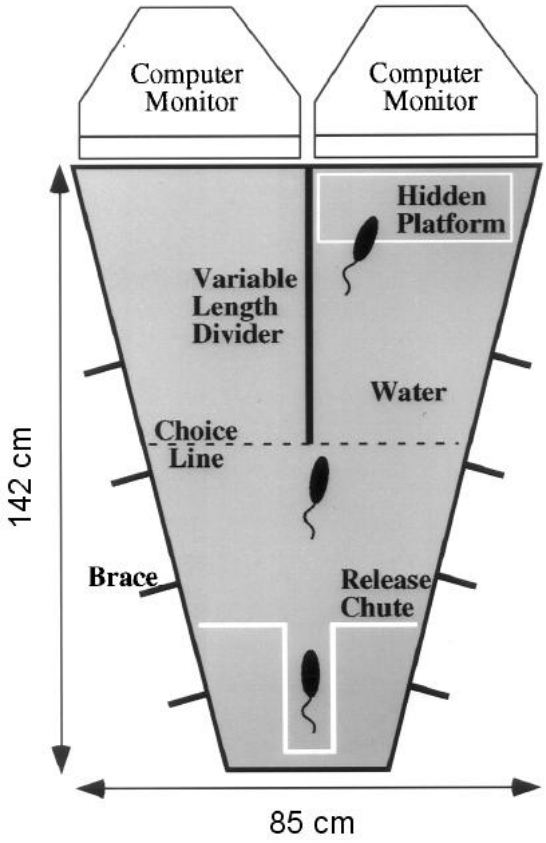

Fig. 1 *- View from above showing the major components of the visual water box including pool, midline divider, platform, starting chute and two monitors. Modified from* Prusky *et al.* 2000.

In the present study a modified version of this task was used to obtain a behavioural measure of the rat visual perceptual learning in order to evaluate the gradual improvement in the ability of the animals to distinguish two vertically oriented gratings of different spatial frequencies.

The apparatus consisted of a trapezoidal-shaped pool with two computer-controlled monitors placed side-by-side at one end of the pool (Fig.1). The pool is made of 6 mm clear Plexiglas, is 142 cm long, is wider at one end (85 cm) than the other (25 cm) and with 56 cm high walls. A midline dividers (45 cm high) of 50 cm length extends from the end wall between the monitors into the pool, bisecting it along its long axis.

The length of the divider setted the choice point and effective spatial frequency of the stimulus. A portable escape platform (35 cm long, 14 cm wide and 14 cm high) was placed below one of the two monitors and the pool was filled with tepid (25°C) water to a depth of 15 cm. White paint mixed with water rendered the platform invisible from water level.

Visual stimuli were presented by the two monitors through two glass windows 31 cm high x 23 cm long (Fig.2).

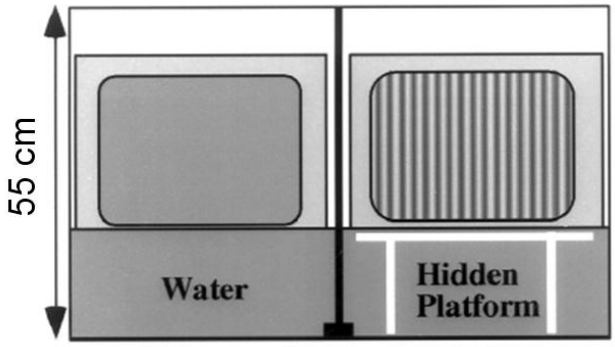

Fig. 2 *- Front view showing monitor screens, submerged platform and midline divider. Modified from* Prusky *et al.* 2000.

The lower side of the monitors was correspondent with the level of the water. A specific software, realized in the Institute of Neurophysiology of CNR in Pisa, provided the stimuli with a pseudorandom sequence (Gellerman 1993). The sequence was organized so that one of the two monitors showed the target stimulus. Within 10 presentations each of the two monitors showed the same number of presentations of the target stimulus avoiding more than three consecutive repetitions in the same monitor. The two stimuli had the same luminance (40.06 cd/m2) and the same contrast (90%).

Electrophysiology

Rats were anesthetized by isofluorane inhalation. After decapitation brains were removed and immersed in ice-cold oxygenated ($O_2$95% $CO_2$5%) cutting solution containing (in mM): 130 NaCl, 3.1 KCl, 1.0 K_2HPO_4, 4.0 $NaHCO_3$, 5.0 dextrose, 2.0 $MgCl_2$, 1.0 $CaCl_2$, 10 HEPES, 1.0 ascorbic acid, 0.5 myo-Inositol, 2.0 pyruvic acid, and 1.0 kynurenate, pH 7.3. Slices (0.33 mm thick) of visual cortex were obtained using a Leica (Nussloch, Germany) vibratome.

The recording solution was composed as the cutting solution with the following differences (in mM): 1.0 $MgCl_2$, 2.0 $CaCl_2$, 0.01 glycine, and no kynurenate. Slices were perfused at a rate of 2 ml/min with 35°C oxygenated recording solution.

Electrical stimulation (0.1 msec duration) was delivered with a bipolar concentric stimulating electrode (FHC, St. Bowdoinham, ME). Field potentials were recorded by a micropipette (1–3 MΩ) filled with recording solution.

Drug administration

Rats were implanted bilaterally with osmotic minipumps (model 1007D; Alzet, Palo Alto, CA, USA; pumping rate 0.5 µl/hr, for 6–7 days) by which the drug or the vehicle solution was continuously infused in the visual cortex of both hemispheres throughout one week. Minipumps were connected via polyethylene tubing to a stainless steel 30-gauge cannula implanted 1 mm lateral to lambda.

Surgery was done under anaesthesia with i.p. avertin. The minipump cannulas were fixed to the bone with dental acrylate and a screw. After the implantation, the skin was sutured and the wound was treated with antibiotics and local anaesthetics. Animals were allowed to recover in a small cage before being returned to the animal house.

Experimental procedures

Visual discrimination improvement assessment

A group of 10 animals, the Visual Discrimination group (VD animals), was first conditioned to distinguish a 0.117 cycles per degree (c/deg) spatial frequency grating (standard grating) from a higher spatial frequency (0.712 c/deg) grating. Then, keeping the standard grating linked to the presence of the platform, the spatial frequency of the other grating (varying grating) was gradually reduced from 0.712 c/deg to 0.127 c/deg. Visual perceptual learning consisted in the improvement of visual discrimination allowing VD animals to distinguish between the two spatial frequencies values when they became progressively more similar to each other. This discrimination task continued until the limit was achieved.

To control for the effect of the simple association between the platform and the standard grating, another group of 10 animals (control animals) was only trained to distinguish between the standard grating and a homogeneous gray. During the entire course of the experiment, control animals always made this same distinction so that the manipulation, the duration, the number of session and the amount of physical exercise were the same for both VD and control animal groups.

The task consisted of three phases: pretraining shaping; task training; and discrimination learning. There were three sessions per day and each session had 15 trials. Sessions were at least 60 minutes interleaved. In the pretraining phase, animals were shaped gradually to locate the platform hidden below the screen displaying the standard grating. On the first trial, animals were removed from their holding cage and released, facing the screen, into the pool a few centimetres from the platform. Upon being released, most animals swam directly forward and touched the platform, then climbed upon it. They were allowed to remain on the platform for a few seconds and were subsequently removed and returned to their holding cage. On the next trial, the location of the standard grating and the platform was switched to the opposite side and another trial was run. After this routine

was repeated a few times, the release distance from the platform was gradually increased until animals could reliably swim to the platform from the opposite end of the pool.

During the training phase animals were gradually conditioned to distinguish the standard grating from the varying grating (0.712 c/deg). This phase ended when animals acquired this association, reaching the 80% of correct responses for at least three subsequent sessions.

In the discrimination learning phase the standard grating was kept at 0.117 c/deg, while the spatial frequency of the varying grating was gradually reduced from 0.712 c/deg until it became impossible for the animals to distinguish between the two gratings. Initially, the step to a lower frequency occurred after one shot trials for each spatial frequency. In the case of a wrong response for a VD animal, the animal was required to reach at least 75% (3 out of 4) or 70% (7 out of 10) of correct responses. The scale of spatial frequencies utilized during the discrimination learning phase are shown in the following table.

C/deg	Criterion
0.712	1 out of 1
0.684	1 out of 1
0.659	1 out of 1
0.636	1 out of 1
0.593	1 out of 1
0.556	1 out of 1
0.523	1 out of 1
0.494	2 out of 2
0.468	2 out of 2
0.434	2 out of 2
0.404	2 out of 2
0.378	2 out of 2
0.356	2 out of 2
0.329	2 out of 2
0.296	3 out of 4
0.269	3 out of 4
0.234	3 out of 4
0.207	3 out of 4
0.178	7 out of 10
0.148	7 out of 10
0.136	7 out of 10
0.127	7 out of 10

At the end, during discrimination of the last spatial frequencies, the required performance was always 7 out of 10. During the course of sessions, for each spatial frequency statistical analysis was made on the number of correct answers on the total number of trials.

The discrimination learning phase lasted typically one week. The animal performance displayed an oscillation around the criterion level (70% of correct choices) when a VD animal was near its threshold: the performance was more than 70% for a given spatial frequency of the varying grate, became minor than 70% for a further step of decrement in the spatial frequency and returned to be more than 70% when the spatial frequency was reported one step higher. If this oscillation was permanent for at least three days, the discrimination learning phase was assumed to be concluded as VD animals seemed not capable of any further improvement. Generally, during the last days, the lowest spatial frequency of the varying grating which the animals were still able to distinguish from the standard one, oscillated between 0.136 c/deg and 0.148 c/deg.

Change of stimuli orientation

An addicitonal experiment in which the stimulus orientation was changed was performed to estimate the selectivity of the visual discrimination task for orientation. Five animals were used for an experiment of change of stimuli orientation (CSO animals). These animals were submitted exactly to the same three phases of shaping, training and discrimination learning, as described for VD animals. As soon as the varying grating had oscillated between the same spatial frequency values for at least three days, the CSO animals reached the end of their discrimination learning phase. At that moment, both stimuli were 90° rotated in the two monitors. The last spatial frequency value the animals had distinguished at the end of the discrimination learning was kept. New trials were performed in order to obtain the new lowest spatial frequency value distinguished by CSO animals, which had to discriminate the new 90° rotated stimuli. Specifically, if the performance of the CSO animals did not reach the criterion level (70%), the spatial frequency of the vary grating was increased until a new level of oscillation occurred.

Mimicry and occlusion of LTP

VD and control animals slices were used for electrophysiological *in vitro* recordings after one week of discrimination learning phase, when no further improvement in discrimination occurred in VD animals. Post synaptic field potentials in layer II/III of the visual cortex where recorded in order to verify if visual discrimination learning led to an increase of excitability mimicking a LTP effect.

All rats were submitted to two different stimulation conditions. In some slices the stimulating electrode was placed in layer IV in order to activate vertical connections, while in other ones the stimulating electrode was placed in layer II/III in order to stimulate horizontal connections.

After stimulus, electrical artefact was almost instantaneous. Physiological signal began within few milliseconds and could include a presinaptic component in addition to the postsynaptic one. Thus, signals were accepted and recorded only when latency from the artefact was not less then 5 milliseconds. Bath application of kynurenate (general glutamate receptors blocker) at the end of the experiment confirmed that a latency value lower than 5 milliseconds occured when the presynaptic component was likely to be predominant (data not shown). Once a field potential signal was obtained, excitability was assessed by measuring its voltage level in function of the intensity of stimulation (I-V curve). Intensity was increased by steps of 100 μA, until the field potential signal reached a saturation level.

After obtaining the I-V curve, the same signal with the same site of stimulation (layer II/III or layer IV) was kept to begin the LTP experiment finalized to verify the occlusion of potentiation in slices coming from VD animals. Baseline responses were obtained every 30 seconds with a stimulation intensity that yielded a half-maximal response. In order to reach the highest level of cumulative potentiation, three theta burst stimulations (TBS) were delivered. After each TBS field potential amplitude was monitored for 30 minutes. The first TBS was delivered after achievement of a 15 min stable baseline (field potential amplitude within 20% of change and with no evident increasing or decreasing trends). Each TBS consisted in 4 bursts separated by 10 sec intervals. Each burst consisted in 12 trains with 0.2 msec intervals where each train was composed of four pulses of 10 msec intervals.

Pharmacological interference

U0126 is a drug known to block MEK, which is a fundamental step of MAPK pathway. MAPK molecular pathway is essential for LTP to occur and U0126 is able to impair it without altering visual acuity (Di Cristo *et al.* 2001). U0126 (250 mM) was administereted to 5 animals (U0126 animals) to verify whether pharmacological interference blocking LTP also impairs visual discrimination learning. U0126 was dissolved in DMSO and diluted into saline solution. Drug was solved from 100x stock solutions in DMSO to give the desired final concentration of 5%. A group of 6 control animals was administereted only with DMSO 1% (vehicle animals).

To avoid undesired interference with the acquisition of the task, U0126 or vehicle were administereted at the end of the training phase, just before the animals began the visual discrimination learning. After being implantation, animals rested in separated cages for one day. Subsequently, one session of behavioural task was spent to confirm that the animals still remembered the association learned during the training phase. Then, U0126 animals and vehicle animals were ready to begin the discrimination learning phase. Implantation of one week lasting mimipumps ensured that drug administration covered the entire period during which animals were involved in visual discrimination improvement.

Results

The purpose of this study was to verify that LTP in the primary visual cortex is involved in visual discrimination learning. A Visual Discrimination group of 10 animals (VD animals) was submitted to a behavioural test in which they had to learn to distinguish different spatial frequencies. Another group of 10 animals instead, was only involved in a simple association training and was not entered to the subsequent discrimination learning (control animals). As expected, VD animals gave demonstration to effectively improve their visual discrimination ability due to the behavioural learning test.

VD and control animals, after the behavioural learning test, were used for *in vitro* electrophysiological experiments. Field EPSPs were recorded from visual cortex slices and the increased excitability of slices from VD animals suggested that learning mimicked the effects of LTP. TBS protocol applied on the same animal's slices resulted in a lower LTP in VD animals recordings. Compared to controls, further potentation was likely to be prevented by occlusion of LTP occurring as a consequence of visual discrimination learning.

Other two groups of animals were also submitted to the same behavioural test. The first one, at the end of the test, had to discriminate again the last distinguished spatial frequencies after the stimuli were 90° rotated. They showed to be impaired in this task, demonstrating that learning task critically selective for orientation of the stimuli.

The second group was involved in a pharmacological interference experiment. Some were treated with the U0126 (U0126 animals), a drug known to impair LTP induction, while others were only treated with U0126 solvent, DMSO (vehicle animals). Comparison between the behavioural test performances of the two groups showed that pharmacological interference on LTP mechanisms impaired visual discrimination learning in U0126 animals.

Visual discrimination improvement

During the training phase VD animals learned to associate the presence of the platform with the standard low frequency grating (0.117 c/deg) and to discriminate this grating from a high spatial frequency grating (0.712 c/deg), while control animals compared the standard grating with a homogeneous grey stimulus. Thus, during the learning phase, only VD animals learned to distinguish 0.117 c/deg from the different spatial frequencies presented by the varying grating. The different comparison used for control animals during the training phase, however, led them to learn the association significantly faster (Fig.3). A two way repeated measures ANOVA confirmed the presence of a significant effect for the factor of training trials ($p<0.001$) and for the factor of task condition ($p<0.004$).

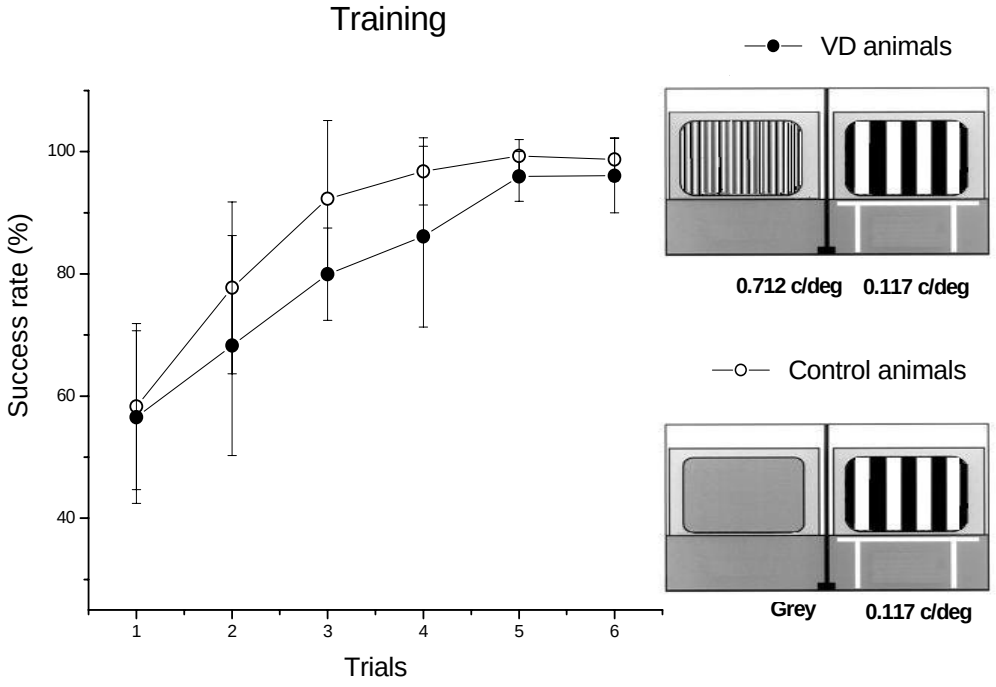

Fig.3 *- Training phase performance of the two groups of animals.*

As soon as the animals reached the criterion level of task execution (80 % of correct choices for at least three sessions), the discrimination learning phase began. Only VD animals were involved in the visual discrimination learning task. The discrimination learning phase required distinction between the standard grating and the varying grating. During the course of the learning sessions, the spatial frequency of the varying grating was gradually decreased from 0.712 c/deg to 0.127 c/deg, according to the progress of improvement. The purpose was to make the varying grating progressively similar to the standard grating, in order to challenge the abilities of the animals.

Animals showed to be able to improve their ability to distinguish gratings of spatial frequencies progressively closer to each other. VD animals could not discriminate some spatial frequency values during the first days of the discrimination learning phase, but they managed during the successive sessions (Fig. 4 and 5). It was possible to delineate a

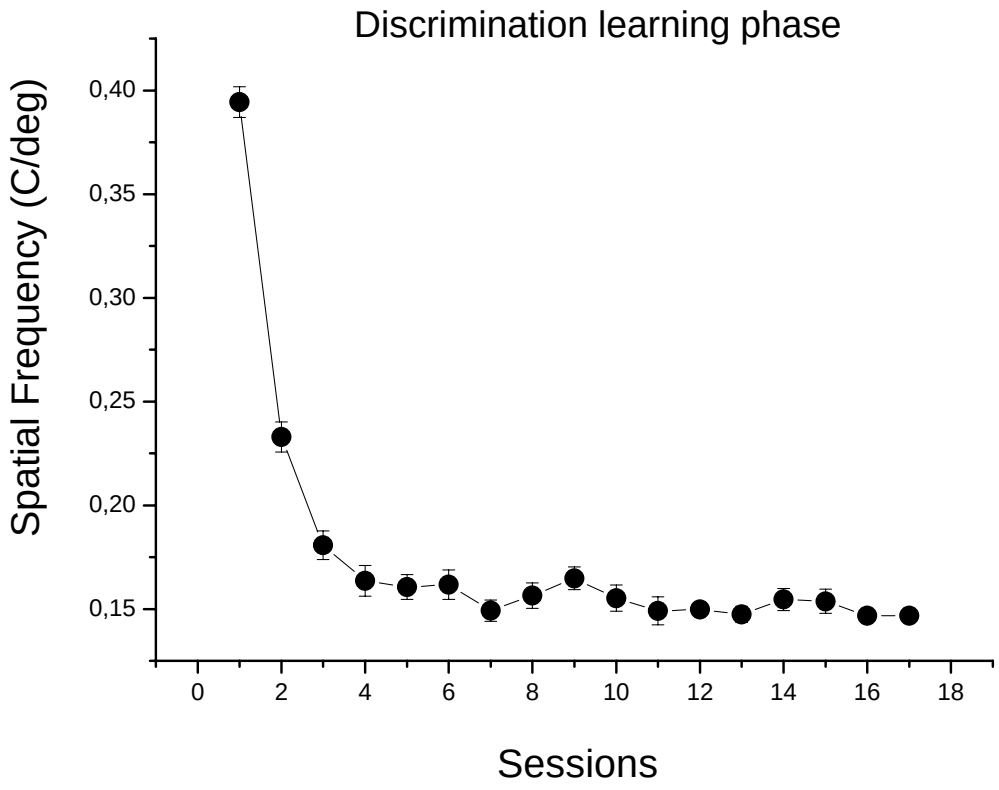

Fig.4 *- Visual discrimination improvement along the sessions. The graph shows the average between 10 VD animals of the lowest spatial frequencies of the varying grating distinguished during the sessions of visual discrimination learning phase.*

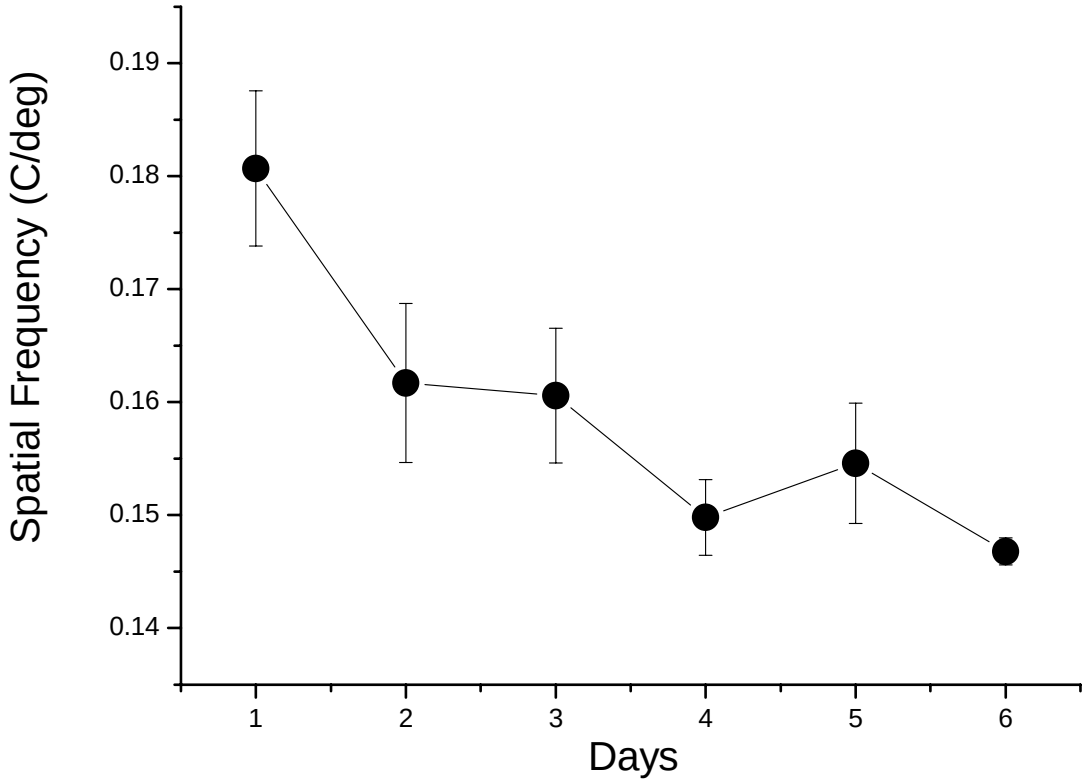

Fig.5 *– A different representation of the same visual discrimination improvement shown in Fig. 4. This graph shows the average between 10 VD animals of the lowest spatial frequencies of the varying grating distinguished during each day of the visual discrimination learning phase.*

general trend: from 0.712 c/deg to 0.207 c/deg animals did not make errors. Subsequently, discrimination became more difficult and the success rate started to oscillate as the animals began to make mistakes (Fig. 4 and 5).

By practice animals became able to discriminate those spatial frequency values they could not distinguish the days before. Moreover, the progressive decrease of difference in terms of c/deg between the spatial frequency values of the two gratings, was itself an evident proof of the occurrence of learning. Indeed, during the first sessions, animals failed to distinguish the spatial frequency value of the standard grating from the value of 0.178 c/deg and of 0.148 c/deg of the varying grating. In the following sessions, by subsequent exposures to these stimuli, a significant improvement was reached and the animals managed to distinguish the two spatial frequency values.

The discrimination learning phase ended when animals reached a stationary situation in which, despite of the continuous presentations, their performance level alternated between the same two spatial frequency values for several sessions. In this situation no more improvement was possible as the animals reached their discrimination limit.

Each trial was composed of ten stimuli presentations and was considered correct if at least the 70% of the animals' answers were right. In that case the spatial frequency of the varying grating was one step decreased in the next trial. When a trial resulted not correct, the spatial frequency of the varying grating was re-increased to reconfirm a good performance for the step before. This alternation between the two levels, one completely overcome, the other repeatedly presented but still to be passed, made animals to finally succeed in solving the task.

On average animals made the first mistakes when the varying grating was setted on the spatial frequency value of 0.186 c/deg, while the lowest value obtained at the end of the improvement was 0.146 c/deg (Fig.6). A paired t-test was performed ($p<0.001$) and showed

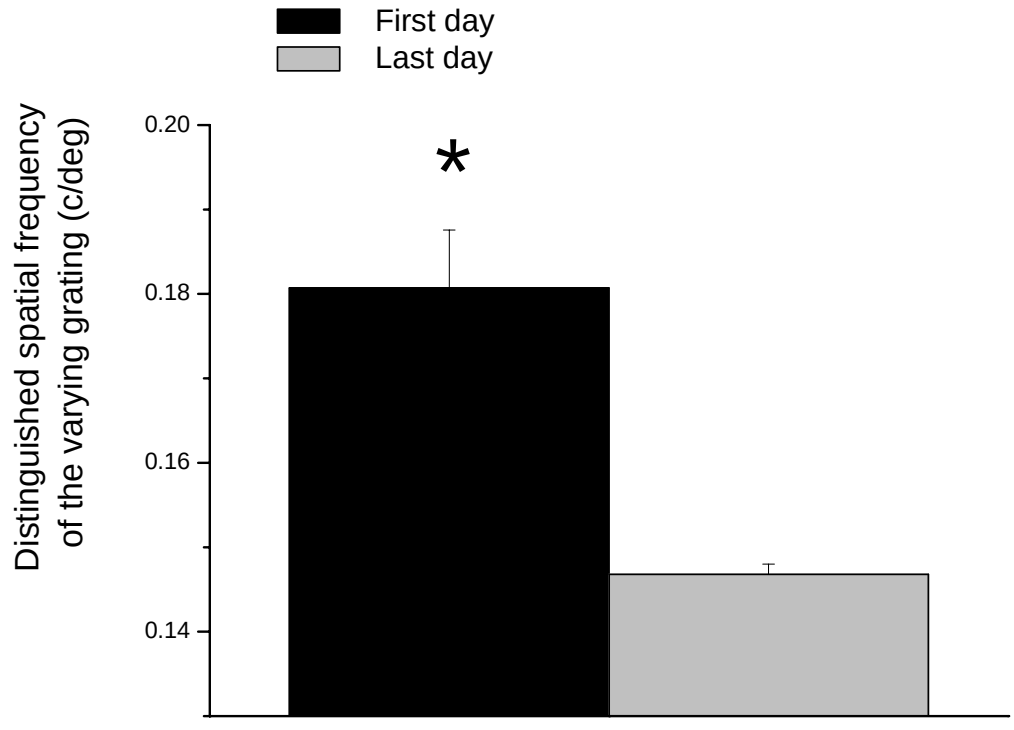

Fig.6 *- Threshold of visual discrimination on the first and on the last day of the visual discrimination learning phase. Difference is significant (paired t-test, $p<0.001$).*

a significant difference between the two spatial frequency thresholds reached respectively the first and the last day of the learning phase.

Another possible way to show the effect of learning is to consider a particular spatial frequency value of the varying grating and to report how the correspondent success rate of discrimination changes over the learning phase. For some spatial frequency values, discrimination improvement was evident (Fig.7 and 8). However, the last spatial frequency value of the scale was never distinguished from the one of the standard grating (Fig.9). A global graph of the average animal performance is shown in Fig.10.

The graph shows the animal success rate during the learning phase when the varying grating had a spatial frequency value of 0.148 c/deg (Fig.10). A Friedman repeated measures ANOVA on Ranks was performed to asses the effect of learning sessions. The analysis confirmed the significant effect ($p<0.001$).

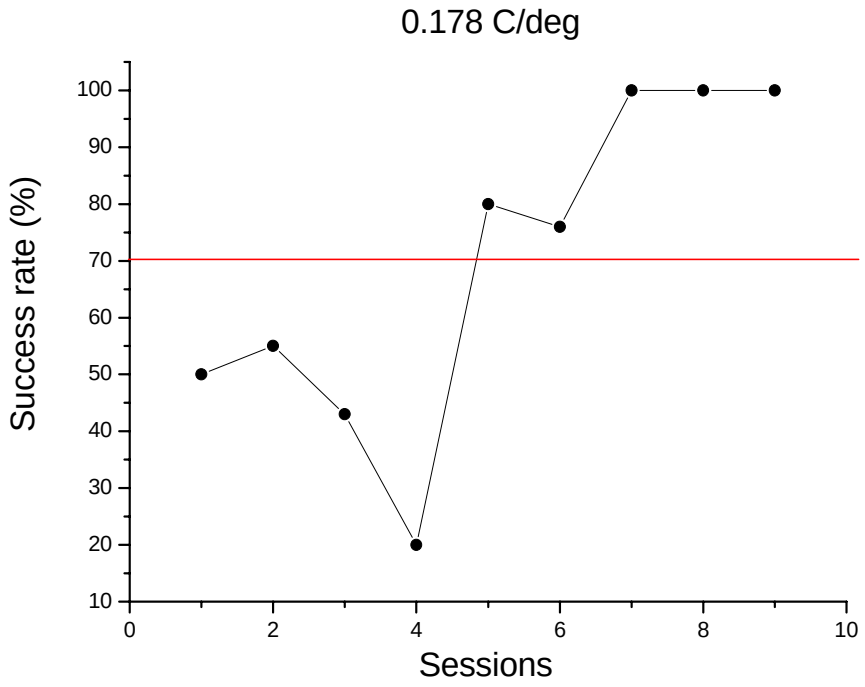

Fig.7 *- An example of improvement in distinguishing the value of 0.178 c/deg from the value of the standard grating.*

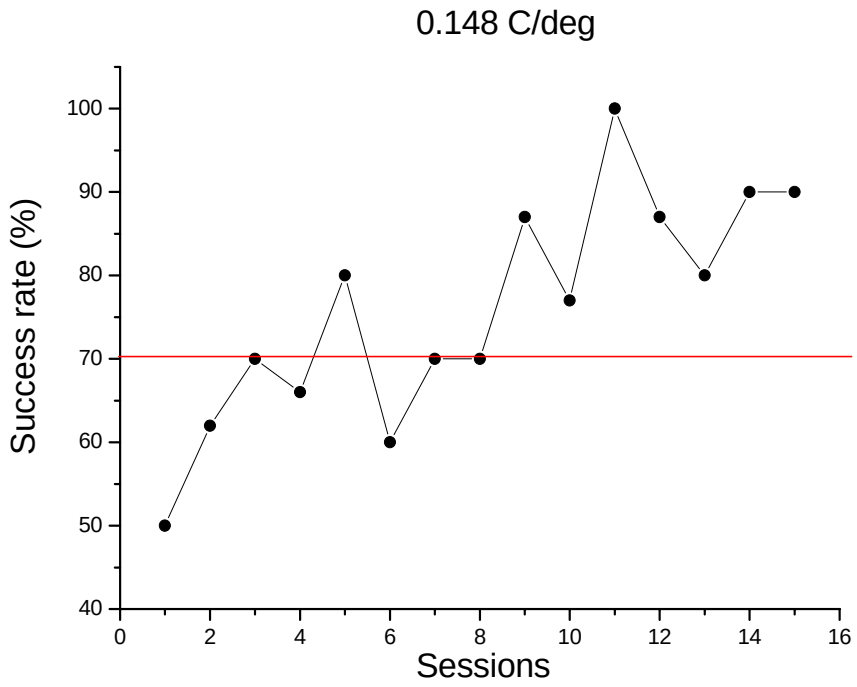

Fig.8 *- An example of improvement in distinguishing the value of 0.148 c/deg from the value of the standard grating.*

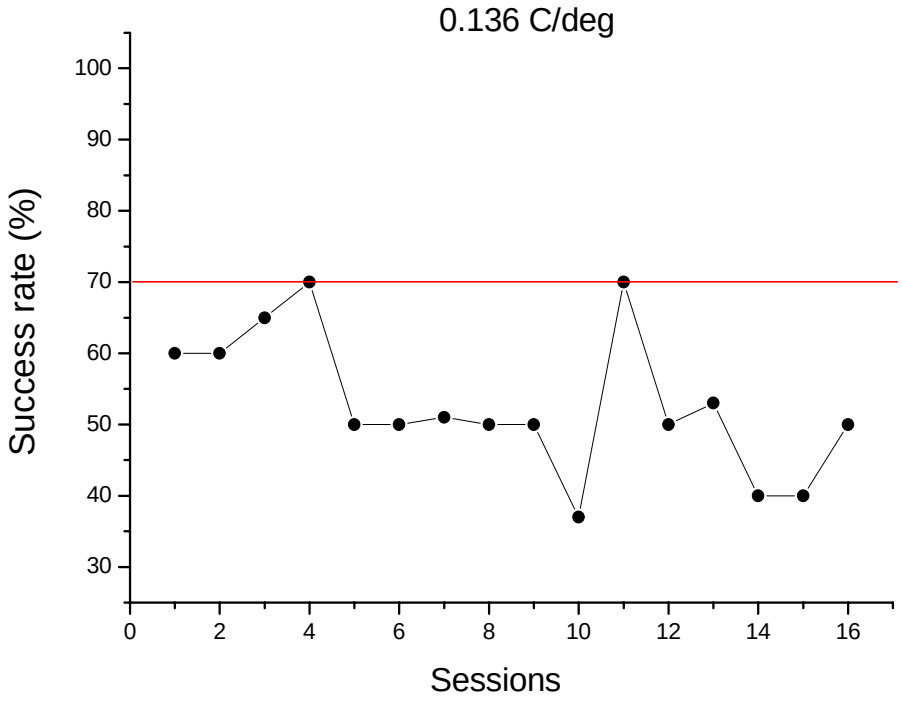

Fig.9 *- An example showing the lack of improvement in distinguishing the value of 0.136 c/deg from the value of the standard grating.*

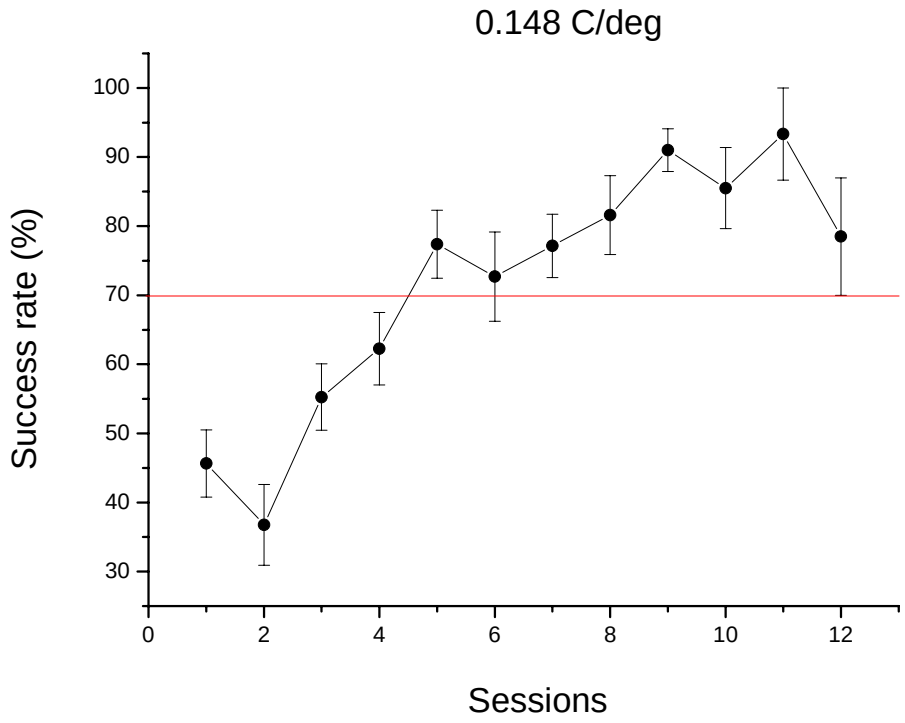

Fig.10 *– A global graph showing the average improvement in distinguishing the value of 0.148 c/deg from the value of the standard grating (0.117 c/deg). The increase of correct choices is significant (Friedman Repeated Measures ANOVA on Ranks* $p<0.001$*).*

Selectivity of orientation

A change from vertical to horizontal gratings was performed to estimate the selectivity of this visual discrimination task for stimuli orientation. Five animals were used for that experiment (CSO animals). Animals were submitted to shaping, training and discrimination learning phases, as described for VD animals. Once the lowest spatial frequency they could distinguish from the standard grating was reached, both stimuli orientation was 90° rotated.

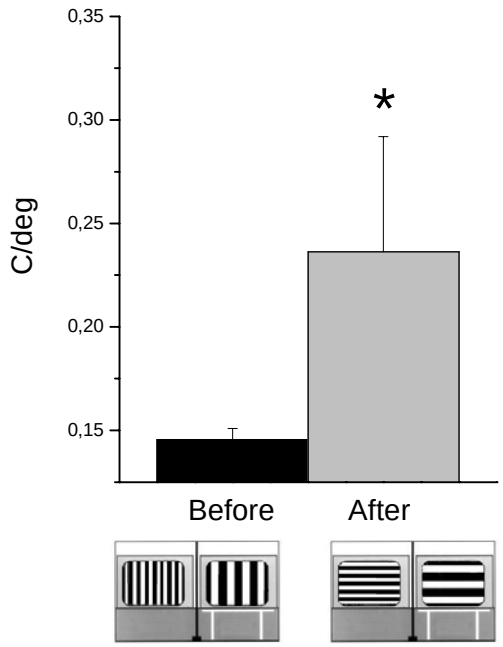

Fig.11 *- The graph shows the lowest spatial frequency value distinguished by CSO animals before and after the orientation change. Five animals were used. Difference is statistically significant (paired t-test p<0.022).*

CSO animals were not able to distinguish the two gratings anymore (Fig.11), so the spatial frequency of the varying grating was increased until they succeeded again in discriminating it from the standard one. The new spatial frequency value of the varying grating was compared with the value obtained before the stimuli were rotated, to show the animal

impairment (Fig.11). There was a significant difference between the two values (paired t-test $p<0.022$). This result shows that the improvement the animals obtained by visual discrimination learning is strictly selective for the orientation of the stimuli.

Visual discrimination learning causes LTP-like changes in primary visual cortex

At the end of the discrimination learning phase, brain slices of visual cortex were obtained from VD and control animals to perform *in vitro* electrophysiological experiments. The recording electrode was placed in layer II/III, while two different stimulation protocols were used: some slices were stimulated in layer IV and others were stimulated in layer II/III. Few milliseconds after the stimulus, signal is translated into a postsynaptic response by neurons of layer II/III. This response is recordable as a field

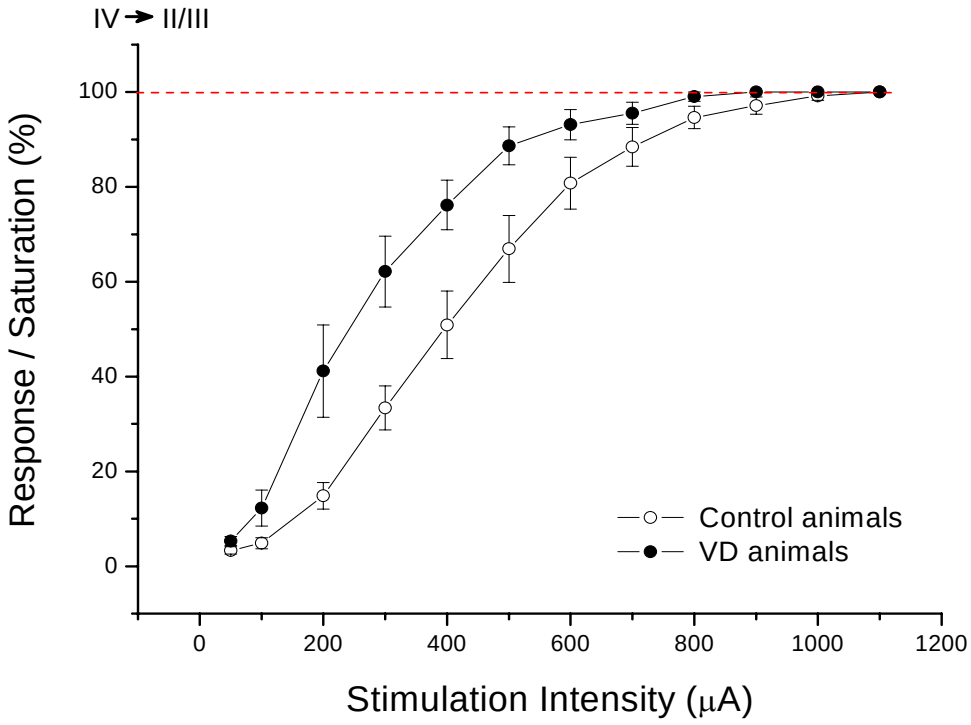

Fig. 12 *– The graph shows the percentage of f-EPSP amplitude with respect to saturation at different layer IV intensities of stimulation. In control animals 7 slices from 2 animals were used. In VD animals 10 slices from 2 animals were used. Difference between VD and control animals is significant (Two Way Repeated Measures ANOVA, $p<0.001$). There is statistically significant interaction between variables "group of animals" and stimulation intensity for values going from 200 μA to 700 μA ($p<0.001$).*

excitatory post synaptic potential (f-EPSP). With an increase in the intensity of the stimulus, the amplitude of f-EPSPs increases following a sigmoidal function until it reaches a saturation level.

The amplitudes of f-EPSPs responses evoked by different intensities of stimulation were recorded. In slices obtained from VD animals, reaching the saturation level required a lower intensity of the stimulus, in comparison with slices obtained from control animals (Fig.12 and 13).

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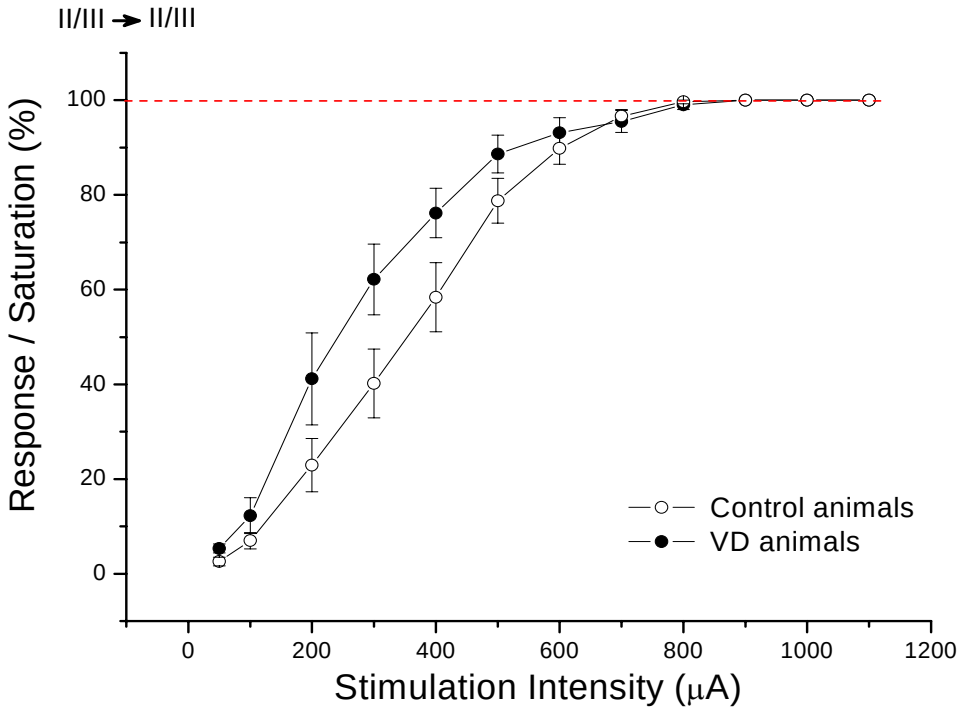

Fig. 13 *- The graph shows the percentage of f-EPSP amplitude with respect to saturation at different layer II/III intensities of stimulation. In control animals 9 slices from 3 animals were used. In VD animals 8 slices from 2 animals were used. There is statistically significant interaction between variables "group of animals" and stimulation intensity for 200 μA, 300 μA and 400 μA values ($p<0.05$).*

Saturation of primary visual cortex LTP by visual discrimination learning

The f-EPSP with an amplitude nearly half the saturation level was monitored for a baseline period of 15 minutes, before the first TBS was delivered. The entire LTP protocol consisted of three successive TBS. After each of them the f-EPSP was monitored for 30 minutes before the following one was triggered. There were two kinds of LTP recordings according to the stimulation site: IV-LTP, in case of layer IV stimulation site and II/III-LTP, in case of layer II/III stimulation site.

IV-LTP

In IV-LTP recordings, potentiation resulted to be occluded: slices from VD animals showed a failure in obtaining a significant LTP after the first, the second and the third TBS. Average of f-EPSP percentage values within the last ten minutes of each period were analysed (Two Way Repeated Measures ANOVA: baseline vs 1^{st} post-TBS period, $p>0.05$; baseline vs 2^{nd} post-TBS period, $p>0.05$; baseline vs 3^{rd} post-TBS period, $p>0.05$) (Fig.14).

In contrast, after the first induction, control animals slices showed a strong IV-LTP (Two Way Repeated Measures ANOVA: baseline vs 1^{st} post theta period, $p<0.05$), that further increased after the two subsequent TBSs (Two Way Repeated Measures ANOVA: baseline vs 2^{nd} post-TBS period, $p<0.05$; baseline vs 3^{rd} pos-TBS period, $p<0.05$) (Fig.15).

Direct comparison between the two groups showed that after the second TBS, in slices derived from VD animals a lower level of IV-LTP was present (Two Way Repeated Measures ANOVA, $p=0.004$) (Fig.16 and Fig.17).

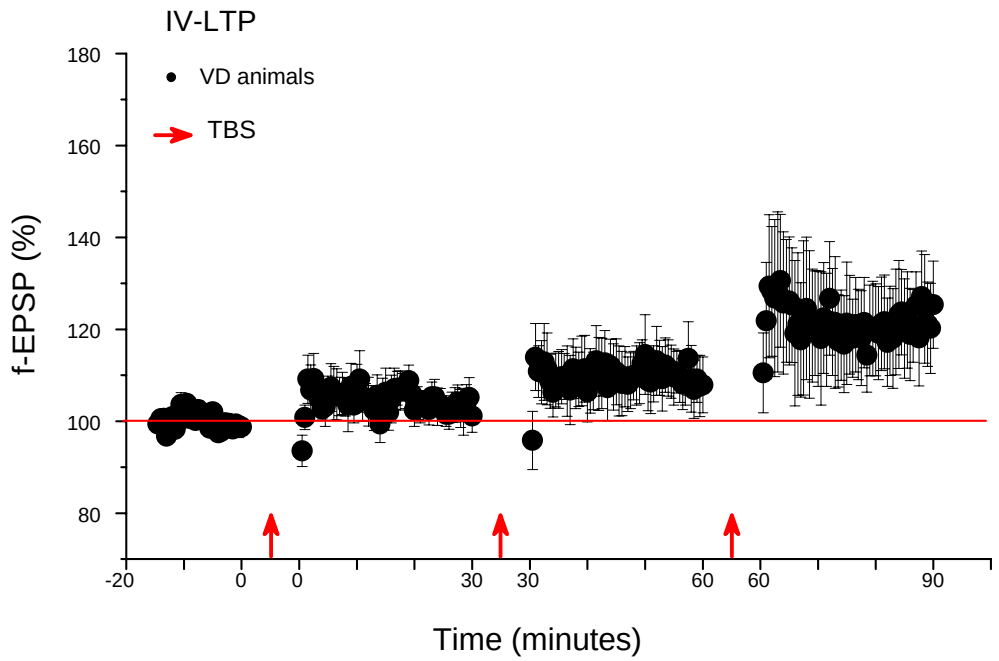

Fig. 14 *- In VD animal slices, no significant IV-LTP was present after each induction (Two Way Repeated Measures ANOVA: baseline vs 1st post-TBS period, p>0.05; baseline vs 2nd post-TBS period, p>0.05; baseline vs 3rd post-TBS period, p>0.05). Baseline and 1st post-TBS period: 8 slices from 5 animals. 2nd post-TBS period: 7 slices from 4 animals. 3rd post-TBS period: 4 slices from 3 animals.*

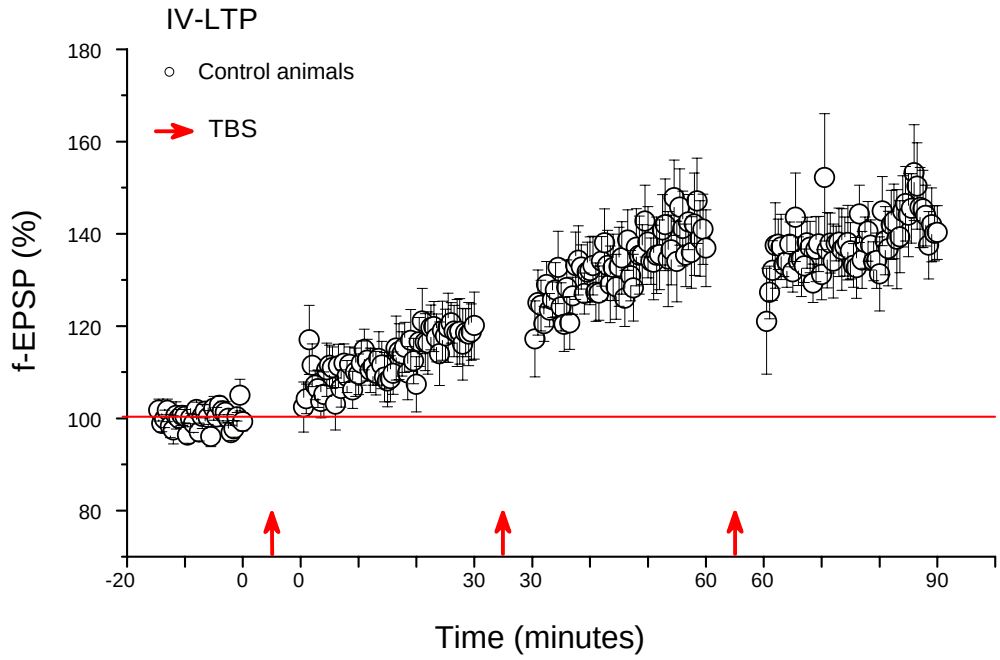

Fig. 15 *- In control animal slices, a significant IV-LTP was present since the first induction. (Two Way Repeated Measures ANOVA: baseline vs 1st post-TBS period, p<0.05; baseline vs 2nd post-TBS period, p<0.05; baseline vs 3rd post-TBS period, p<0.05). Baseline and 1st post-TBS period: 9 slices from 7 animals. 2nd post-TBS period: 7 slices from 7 animals. 3rd post-TBS period: 5 slices from 5 animals.*

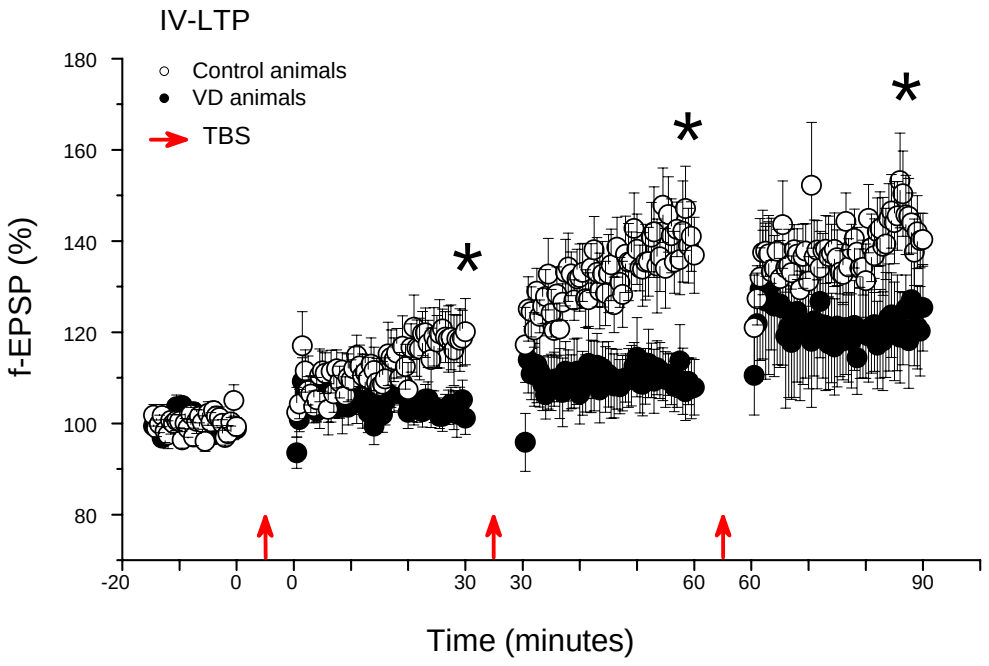

Fig. 16 *- IV-LTP in control and VD animals slices. The difference of LTP level between the two groups was statistically significant (Two way Repeated Measures ANOVA, p=0.004).*

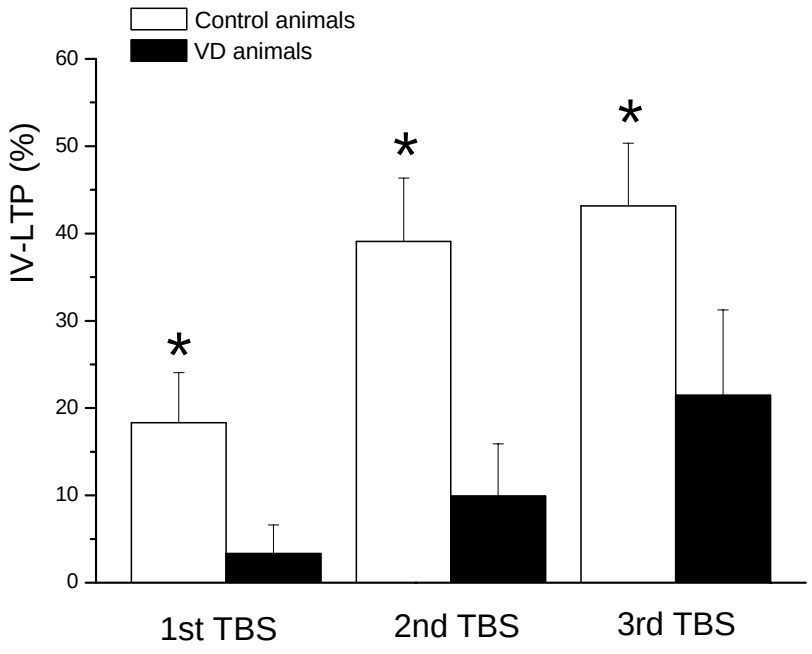

Fig. 17 *- IV-LTP average of the last 20 values (10 minutes) of each post-TBS period. Difference between VD and control animals was statistically significant (Two Way Repeated Measures ANOVA, p=0.004).*

II/III-LTP

In II-III-LTP recordings, potentiation showed occlusion as the f-EPSP percentage level was never significantly different from the percentage baseline level (Two Way Repeated Measures ANOVA: baseline vs 1st post-TBS period, p>0.05; baseline vs 2nd post-TBS period, p>0.05; baseline vs 3rd post-TBS period, p>0.05) (Fig.18). A significant increase instead was evident analysing II/III-LTP experiments of control animals slices (Two Way Repeated Measures ANOVA: baseline vs 1st post theta period, p<0.05; baseline vs 2nd post-TBS period, p<0.05; baseline vs 3rd pos-TBS period, p<0.05) (Fig.19).

A direct comparison between the two groups showed a significant difference in potentiation level: after the second and the third TBS, slices derived from VD animals showed a lower level of II/III-LTP, in comparison with slices derived from control animals (Two Way Repeated Measures ANOVA, p=0.041) (Fig.20 and Fig.21).

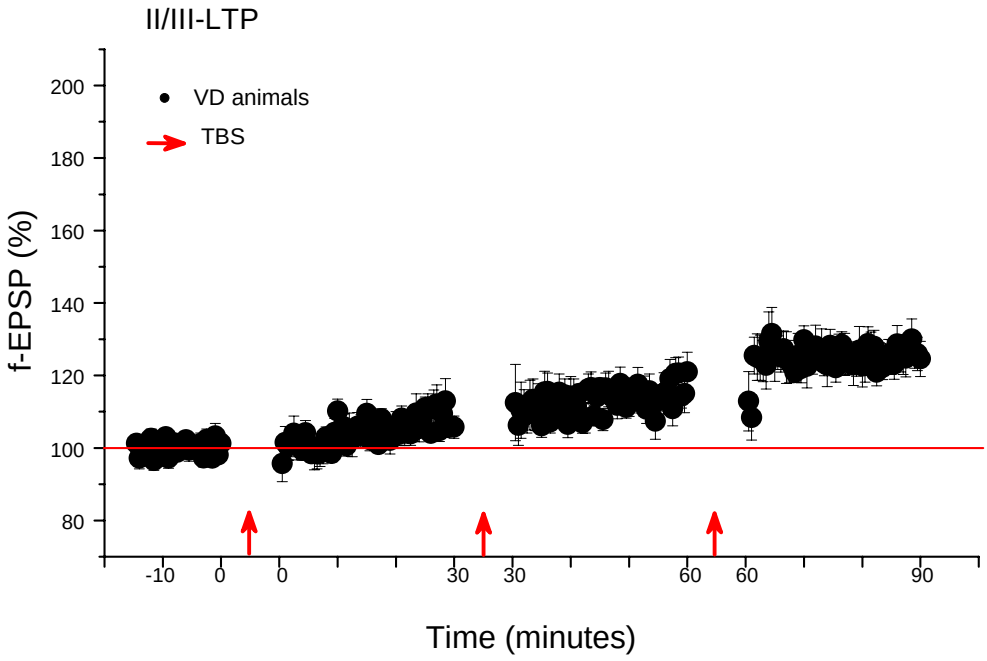

Fig. 18 *- In VD animal slices, no significant IV-LTP was present after each induction (Two Way Repeated Measures ANOVA: baseline vs 1st post theta period LTP, p>0.05; baseline vs 2nd post theta period LTP, p>0.05; baseline vs 3rd post theta period LTP, p>0.05). Baseline and 1st post theta period: 8 slices from 6 animals. 2nd theta period: 8 slices from 6 animals. 3rd theta period: 5 slices from 4 animals.*

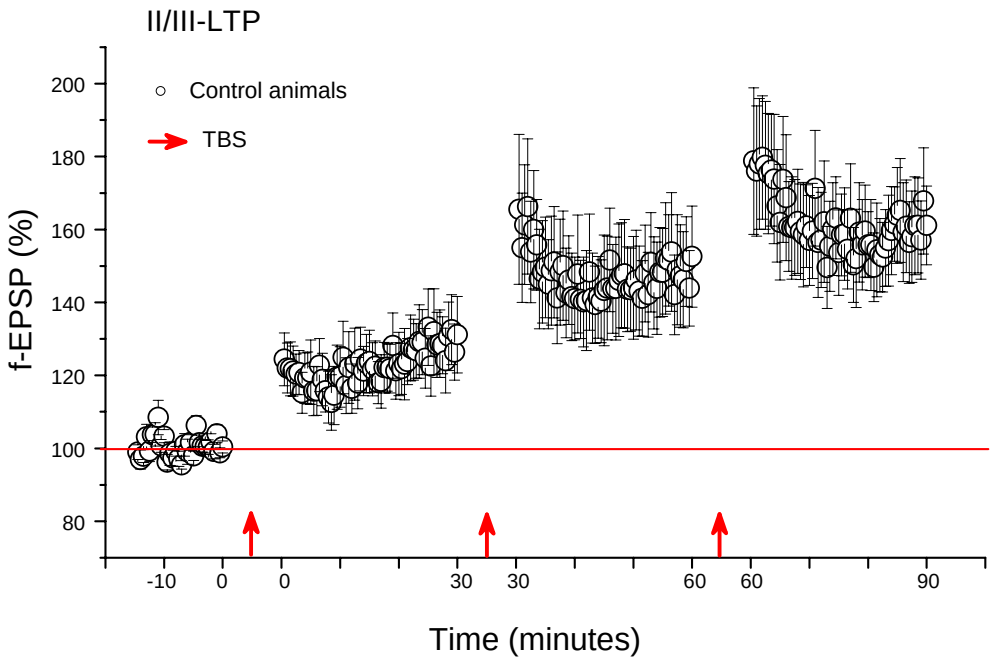

Fig.19 *– In control animal slices a consistent II/III-LTP was evident (Two Way Repeated Measures ANOVA: baseline vs 1st post theta period LTP, p<0.05; baseline vs 2nd post theta period LTP, p<0.05; baseline vs 3rd post theta period LTP, p<0.05). Baseline and 1st post theta period: 12 slices from 7 animals. 2nd theta period: 11 slices from 7 animals. 3rd theta period: 10 slices from 6 animals.*

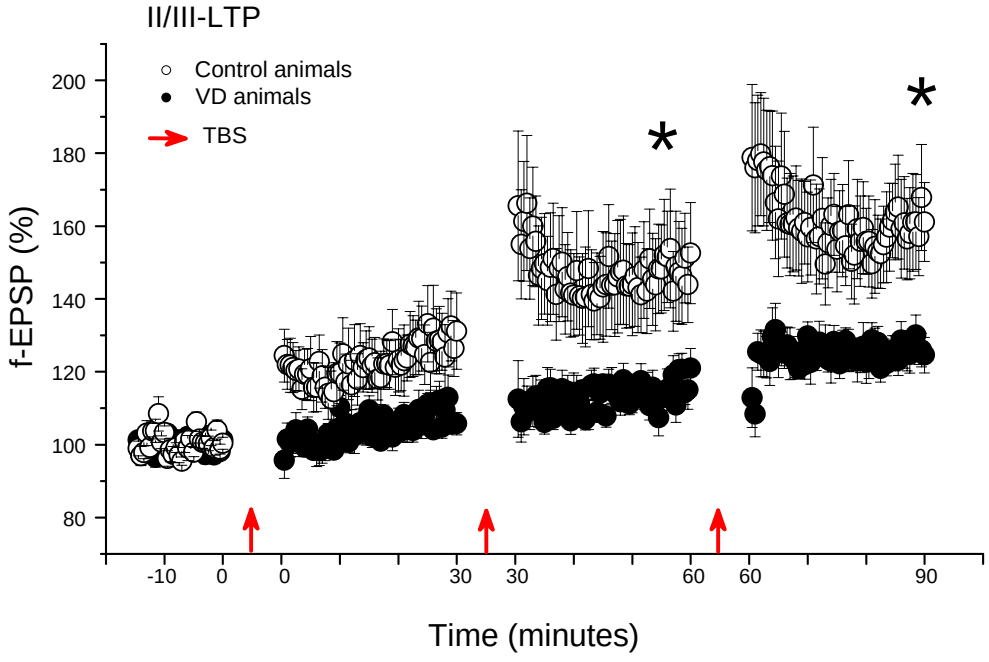

Fig. 20 *- II/III-LTP in control and VD animal slices. The difference of LTP level between the two groups is statistically significant (Two Way Repeated Measures ANOVA, p=0.041).*

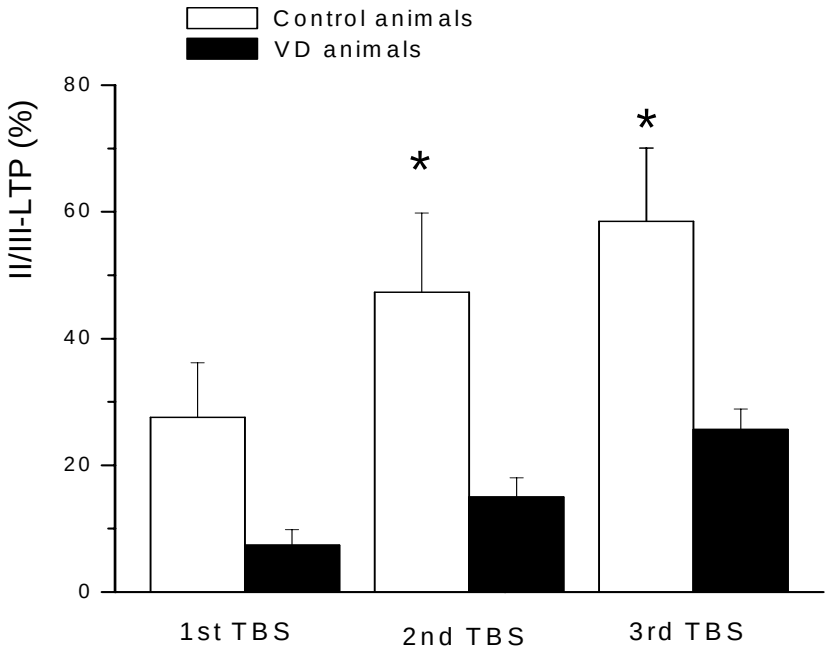

Fig.21 *- II/III-LTP average of the last 20 values (10 minutes) of each post-TBS period. Difference between VD and control animals is statistically significant (Two Way Repeated Measures ANOVA, p=0.041).*

Pharmacological blockade of LTP impairs visual discrimination learning

A parmacological approach was performed to further investigate the involvement of LTP in visual discrimination learning. The aim was to investigate whether a pharmacological administration of a drug known to block the LTP induction also caused the occurrence of deficits in this kind of learning. To achieve this purpose, the pharmacological interference took place during the discrimination learning phase of the visual discrimination learning task. A group of animals was implanted with osmotic minipumps filled with U0126 (U0126 animals), a drug known to interfere with molecular mechanisms of LTP induction, while another group of animals was simply administereted with vehicle (vehicle animals).

After minipump implant, basic association learned during the training was successfully confirmed in vehicle and U0126 animals by a post-implant training session. All implanted animals had no difficulties in remembering the association between the standard grating and the presence of the platform. No difference was present comparing the success rate of the session occurred before the implant with the one of the session occurred afterward (Data not shown). Post-implant performance also showed no difference from the last training performance of non-implanted VD animals. This suggested that neither the invasive implant of minipumps, nor the diffusion of U0126 throughout the primary visual cortex, damaged structures required to remember the simple association task.

During the discrimination learning phase, there was no significant difference between the pace of improvement of vehicle animals and VD animals. U0126, instead showed to be significantly delayed in comparison with the other two groups (Fig.22). Indeed, while the lowest spatial frequency (0.148 c/deg) of the varying grating distinguished by VD and vehicle animals was reached on the forth day of the discrimination learning phase, U0126 animals reached the same value only the day after. A two ways repeated measures ANOVA was performed. Among the different three groups there was a statistically significant difference ($p<0,001$). An all pair wise multiple

comparison procedures (Holm-Sidak method) showed that this effect depended on which days were considered. In particular, between U0126 animals and VD animals a difference was present on the first four days. U0126 animals and vehicle animals performances were statistically different only in day three and four, while no difference was present between VD and vehicle animals during all the six days period.

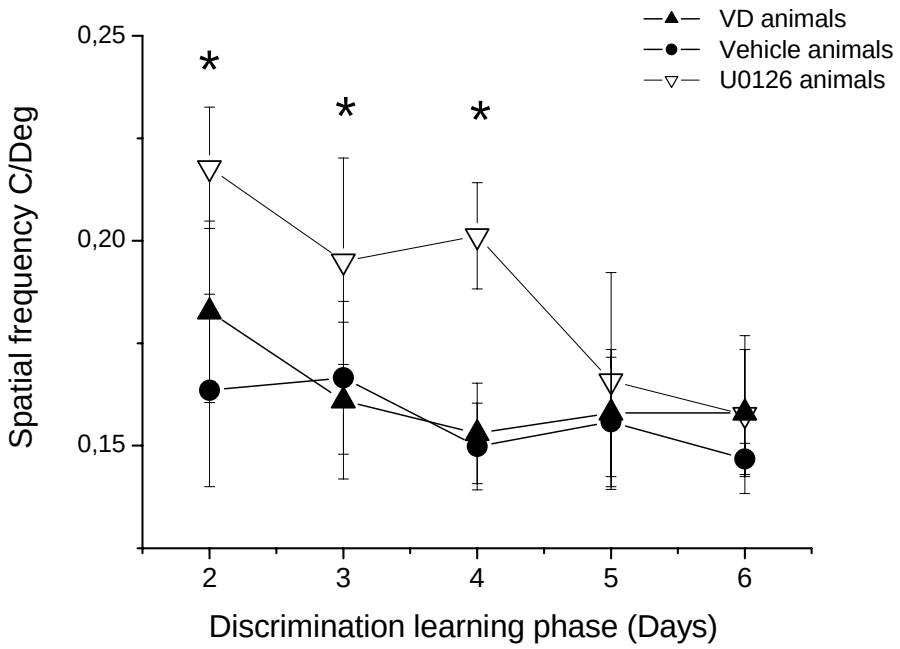

Fig.22 *– The graph shows the varying grating spatial frequency values distinguished by VD, Vehicle and U0126 animals across the days of visual discrimination. U0126 animals performance is significantly delayed. There is a significant difference among U0126 animals and the other two groups (Two Ways Repeated Measures ANOVA $p<0.05$) but not between VD animals and vehicle animals. Difference is present between U0126 animals and VD animals from the second to the fourth day. U0126 animals and vehicle animals performances are statistically different in day three and four (Holm-Sidak method $p<0.05$).*

Discussion

During the last few decades many studies have focused on visual discrimination learning occurring after repeated expositions to visual stimuli (Kinchla & Smyzer 1967, Laming & Scheiwiller 1985, Regan 1985, Burbeck 1987, Magnussen *et al.* 1990, Heeley *et al.* 1993, Magnussen & Greenlee 1999 and Magnussen 2000) and strong evidence suggested the involvement of modifications in primary visual cortex (Schoups *et al.* 2001, Furmansky *et al.* 2004, Maertens & Pollmann 2005, Frenkel *et al.* 2006, Pourtois *et al.* 2007 and Yotsumoto *et al.* 2008). Improvement seems to principally rely upon mechanisms of visual perceptual learning (Kinchla & Smyzer 1967, Laming & Scheiwiller 1985, Regan 1985, Burbeck 1987, visual declarative memory might also be an involved component.

Visual memory is known to be mediated by hippocampus and MTL, but long term modifications seem also to occur in other neocortical areas, including the primary visual cortex (Alvarez & Squire 1994, Roland & Gulyàs 1995, Mc Gaugh 2000, Osipova *et al.* 2006 and Takashima *et al.* 2006).

Visual discrimination improvement however is likely to principally rely upon a process of implicit perceptual learning. Visual perceptual learning is the result of complex and various neural activities (Grossberg 1999, Gilbert *et al.* 2000) including top-down interactions (Gilbert & Sigman 2007). Its occurrence is well known to principally require changes directly in the primary visual cortex (Gilbert 1994, Furmansky *et al.* 2004 and Schoups *et al.* 2001). In V1, neuronal responses are strictly specific for stimulus properties, like spatial position and orientation, which are known to be a target of modification in visual perceptual learning (Schoups *et al.* 2001).

Both visual memory and visual perceptual learning rely upon changes that are thought to be due to alterations in neuronal synaptic efficacy. The possibility of synaptic efficacy changes is a phenomenon that allows modifications in the processes of elaboration occurring between the components of neural networks. This process, which is well known in neurobiology, is commonly called synaptic plasticity. Synaptic plasticity has always been a field of great interest since the beginning of brain research. Modification of neuronal

synaptic efficacy requires functional and structural alterations, which are potentially present all over the nervous system as a basic physiological property. Synaptic plasticity operates wherever a change in the process of neural information is needed, so it is particularly enhanced in those areas where information is processed continuously. This happens in the circuitry of neural structures whose maturation has to take into account signals coming from the environment or, especially in adulthood, in those structures underlying memory and learning. Therefore, visual discrimination learning is likely to be mediated by synaptic plasticity mechanisms. The present study had the purpose to verify the recruitment of long term potentiation (LTP) in the primary visual cortex in this kind of learning. LTP is the most reliable model of synaptic plasticity and is largely used to study the neurophysiology of memory and learning (Bliss & Lømo 1973).

The LTP phenomenon is an activity dependent increase of synaptic efficacy. LTP has properties that have made it the principal model to study possible memory mechanisms (Bliss & Collingridge 2003). It is induced by stimulation that appears physiological, it has properties that enable association of temporally contiguous events and it can be stable and long lasting (Morris *et al.* 2003). Its involvement in the hippocampus processes underlying memory and learning has been well established (Barnes 1979) for decades. Nowadays, the interest of many researchers has been directed on trying to relate LTP with various forms of memory and learning. There are different kind of approaches. The most commons are interference, mimicry, occlusion of learning and occlusion of LTP. Interference uses drug administration to impede LTP induction or expression, in order to verify whether this treatment impairs a specific learning task (Morris *et al.* 1986, Richter-Levin *et al.* 1994 and McNaughton *et al.* 1995). Occlusion of learning is realized by first submitting the analysed neural structure to a high frequency stimulation (HFS) protocol that saturates the level of potentiation. Then, the procedure is completed by verifying whether learning task performance results to be impaired (McNaughton *et al.* 1986, Castro *et al.* 1989, Barnes *et al.* 1994, Rogan *et al.* 1997, Moser *et al.* 1998, Rioult-Pedotti *et al.* 1998 and Whitlock *and al.* 2006). Mimicry can be verified by observing whether properties of neural structures involved in a previous learning task show changes that are similar to modifications known to be caused by LTP (Rogan *et al.* 1997, Rioult-Pedotti *et al.* 1998 and Whitlock *and al.* 2006). After several intensive sessions of learning, LTP occlusion can be tested trying to

induce LTP in the neural structure involved. The intent is to demonstrate that LTP is occluded or reduced, because most of the available potentiation had been already used to permit learning to take its course (Rioult-Pedotti *et al.* 2000 and Whitlock *et al.* 2006).

In the present thesis the general hypothesis we tested was that visual discrimination learning provoked LTP-like changes in primary visual cortex. According to this hypothesis, the animal improvement in discriminating visual stimuli could be explained in terms of potentiation of synaptic efficacy in the same cortical area at work during perception. In this visual discrimination task, involvement of the earliest cortical levels of perception was successfully assured by demonstrating the selectivity of learning for stimuli orientation. Indeed, once the animals had learned the behavioural task, if the orientation of the stimuli was changed, their performance was severely impaired.

This study exploited three of the four most common approaches used to relate LTP with learning: interference, mimicry and occlusion of LTP. One assumption was that whether the connections of primary visual cortex were involved in learning the task, there would have been an increase of synaptic efficacy (mimicry) and a gradual approach to a maximum level of potentiation. Consequently to this increase, a following induction of LTP would have been impaired or markedly reduced (occlusion of LTP). These two strategies tried to verify this learning induced potentiation by the use of f-EPSP recordings. A pharmacological approach was further applied to verify whether LTP was responsible of such a potentiation, by using the LTP blocker U0126 during the animal behavioural tests (interference).

The principal concern in the use of f-EPSP experiments to investigate experience-dependent LTP was whether during learning is realistic to expect a synaptic change of the magnitude necessary to be detectable in a f-EPSP experiment. It is indeed possible that the proportion of synapses that change during the learning experience is so small that it becomes difficult to detect them by recording a f-EPSP response of a large population of cells. This is the general prediction of the theory of distributed associative memory (Marr 1971), which suggests that if a small amount of learning leads to a durable modification of a significant proportion of synapses, then the storage capacity of the network would be very low. This would imply that the storage capacity in primary visual cortex is likely to be low.

To interpret the results reported in the current thesis study, a different kind of coding strategy for information storage could be taken into account. It is reasonable to think that a detectable proportion of synapses might have been devoted to the storage of a few items of information following a sparse coding scheme. In layer II/III of the rat primary visual cortex, most cells responses are selective for a given spatial frequency (Girman *et al.* 1999). Synaptic modifications probably occurred in most of the cellular units of neuronal populations selective for the spatial frequencies' scale used during the entire visual discrimination learning task. According to a sparse coding modality, learning was likely to have involved a large spectrum of spatial frequency selective neurons. In this case it would be possible to detect the visual discrimination improvement able by measuring changes in cortical f-EPSP.

These expectations have been confirmed by our result in which both mimicry and occlusion of LTP were successfully verified in f-EPSP electrophysiological experiments. Experiments followed two different stimulation protocols. In the first one, layer IV was stimulated in order to activate vertical connections arriving to layer II/III. In the second case, stimulation occurred in layer II/III in order to record signals arriving from the same layer through horizontal connections.

The mimicry LTP-like results were significant in VD animal slices compared to control animal slices, even if the effect was less marked when stimulation of horizontal connections was applied. This discrepancy could be explained by arguing that a consistent part of the LTP-like effect in layer II/III might have been due to an increase in the neurotransmitter release from presynaptic terminals of excitatory projections coming from layer IV. Indeed, since visual information reaches layer II/III through connections coming from layer IV, it is possible that, during learning, a LTP-like process also occurres in layer IV neurons. This process would add to the potentiation in layer II/III making the synaptic change more detectable when mimicry is assessed by stimulation from layer IV.

On the other hand, LTP showed a significant effect in both stimulation conditions: IV-LTP and II/III-LTP were both significantly reduced in VD animal slices. The increase of the potentiation level until saturation was reached implied the possibility to force synapses to reach their maximal involvement. This strategy was probably able to detect differences that were difficult to find out by using the mimicry approach. However, a sort

of difference depending on the point of stimulation was anyway present. A significant difference between slices from VD animals and control animals was present from the first till the third TBS, when stimulation was applied to layer IV. On the other side, when stimulating layer II/III, significant difference is present after the second and after the third TBS. This seems to resemble a different implication of IV-LTP and II/III LTP in this kind of visual discrimination learning.

The third approach was based on a pharmacological interference experiment. This has been done by administrating U0126, a drug known to block the molecular mechanism underlying LTP. U0126 administration resulted in a delayed progress of the animal performance in the visual discrimination learning. This effect was selectively measured during the period of visual discrimination improvement, as U0126 was not administrated during the previous basal association learning. U0126, which has been largely used to selectively block plasticity, it has also been recognised to be selective only for the specific MEK kinase (Favata *et al.* 1998) and it has been shown that it does not affect visual functions and normal brain processes (Di Cristo *et al.* 2001).

Even if U0126 had a significantly negative influence on animal performance, it did not completely prevent visual discrimination learning to occur. However, drug interference rarely has a dramatic effect when delivered to a single neural structure that operates during the analysed learning task (Morris *et al.* 1986, Butcher *et al.* 1991, Riedel *et al.* 1994 Riedel *et al.* 1995). It is unlikely that this learning task is only managed by this single impaired area. On the contrary, most often, other involved brain structures are able to compensate for the lacking part of the synergic system. In the case of U0126 and the visual cortex, it might be that some portions of the visual cortex were spared by drug diffusion and these portions alone were anyway sufficient to furnish the required support for the entire learning task to complete.

The most compelling evidence proving that an LTP-like process operates during visual discrimination learning came form the electrophysiological experiments. Results of electrophysiological recordings effectively demonstrated that visual discrimination learning was accompanied by an LTP-like increased synaptic efficacy in primary visual cortex. Electrophysiological experiments of LTP mimicry and occlusion suggested that visual discrimination learning and LTP share a similar mechanism. These effects were particularly

evident for vertical connections between layer II/III neurons and projections coming from layer IV neurons.

These findings are comparable with changes observed in the amygdala, in the hippocampus and in the primary motor cortex following, respectively, fear conditioning, spatial learning and motor skill learning (Rogan *et. al* 1997, Rioult-Pedotti *et al*. 1998 and Whitlock *et al.* 2006).

Another important source of discussion derives from the possibility that long term depression (LTD) of synaptic strength might have been involved in V1 for visual discrimination learning to occur. Like LTP, LTD has also been largely investigated to relate synaptic plasticity with various forms of memory and learning (Massey & Bashir 2007). However, in the current thesis work time and resources constrains demanded to choose and focus on one form of plasticity. The matter was to point out which was more reasonable to underlie an improvement in detecting differences between cortical representations of visual stimuli. A potentiation process could amplify these differences in order to make them more detectable suggesting that an LTP-like mechanism is more suitable then a depression process. This is a sufficient motivation to investigate LTP rather than LTD. Moreover, a recent investigation showed that repeated exposure to a visual stimulus leads to a frequency dependent increase of visual evoked potentials (Frenkel *et al.* 2006). The results reported in the present thesis are in agreement with these findings. In slices of animals involved in learning, neuronal response with respect to the saturation level was found to be increased after layer IV stimulation and the two forms of LTP investigated resulted decreased. This means that mechanisms of potentiation are likely to be predominant over mechanisms of depression. Arguing a stronger involvement of LTD, in slices of VD animals one would expect to have found a decreased LTP-like effect (i.e. less potentiation in the mimicry experiment) and an increased LTP level in comparison with slices of control animals. Indeed, if synaptic depression had occurred, potential levels of VD animal slices would have displayed more susceptibility to increment being farther from the saturation level. A reasonable control experiment could concern investigation of LTD saturation level in both groups of animals by inducing low frequencies stimulation (LFS) instead of TBS. Following the previous reasoning, what one would expect to find out is that saturation comes first in control animal slices, while VD animal slices need further LFS to reach

occlusion. It is reasonable to suppose that with time depression becomes necessary in order to substitute old information with the new one. Investigation of LTD

In summary, this thesis study provided evidence supporting the theory which suggests that visual discrimination learning relies upon plastic modifications including an LTP-like increase in synaptic efficacy. These findings can be used to depict a general theoretic model concerning the processes underlying visual discrimination learning in the primary visual cortex. Such a model requires to take into account the behavioural strategy employed by the animals: animals managed to improve their performance because they were strongly motivated to find a hidden platform. Consequently, neural modifications occurring in V1 are very likely to be allowed by the influence of extra-V1 projections. These projections carry information about the behavioural and motivational state. Their modulation sets the early visual areas in a specific working mode according to expectation and behavioural requirements. This allows the visual system to compare stored representations against bottom-up information on stimulus characteristics. This loop of interactions may have a fundamental role in plasticity underlying the visual discrimination learning analysed by the current thesis study.

An interaction between the appropriate V1 intrinsic connections and the top-down feedback signals associated with the expectations of the behavioural task is a possible explanation for the induction of a potentiation process. The primary visual cortex receives feedback projections from higher order areas like V2, secondary motor cortex, temporal association cortex and perirhinal cortex (Coogan & Burkhalter 1993 and Bai *et al.* 2004). These feedback connections are known to provide strong excitatory input to forward projecting cells (Johnsonn & Burkhalter 1997). The connections of neurons more selective for the spatial frequency values of the training stimuli are the most likely to be strengthened. During the behavioural test, the two events (presence or absence of the platform) could be progressively associated with the trained spatial frequency values. Specific neurons of these higher order areas and specific neurons of V1 would simultaneously fire and this might allow a selective reinforcement in the neural circuitry. Frequent and persistent activity of these circuits during discrimination improvement might further increase their synaptic strength by a positive feedback control. According to the Hebbian rule, these neurons would strengthen their mutual interaction by potentiating the

efficacy of synapses they form with each other and with neurons of feed back projections coming down from the higer order areas. LTP-like mechanisms might have been necessary to reach such a purpose.

This hypothetical theory could provide a general idea about how visual discrimination learning relies upon plastic modifications including an LTP-like increase in synaptic efficacy in layer II/III. What neuronal activity might represent in terms of specific cognitive features is still undefined. Whether a distributed cellular potentiation throughout the cortical circuitry really represent “what has been learned” has to be further investigated. Answers are not likely to come by studying general levels of potentiation, but surely more advanced approaches are needed.

Bibliography

Aiba A., Chen C., Herrup K., Rosenmund C., Stevens C.F. and Tonegawa S. (1993)
Reduced hippocampal long-term potentiation and context-specific deficit in associative learning
in mGluR1 mutant mice.
Cell 79(2): 365-375

Aizenman C.D., Kirkwood A. & Bear M.F. (1996)
A current source density analysis of evoked responses in slices of adult rat visual cortex: implications for the regulation of long-term potentiation.
Cereb. Cortex. 6(6):751-758

Akaneya Y. & Tsumoto T. (2006)
Bidirectional trafficking of prostaglandin E_2 receptors involved in long-term potentiation in visual cortex.
J. Neurosci. 26(40):10209-10221

Alvarez P. & Squire L.R. (1994)
Memory consolidation and the medial temporal lobe: a simple network model.
Proc. Natl. Acad. Sci. USA 91:7041-7045

Anagnostaras S.G., Maren S. and Fanselow M.S. (1999)
Temporally graded retrograde amnesia of contextual fear after hippocampal damage in rats: within-subjects examination.
J. Neurosci. 19:1106-1114

Artola A., Brocher S. & Singer W. (1990)
Different voltage-dependent thresholds for inducing long-term depression and long-term potentiation in slices of rat visual cortex.
Nature (London) 347:69-72

Ashby F.G. & Waldron E.M. (1999)
On the nature of implicit categorization.
Psychon. Bull. Rev. 6(3):363-378 Review

Baker C.L. Jr & Mareschal I. (2001)
Processing of second-order stimuli in the visual cortex.
*Prog. Brain Res.*134:171-191. Review

Bai W.Z., Ishida M. and Arimatsu Y. (2004)
Chemically defined feedback connections from infragranular layers of sensory association cortices in the rat.
Neuroscience 123(1):257-267

Bailey, C. H. & Kandel, E. R. (1993)
Structural changes accompanying memory storage.
Annu. Rev. Physiol. 55: 397-426

Bannerman D.M., Good M.A., Butcher S.P., Ramsay M. and Morris R.G. (1995)
Distinct components of spatial learning revealed by prior training and NMDA receptor blockade *Nature* 378(6553):182-186

Barco A., Alarcon J.M.and Kandel E.R. (2002)
Expression of constitutively active CREB protein facilitates the late phase of long-term potentiation by enhancing synaptic capture.
Cell. 108:689 -703

Barnes C.A. (1979)
Memory deficits associated with senescence: A neurophysiological and behavioral study in the rat.
J. Comp. Physiol. Psychol. 93:74-104

Barnes C.A., Jung M.W., McNaughton B.L., Korol D.L., Andreasson K. and Worley P.F. (1994)
LTP saturation and spatial learning disruption: effects of task variables and saturation levels.
J. Neurosci. 14(10): 5793-5806

Barondes SH. (1970)
Cerebral protein synthesis inhibitors block long-term memory.
Int. Rev. Neurobiol. 12:177-205 Review

Bauer R.H.& Fuster J.M. (1976)
Delayed-matching and delayed-response deficit from cooling dorsolateral prefrontal cortex in monkeys.
J. Comp. Physiol. Psychol. 90(3):293-302

Bayley P.J., Hopkins R.O., and Squire L.R. (2003)
Successful recollection of remote autobiographical memories by amnesic patients with medial temporal lobe lesions.
Neuron 38:135-144

Bear M.F. & Kirkwood A. (1993)
Neocortical long-term potentiation.
Curr. Opin. Neurobiol. 3**:**197-202

Benke T.A., Lüthi A., Isaac J.T. and Collingridge GL. (1998)
Modulation of AMPA receptor unitary conductance by synaptic activity.
Nature. 393:793-797

Bennett E.L., Diamond M.C., Krech D. and Rosenzweig M.R. (1964)
Chemical and anatomical plasticity of brain.
Science 146:610-619

Berardi N., Pizzorusso T., Ratto G.M. and Maffei L. (2003)
Molecular basis of plasticity in the visual cortex
Trends in Neurosci. 26(7):367-377

Berger T.W. (1984)
Long-term potentiation of hippocampal synaptic transmission affects rate of behavioral learning.
Science 224(4649):627-30

Bilkey D.K. (1996)
Long-term potentiation in the in vitro perirhinal cortex displays associative properties.
Brain Res. 733(2):297-300

Bliss T.V.P. & Lomo T. (1973)
Long-lasting potentiation of synaptic transmission in the dentate area of the anesthetized rabbit following stimulation of the perforant path.
J. Physiol. 232:331-56

Bliss T.V.P. & Lynch M. (1988)
Long-term potentriation of synaptic transmission in the hippocampus: properties and mechanisms.
In: Landfield PW, Deadwyler SA, editors. Long-term potentiation in the hippocampus: from biophysics to behavior.
New York: Liss,. pp. 3.

Bliss T.V.P. & Collingridge G.L. (1993)
A synaptic model of memory: long-term potentiation

in the hippocampus.
Nature 361:31-39

Bonhoeffer T., Staiger V. and Aertsen A. (1989)
Synaptic plasticity in rat hippocampal slice cultures: local “Hebbian” conjunction of pre- and postsynaptic stimulation leads to distributed synaptic enhancement.
Proc. Natl. Acad. Sci. U.S.A. 86(20):8113–8117

Bozon B, Kelly A., Josselyn S.A., Silva A.J., Davis S. and Laroche S. (2003)
MAPK, CREB and zif268 are all required for the consolidation of recognition memory.
Philos. Trans. R. Soc. Lond. B. Biol. Sci. 358:805-814

Brinton R.E. (1991)
Biochemical correlates of learning and memory.
See Martinez & Kesner 1991, pp. 199–257

Brown T.H., Chapman P.F., Kairiss E.W. and Keenan C. L. (1988)
Long-term synaptic potentiation.
Science 242:724-728

Buckmaster P.S. & Schwartzkroin P.A. (1995)
Interneurons and inhibition in the dentate gyrus of the rat in vivo.
J. Neurosci. 15:774-789

Butcher S.P., Hamberger A. and Morris R.G. (1991)
Intracerebral distribution of DL-2-amino-phosphonopentanoic acid (AP5) and the dissociation of different types of learning.
Exp Brain Res. 83(3):521-526

Burbeck C.A. (1987)
Locus of spatial frequency discrimination.
J. Opt. Soc. Am. A 4:807-813

Burnham W.H. (1903)
Retroactive amnesia: illustrative cases and a tentative explanation.
Am. J. Psychol. 14:382-396.

Buzsaki G. (1989)
Two-stage model of memory trace formation: A role for "noisy" brain states.
Neuroscience 31:551-570

Cain D.P., Hargreaves E.L., Boon F. and Dennison Z. (1993)
An examination of the relations between hippocampal long-term potentiation, kindling, afterdischarge, and place learning in the water-maze.
Hippocampus 3:153-164

Cancedda L., Putignano E., Pizzorusso T., Ratto G.M. and Maffei L. (2002)
Visual stimulation induces CREB-mediated gene expression in visual cortical neurons through ERK activation.
Soc. Neurosci. Abstr. 731-735

Carew T.J., Castellucci V.F. and Kandel E.R. (1971)
An analysis of dishabituation and sensitization of the gill-withdrawal reflex in Aplysia.
Int. J. Neurosci. 2(2):79-98

Carew T.J. (1989)
Development assembly of learning in Aplysia.
Trends Neurosci. 12(10):389-394 Review

Castellucci V., Pinsker H., Kupfermann I. and Kandel E.R. (1970)
Neuronal mechanisms of habituation and dishabituation of the gill-withdrawal reflex in Aplysia.
Science 167(926):1745-1748

Castro C.A., Silbert L.H., McNaughton B.L. and Barnes C.A. (1989)
Recovery of learning following decay of experimental saturation of LTE at perforant path synapses.
Nature 342:545-48

Clark R.E., Broadbent, N.J., Zola S.M. and Squire L.R. (2002)
Anterograde amnesia and temporally graded retrograde amnesia for a nonspatial memory task after lesions of hippocampus and subiculum.
J. Neurosci. 22:4663-4669

Collingridge G.L., Kehl S.J. and McLennan H. (1983)
Excitatory amino acids in synaptic transmission in the Schaffer collateral-commissural pathway of the rat hippocampus.
J. Physiol. 334:33-46

Collingridge G.L. & Bliss T.V. (1995)
Memories of NMDA receptors and LTP.
Trends Neurosci. 18:54-56

Conquet F., Bashir Z.I., Davies C.H., Daniel H., Ferraguti F., et al. (1994)
Motor deficit and impairment of synaptic plasticity in mice lacking mGluR1.
Nature 372(6503): 237-243

Coogan T.A. & Burkhalter A. (1993)
Hierarchical organization of areas in rat visual cortex.
J. Neurosci. 13(9):3749-3772

Corkin S. (1984)
Lasting consequences of bilateral medial temporal lobectomy: clinical course and experimental findings in H.M.
Semin. Neurol. 4:249-259

Cornette L., Dupont P., Rosier A., Sunaert S., Van Hecke P., Michiels J., Mortelmans L and Orban G.A. (1998)
Human brain regions involved in direction discrimination.
J. Neurophysiol. 79:2749-2765

Costa-Mattioli M., Gobert D., Harding H., Herdy B., Azzi M., Bruno M., Bidinosti M., Ben Mamou C., Marcinkiewicz E., Yoshida M., Imataka H., Cuello A.C., Seidah N., Sossin W., Lacaille J.C., Ron D., Nader K. and Sonenberg N. (2005)
Translational control of hippocampal synaptic plasticity and memory by the eIF2alpha kinase GCN2.
Nature 436(7054):1166-1173

Crist R.E., Li W. and Gilbert C.D. (2001)
Learning to see: experience and attention in primary visual cortex.
Nat. Neurosci. 4(5):519-525

Cui Z., Wang H., Tan Y., Zaia K.A., Zhang S., and Tsien J.Z. (2004)
Inducible and reversible NR1 knockout reveals crucial role of the NMDA receptor in preserving remote memories in the brain.
Neuron 41:781-793

Derkach V., Barria A. and Soderling T.R. (1999)
Ca2+/calmodulin-kinase II enhances channel conductance of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate type glutamate receptors.
Proc. Natl. Acad. Sci. U.S.A. 96:3269-3274

Derrington A.M., Allen H.A. and Delicato L.S. (2004)
Visual mechanisms of motion analysis and motion perception.
Annu. Rev. Psychol. 55:181-205 Review

Desimone R. (1996)
Neural mechanisms for visual memory and their role in attention.
Proc. Natl. Acad. Sci. U. S. A. 93(24):13494-13499 Review

Di Cristo G., Berardi N., Cancedda L., Pizzorusso T., Putignano E., Ratto G.M. and Maffei L. (2001)
Requirement of ERK activation for visual cortical plasticity.
Science 292:2337-2340

Dunaevsky A., Tashiro A., Majewska A., Mason C. and Yuste R. (1999)
Developmental regulation of spine motility in the mammalian central nervous system.
Proc. Natl. Acad. Sci. U.S.A. 96:13438-13443.

Dupont P., Vogels R., Vandenberghe R., Rosier A., Cornette L., Bormans G., Mortelmans L. and Orban G.A. (1998)
Regions in the human brain activated by simultaneous orientation discrimination: a study with positron emission tomography.
Eur. J. Neurosci. 10:3689-3699

Durand G.M., Kovalchuk Y and Konnerth A. (1996)
Long-term potentiation and functional synapse induction in developing hippocampus.
Nature 381(6577):71-75

Faber D.S., **Lin J.W. and Korn H**. (1991)
Silent synaptic connections and their modifiability.
Ann. New. York. Acad. Scie. 627:151-164

Fahle M. & Edelman S. (1993)
Long-term learning in vernier acuity: effects of stimulus orientation, range and of feedback.
Vis. Res. 33:397-412

Fahle M., Edelman S. and Poggio T. (1995)
Fast perceptual learning in hyperacuity.
Vis. Res. 35:3003-3013

Fischer M., Kaech S., Knutti D. and Matus A. (1998)
Rapid actinbased plasticity in dendritic spines.
Neuron 20:847-854
.

Frankland P.W., O'Brien C., Ohno, M., Kirkwood A. and Silva A.J. (2001)
Alpha-CaMKII-dependent plasticity in the cortex is required for permanent memory.
Nature 411:309-313

Frankland P.W., Bontempi B., Talton L.E., Kaczmarek L. and Silva A.J. (2004)
The involvement of the cortex in remote contextual fear memory.
Science 304:881-883

Frenkel M.Y., Sawtell N.B., Diogo A.C., Yoon B., Neve R.L. and Bear M.F. (2006)
Instructive effect of visual experience in mouse visual cortex.
Neuron 51(3):339-349

Frey U. & Morris R.G. (1997)
Synaptic tagging and long-term potentiation.
Nature 385:533-536

Frey U. & Morris R.G. (1998a)
Synaptic tagging: Implications for late maintenance of hippocampal long-term potentiation.
Trends Neurosci. 21:181-188

Frey U. & Morris R.G. (1998b)
Weak before strong: Dissociating synaptic tagging and plasticity-factor accounts of late-LTP.
Neuropharmacology 37:545-552

Furmansky C., Schluppcek D. and Engel S.A. (2004)
Learning strenghtens the response of primary visual cortex to simple patterns.
Curr. Biol. 14:573-578

Ghose G.M., Yang T. and Maunsell J.H. (2002)
Physiological correlates of perceptual learning in monkey V1 and V2.
J. Neurophysiol. 87(4):1867-1888

Gilbert C.D. (1994)
Early perceptual learning.
Nature 356:150-152

Gilbert C.D. Ito M., Kapadia M. and Westheimer G. (2000)
Interactions between attention, context and learning in primary visual cortex.
Vision Res. 40(10-12):1217-1226 Review.

Gilbert C.D. & Sigman M. (2007)
Brain states: top-down influences in sensory processing.
Neuron 54(5):677-696

Graham K.S. & Hodges J.R. (1997)
Differentiating the roles of the hippocampal complex and the neocortex in long-term memory storage: evidence from the study of semantic dementia and Alzheimer's disease.
Neuropsychology 11:77-89

Grant S.G., O'Dell T.J., Karl K.A., Stein P.L., Soriano P. and Kandel E.R.. (1992)
Impaired longterm potentiation, spatial learning, and hippocampal development in *fyn* mutant mice.
Science 258(5090):1903-1910

Greenlee M.W. Reinvang I., Magnussen S., Kraemer F .M. and Hennig J. (1998)
Neocortical areas underlying visual short-term memory: evidence from fMRI.
NeuroImage 7, S846

Grossberg S. (1999)
How does the cerebral cortex work? Learning, attention and grouping by the laminar circuits of visual cortex.
Spat. Neurol. 12(2):371-378

Han Z-S., Buhl E., Lorinczi Z. and Somogyi P. (1993)
A high degree of spatial selectivity in the axonal and dendritic domains of physiologically identified local-circuit neurons in the dentate gyrus of the rat hippocampus.
Eur J Neurosci 5:395-410

Hanse E. & Gustafsson B. (1992)
Postsynaptic but not presynaptic activity controls the early time course of long-term potentiation in the dentate gyrus.
J. Neurosci. 12:3226-3240

Hasselmo M.E. (1999)
Neuromodulation: acetylcholine and memory consolidation.
Trends Cogn. Sci. 3:351-359

Hayashi Y., Shi S.H., Esteban J.A., Piccini A., Poncer J.C. and Malinow R. (2000)
Driving AMPA receptors into synapses by LTP and CaMKII: Requirement for GluR1 and PDZ domain interaction.

Science 287:2262-2267

Hebb D.O. (1949)
The organization of behaviour.
New York: Wiley

Heeley D.W., Buchanan-Smith H.M. and Heywood S. (1993)
Orientation acuity for sine-wave gratings with random variation of spatial frequency.
Vis. Res. 33:2509-2513

Hensch T.K. (2005)
Critical period mechanisms in developing visual cortex.
Curr. Top. Dev. Biol. 69:215-237

Hess G. & Donoghue J.P. (1994)
Long-term potentiation of horizontal connections provides a mechanism to reorganize cortical motor maps.
J. Neurophysiol. 71(6):2543-2547

Hubel D.H. & Wiesel T.N. (1959)
Receptive fields of single neurones in the cat's striate cortex.
J. Physiol. 148:574-591

Hubel D.H. & Wiesel T.N. (1977)
Ferrier lecture. Functional architecture of macaque monkey visual cortex.
Proc. R. Soc. Lond B. Biol. Sci. 198(1130):1-59

Impey S., Mark M., Villacres E.C., Poser S., Chavkin C. and Storm D.R. (1996)
Induction of CRE-mediated gene expression by stimuli that generate long-lasting LTP in area CA1 of the hippocampus.
Neuron 16:973-982

Isaac J.T., Nicoll R.A. and Malenka R.C. (1995)
Evidence for silent synapses: implications for the expression of LTP.
Neuron 15(2):427-434

Iversen S.D. (1976)
Do hippocampal lesions produce amnesia in animals.
International Review of Neurobiology 19:1-49

Izquierdo I. & Medina J.H. (1997)
Memory formation: the sequence of biochemical events in the hippocampus and its connection to activity in other brain structures.
Neurobiol. Learn. Mem. 68(3):285-316 Review

Jeffery K.J. & Morris R.G.M. (1993)
Cumulative long-term potentiation in the rat dentate gyrus correlates with, but does not modify, performance in the water-maze.
Hippocampus 3:133-140

Ji D. & Wilson M.A. (2007)
Coordinated memory replay in the visual cortex and hippocampus during sleep.
Nature Neurosci. 10(1):100-107

Johnson R.R. & Burkhalter A. (1997)
A polysynaptic feedback circuit in rat visual cortex.
J. Neurosci. 17(18):7129-7140

Jonides J., Schumacher E.H., Smith E.E., Koeppe R.A., Awh E., Reuter-Lorenz P.A., Marshuetz C. and Willis C.R. (1998)
The role of parietal cortex in verbal working memory.
J. Neurosci. 18(13):5026-5034

Kandell E.R. (2001)
The molecular biology of memory storage: a dialogue between genes and synapses.
Science 21(5):565-611

Kato N., Artola A. and Singer W. (1991)
Developmental changes in the susceptibility to long-term potentiation of neurones in rat visual cortex slices.
Brain Res. Dev. Brain Res. 60(1):43-50

Kauer J.A., Malenka R.C. and Nicoll R.A. (1988)
NMDA application potentiates synaptic transmission in the hippocampus.
Nature 334:250-252

Kelleher RJ 3rd, **Govindarajan A**, **Jung HY**, **Kang H**, **Tonegawa S**. (2004)
Translational control by MAPK signaling in long-term synaptic plasticity and memory.
Cell 116(3):467-79.

Kelso S.R. & Brown T.H. (1986)
Differential conditioning of associative synaptic enhancement in hippocampal brain slices.
Science 232(4746):85-87

Kennedy M.B. (1997)
The postsynaptic density at glutamatergic synapses.
Trends Neurosci. 20:264-268

Kesner R.P. & Connor H.S. (1972)
Independence of short- and long-term memory: a neural system analysis.
Science 176(33):432-434

Kesner R.P., Evans R.B. and Hunt M.A. (1987-1988)

Further evidence in support of the neurobiological bases of an attribute model of memory: role of the hippocampus.
Int. J. Neurol. 21-22:184-196 Review

Kim J.J. & Fanselow M.S. (1992)
Modality-specific retrograde hyperamnesia of fear.
Science 256:675-677

Kim J.J., Clark R.E. and Thompson R.F. (1995)
Hippocampectomy impairs the memory of recently, but not remotely, acquired trace eyeblink conditioned responses.
Behav. Neurosci. 109:195-203

Kinchla R. A. & Smyzer F. (1967)
A diffusion model of perceptual memory.
Perception and Psychophysics 2:219-229

Kirkwood A., Dudek S.M., Gold J.T., Aizenman C.D. and Bear M.F. (1993)
Common forms of synaptic plasticity in the hippocampus and neocortex in vitro.
Science 260(5113):1518-1521

Kirkwood A. & Bear M.F. (1994)
Hebbian synapses in visual cortex.
J. Neurosci. 14(3 Pt 2):1634-1645

Kirkwood A., Silva A. and Bear M.F. (1997)
Age-dependent decrease of synaptic plasticity in the neocortex of αCaMKII mutant mice.
Proc. Natl. Acad. Sci. U.S.A. 94:3380-3383

Korol D.L., Abel T.W., Church L.T., Barnes C.A. and McNaughton B.L. (1993)
Hippocampal synaptic enhancement and spatial learning in the Morris swim task.

Hippocampus. 3:127-132

Kosower E.M. (1972)
A molecular basis for learning and memory.
Proc. Natl. Acad. Sci. U.S.A. 69(11):3292-3296 Review

Krichevsky A.M. & Kosik K.S. (2001)
Neuronal RNA granules: A link between RNA localization and stimulation-dependent translation. *Neuron* 32:683-696

Kullmann D.M. (1994)
Amplitude fluctuations of dual-component EPSCs in hippocampal pyramidal cells: implications for long-term potentiation.
Neuron 12 :1111-1120

Laming D. & Laming J. (1992)
F. Hegelmaier: on memory for the length of a line.
Psychological Research 54: 233-239

Lavenex P. & Amaral D.G. (2000)
Hippocampal-neocortical interaction: a hierarchy of associativity.
Hippocampus 10(4):420-430 Review

Lawrence D.H. (1949)
Acquired distinctiveness of cue. I. Transfer between discriminations on the basis of familiarity with the stimulus.
J. Exp. Psychol. 39:770-784

LeDoux J.E. (1993)
Emotional memory systems in the brain.
Behav. Brain Res. 58(1-2):69-79 Review

Lee, B. and Harris, J. (1996)
Contrast transfer characteristics of visualshort-term memory.
Vis. Res. 36: 2159-2166

Lee H.K., Barbarosie M., Kameyama K., Bear M.F. and Huganir R.L. (2000)
Regulation of distinct AMPA receptor phosphorylation sites during bidirectional synaptic plasticity. *Nature* 405:955-959

Lee H.K., Takamiya K., Han J.S., Man H., Kim C.H., Rumbaugh G., Yu S., Ding L., He C., Petralia R.S., Wenthold R.J., Gallagher M. and Huganir R.L. (2003)
Phosphorylation of the AMPA receptor GluR1 subunit is required for synaptic plasticity and retention of spatial memory.
Cell. 112(5):631-643

Leritz E.C., Grande L.J. and Bauer R.M. (2006)
Temporal lobe epilepsy as a model to understand human memory: the distinction between explicit and implicit memory.
Epilepsy Behav. 9(1):1-13 Review

Levy W.B. & Steward O. (1979)
Synapses as associative memory elements in the hippocampal formation.
Brain Res. 175(2):233-245

Li W., Piëch V. and Gilbert C.D. (2004)
Perceptual learning and top-down influences in primary visual cortex.
Nat Neurosci. 7(6):651-657

Liao D., Hessler N.A. and Malinow R. (1995)
Activation of postsynaptically silent synapses during pairing-induced LTP in CA1 region of hippocampal slice.

Nature 375(6530):400-404

Liao D.S., Mower A.F., Neve R.L., Sato-Bigbee C. and Ramoa A.S. (2002)
Different mechanisms for loss and recovery of binocularity in the visual cortex.
J. Neurosci. 22:9015-9023

Linden D.J. & Connor J.A. (1995)
Long-term synaptic depression.
Annu. Rev. Neurosci. 8:319-357

Liu S., Rao Y., and Daw N. (2003)
Roles of protein kinase A and protein kinase G in synaptic plasticity in the visual cortex.
Cerebral Cortex 13(8):864-869

Lynch G. & Granger R. (1986)
Variations in synaptic plasticity and types of memory in corticohippocampal networks.
J. Cogn. Neurosci. 4(3):189-199

Maertens M. & Pollmann S. (2005)
fMRI reveals a common neural substrate of illusory and real contours in V1 after perceptual learning.
J. Cogn. Neurosci. (10):1553-1564

Magee J.C. & Johnston D. (1997)
A synaptically controlled, associative signal for Hebbian plasticity in hippocampal neurons.
Science 275:209-213

Magnussen S., Greenlee M.W., Asplund R. and Dyrnes S. (1990)
Perfect short-term memory for periodic patterns.
European J. Cogn. Psychol. 2:245-262

Magnussen S. & Greenlee M.W. (1992)
Retention and disruption of motion information in visual short-term memory.
J. Exp. Psychol. Learn. Mem. Cogn. 18:151-156

Magnussen S., Idås E. and Myhre S.H. (1998)
Representation of orientation and spatial frequency in perception and memory: a choice reaction time analysis.
J. Exp. Psychol. Hum. Percept. Perform. 24:707-718

Magnussen S. & Greenlee M.W. (1999)
The psychophysics of perceptual memory.
Psychological Research. 62:81-92

Magnussen S. (2000)
Low-level memory processes in vision.
Trends Neurosci. 23(6):247-251 Review

Makintosh N.J. (1974)
The psychology of animal learning.
London: Academic

Malleret G., Haditsch U., Genoux D., Jones M.W., Bliss T.V., Vanhoose A.M., Weitlauf C., Kandel E.R., Winder D.G. and Mansuy I.M. (2001)
Inducible and reversible enhancement of learning, memory, and long-term potentiation by genetic inhibition of calcineurin.
Cell 104(5):675-686

Mansuy I.M., Winder D.G., Moallem T.M., Osman M., Mayford M., Hawkins R.D. and Kandel ER. (1998)
Inducible and reversible gene expression with the rtTA system for the study of memory.
Neuron 21(2):257-265

Markowitsch H.J. (1985)
Hypotheses on mnemonic information processing by the brain.
Int. J. Neurosci. 27(3-4):191-227 Review

Marr D. (1970)
A theory for cerebral neocortex.
Proc. R. Soc. Lond. B. Biol. Sci. 176:161-234

Marr D. (1971)
Simple memory: a theory of archicortex.
Philos. Trans. R. Soc. London Ser. B 262:23-81

Martinez J.L. Jr, Schulteis G. and Weinberger S.B. (1991)
How to increase and decrease the strength of memory traces: the effects of drugs and hormones.
See Martinez & Kesner 1991, pp. 149-287

Massey P.V. & Bashir Z.I. (2007)
Long-term depression: multiple forms and implications for brain function.
Trends Neurosci. 30(4):176-184

Matthies H., Ruethrich H., Ott T., Matthies H.K. and Matthies R. (1986)
Low-frequency perforant path stimulation as a conditioned stimulus demonstrates correlations between long-term synaptic potentiation and learning.
Physiol. Behav. 36(5):811-821

Matus A. (2000)
Actin-based plasticity in dendritic spines.
Science 290:754-778

Maviel T., Durkin, T.P., Menzaghi F., and Bontempi B. (2004)
Sites of neocortical reorganization critical for remote spatial memory.
Science 305:96-99.

Mayr B. & Montminy M. (2001)
Transcriptional regulation by the phosphorylation-dependent factor CREB.
Nat. Rev. Mol. Cell. Biol. 2:599-609

McClelland J.L., McNaughton B.L. and O'Reilly R.C. (1995)
Why there are complementary learning systems in the hippocampus and neocortex: insights from the successes and failures of connectionist models of learning and memory.
Psychol. Rev. 102:419-457

McGaugh J.L. (1966)
Time-dependent processes in memory storage.
Science 153(742):1351-1358

McGaugh J.L. (2000)
Memory a century of consolidation.
Science 287(5451):248-251 Review

McGaugh J.L. & Izquierdo I. (2000)
The contribution of pharmacology to research on the mechanisms of memory formation.
Trends Pharmacol. Sci. 21(6):208-210

McNaughton B.L., Douglas R.M. and Goddard G.V. (1978)
Synaptic enhancement in fascia dentata: cooperativity among coactive afferents.
Brain Res. 157:277-93

McNaughton B.L., Barnes C.A., Rao G., Baldwin J. and Rasmussen M. (1986)

Long-term enhancement of hippocampal synaptic transmission and the acquisition of spatial information.
J. Neurosci. 6(2):563-571

McNaughton B.L. & Morris R.G.M. (1987)
Hippocampal synaptic enhancement and information storage within a distributed memory system. *Trends Neurosci.* 10:408-415

McNaughton B.L., Barnes C.A., Meltzer J., Sutherland R.J. (1989)
Hippocampal granule cells are necessary for normal spatial learning but not for spatially-selective pyramidal cell discharge.
Exp. Brain Res. 76(3): 485-96

McNaughton B.L., Barnes C.A., Gothard K., Jung M.W., Knierim J.J., Kudrimoti H., Qin Y.-L., Skaggs W.E., Gerrard J.L., Suster M. and Weaver K.L. (1995)
J. Exp. Biol. in press.

Mehta M.R. (2007)
Cortico-hippocampal interaction during up-down states and memory consolidation.
Nature Neurosci. 10(1):13-15

Miller S, **Yasuda M**, **Coats JK**, **Jones Y**, **Martone ME**, **Mayford M**. (2002)
Translational control by MAPK signaling in long-term synaptic plasticity and memory.
Neuron 36(3):507-519

Mitsuno K., Sasa M., Ishihara K., Ishikawa M. and Kikuchi H. (1994)
LTP of mossy fiberstimulated potentials in CA3 during learning in rats.
Physiol. Behav. 55(4):633-638

Montague P.R. & Sejnowski T.J. (1994)
The predictive brain: temporal coincidence and temporal order in synaptic learning mechanisms.
Learn. Mem. 1:1-33

Montgomery J.M., Pavlidis P. and Madison D.V. (2001)
Pair recordings reveal all-silent synaptic connections and the postsynaptic expression of long-term potentiation.
Neuron 29(3):691-701

Moosmang S., **Haider N**., **Klugbauer N**., **Adelsberger H**., **Langwieser N**., **Müller J**., **Stiess M**., **Marais E**., **Schulla V**., **Lacinova L**., **Goebbels S**., **Nave K.A**., **Storm D.R**., **Hofmann F., and Kleppisch T**. (2005)
Role of hippocampal Cav1.2 Ca2+ channels in NMDA receptor-independent synaptic plasticity and spatial memory.
J. Neurosci. 25(43):9883-9892

Moretti P., Levenson J.M., Battaglia F., Atkinson R., Teague R., Antalffy B., Armstrong D., Arancio O., Sweatt J.D. and Zoghbi H.Y. (2006)
Learning and memory and synaptic plasticity are impaired in a mouse model of Rett syndrome.
J. Neurosci. 26(1):319-27

Morozov A, **Muzzio IA**, **Bourtchouladze R**, **Van-Strien N**, **Lapidus K**, **Yin D**, **Winder DG**, **Adams JP**, **Sweatt JD**, **Kandel ER**. (2003)
Rap1 couples cAMP signaling to a distinct pool of p42/44MAPK regulating excitability, synaptic plasticity, learning, and memory.
Neuron 39(2):309-325

Morris R.G.M., Garrud P., Rawlins J.N., O'Keefe J. (1982)
Place navigation impaired in rats with hippocampal les ions.
Nature 297(5868):681-83

Morris R.G.M., Anderson E., Lynch G.S. and Baudry M. (1986)
Selective impairment of learning and blockade of long-term potentiation by an *N*-methyl-D-aspartate receptor antagonist AP5.
Nature 319(6056):774-76

Morris R.G.M. (1989)
Does synaptic plasticity play a role in information storage in the vertebrate brain?. In: MorrisRGM, editor. Parallel distributed processing: Implications for psychology and neurobiology
Oxford: Clarendon Press,. pp.248.

Moser E.I., Krobert K.A., Moser M-B. and Morris R.G.M. (1998)
Impaired spatial learning after saturation of long-term potentiation.
Science 281:2038-2042

Mower A.F., Liao D.S., Nestler E.J., Neve R.L. and Ramoa A.S. (2002)
cAMP/Ca2þ response element-binding protein function is essential for ocular dominance plasticity.
J. Neurosci. 22:2237-2245

Muller G.E. & Pilzecker A. (1900)
Experimentelle Beitrage zur Lehre von Geda¨ chtnis.
Z. Psychol. 1:1-300

Muller D., Nikonenko I., Jourdain P. and Alberi S. (2002)
LTP, memory and structural plasticity.
Curr. Mol. Med. 2:605-611

Murray E.A., Bussey T.J. and Saksida L.M. (2007)
Visual perception and memory: a new view of medial temporal lobe function in primates and rodents.
Annu. Rev. Neurosci. 30:99-122 Review

Nadel L. & Moscovitch M. (1997)
Memory consolidation, retrogene grade amnesia and the hippocampal complex.
Curr. Opin. Neurobiol. 7:217-227

Nicoll, R. A. & Malenka, R. C. (1995)
Contrasting properties of two forms of long-term potentiation in the hippocampus.
Nature 377:115-118

Nikonenko I., Jourdain P., Alberi S., Toni N. and Muller D. (2002)
Activity-induced changes of spine morphology.
Hippocampus 12:585-591

Nilsson T.H. & Nelson T.M. (1981)
Delayed monochromatic hue matches indicate characteristics of visual memory
J. Exp. Psychol. Hum. Percept. Perform. 7:141-150

Nimchinsky E.A., Sabatini B.L. and Svoboda K. (2002)
Structure and function of dendritic spines.
Annu. Rev. Physiol. 64:313-353

Nosofsky R.M. (1991)
Tests of an exemplar model for relating perceptual classification and recognition memory.
J. Exp. Psychol. Hum. Percept. Perform. 17:3-27

O'Dell T.J., Kandel E.R. andGrant S.G. (1991)
Long-term potentiation in the hippocampus is blocked by tyrosine kinase inhibitors.

Nature 353(6344):558-60

Olzak L. & Thomas J.P. (1999)
Neural recoding in human pattern vision: model and mechanisms.
Vis. Res. 39:231-256

Orban G.A., Dupont P., Vogels R., Bormans G. and Mortelmans L. (1997)
Human brain activity related to orientation discrimination.
Eur. J. Neurosci. 9:246-259

Orban G.A., Dupont P., De Bruyn B., Vandenberghe R., Rosier A. and Mortelmans L. (1998) Human brain activity related to speed discrimination tasks.
Exp. Brain Res. 122:9-22

Ott T., Ruthrich K., Reymann L., Lindenau L. and Matthies H. (1982)
Direct evidence for the participation of changes in synaptic efficacy in the development of behavioral plasticity.
In *Neuronal Plasticity and Memory Formation*, ed. CA Marsan, H Matthies,
New York: Raven pp. 441-452.

Ordy J.M. & Schjeide O.A. (1973)
Univariate and multivariate models for evaluating long-term changes in neurobiological development, maturity and aging.
Prog. Brain Res. 40(0):25-51 Review

Osipova D., Takashima A., Oostenveld R., Fernández G., Maris E., Jensen O. (2006)
Theta and gamma oscillations predict encoding and retrieval of declarative memory.
J. Neurosci. 26:7523-7531

Pavlov I. P. (1927)
Conditional Reflexes

New York: Dover Publications

Penfield W. & Milner B. (1958)
Memory deficit produced by bilateral lesions in the hippocampal zone.
AMA Arch. Neurol. Psychiatry 79:475-497

Pham T.A., Impey S., Storm D.R. and Stryker M.P. (1999)
CRE-mediated gene transcription in neocortical neuronal plasticity during the developmental critical period.
Neuron 22:63-72

Poggio T., Fahle M. and Edelman S. (1992)
Fast perceptual learning in visual hyperacuity.
Science 256:1018-1021

Prusky G.T., West P.W. and Douglas R.M. (2000)
Behavioral assessment of visual acuity in mice and rats.
Vision Res. 40(16):2201-2209

Regan D. (1985)
Storage of spatial-frequency information and spatial frequency discrimination.
J. Opt. Soc. Am. A 2:619-621

Reinvang I., Magnussen S., Greenlee M.W. and Larsson P.G. (1998)
Electrophysiological localization of brain regions involved in perceptual memory.
Exp. Brain Res. 123:481-484

Reymann K.G., Ruthrich H., Lindenau L., Ott T. and Matthies H. (1982)
Monosynaptic activation of the hippocampus as a conditioned stimulus: behavioral effects.
Physiol. Behav. 29(6):1007-1012

Ribot T.A. (1882)
Diseases of Memory
New York: Appleton-Cen-tury-Crofts

Richter-Levin G., Errington M.L., Maegawa H. and Bliss T.V. (1994)
Activation of metabotropic glutamate receptors is necessary for long-term potentiation in the dentate gyrus and for spatial learning.
Neuropharmacology 33(7):853-857

Riedel G., **Wetzel W. and Reymann K.G.** (1994)
4.(R,S)-alpha-methyl-4-carboxyphenylglycine (MCPG) blocks spatial learning in rats and long-term potentiation in the dentate gyrus in vivo.
Neurosci Lett. 167(1-2):141-144

Riedel G., Wetzel W., Kozikowski A.P. and Reymann K.G. (1995)
Block of spatial learning by mGluR agonist tADA in rats.
Neuropharmacology 34(5):559-561

Rioult-Pedotti M-S., Friedman D., Hess G. and Donoghue P. (1998)
Strenghtening of horizontal cortical connections following skill learning.
Nature Neurosci. 1(3):230-234

Rioult-Pedotti M-S., Friedman D. and Donoghue P. (2000)
Learning-Induced LTP in Neocortex.
Science 290:533-536

Roberts L.A., Large C.H., Higgins M.J., Stone T.W., O'Shaughnessy C.T. and Morris B.J. (1998)
Increased expression of dendritic mRNA following the induction of long-term potentiation.
Brain Res. Mol. Brain Res. 56:38-44

Roediger H.L. 3rd. (1990)
Implicit memory. Retention without remembering.
Am. Psychol. 45(9):1043-1056 Review

Rogan M.T., Staubli V.T., Hess G. and LeDoux J.E. (1997)
Fear conditioning induces associative long-term potentiation in the amygdala.
Nature 390:604-607

Robinson G.B. (1992)
Maintained saturation of hippocampal long-term potentiation does not disrupt acquisition of the eight-arm radial maze.
Hippocampus 2:389-395

Roland P.E. & Gulyàs B (1995)
Visual memory, visual imagery, and visual recognition of large field patterns by the human brain: functional anatomy by positron emission tomography.
Cereb. Cortex. 5(1):79-93

Rosenzweig M.R., Krech D., Bennett E.L. and Diamond M.C. (1962)
Effects of environmental complexity and training on brain chemistry and anatomy: a replication and extension.
J. Comp. Physiol. Psychol. 55:429-37

Sakimura K., Kutsuwada T., Ito I., Manabe T., Takayama C., et al. (1995)
Reduced hippocampal LTP and spatial learning in mice lacking NMDA receptor epsilon 1 subunit.
Nature 373(6510):151-155

Schoups A.A., Vogels R. and Orban G.A. (1995)
Human perceptual learning in identifying the oblique orientation: retinotopy, orientation specificity and monocularity.

J. Physiol. 483 (Pt 3):797-810

Schoups A.A., **Vogels R.**, **Qian N. and Orban G.A.** (2001)
Practising orientation identification improves orientation coding in V1 neurons.
Nature 412(6846):549-553

Schuman E.M. & Madison D.V. (1991)
A requirement for the intercellular messenger nitric oxide in long-term potentiation.
Science 254(5037):1503–6

Scoville W.B. & Milner B. (1957)
Loss of recent memory after bilateral hippocampal lesions.
J. Neurochem. 20:11-21

Sejnowski T.J. (1977)
Storing covariance with nonlinearly interacting neurons.
J. Math. Biol. 4(4):303-321

Schacter D.L. (1990)
Perceptual representation systems and implicit memory. Toward a resolution of the multiple memory systems debate.
Ann. N. Y. Acad. 608:543-571 Review

Schacter D.L. (1992)
Understanding implicit memory. A cognitive neuroscience approach.
Am. Psychol. 47(4):559-569 Review

Seeger T., Fedorova I., Zheng F., Miyakawa T., Koustova E., Gomeza J., Basile A.S., Alzheimer C. and Wess J. (2004)
M2 muscarinic acetylcholine receptor knock-out mice show deficits in behavioral flexibility, working memory, and hippocampal plasticity.
J. Neurosci. 24(45):10117-10127

Shapley R. & Lennie P. (1985)
Spatial frequency analysis in the visual system.
Annu. Rev. Neurosci. 8:547-583 Review

Sheng M. & Kim M.J. (2002)
Postsynaptic signaling and plasticity mechanisms.
Science 298:776-780

Shimizu E., Tang Y.P., Rampon, C., and Tsien J.Z. (2000)
NMDA receptor-dependent synaptic reinforcement as a crucial process for memory consolidation. *Science* 290:1170-1174

Shiu L.P. & Pashler H. (1992)
Improvement in line orientation discrimination is retinally local but dependent on cognitive set.
Percept. Psychophys. 52(5):582-588

Sik A., Ylinen A., Penttonen M. and Buzsa´ki G. (1994)
Inhibitory CA1-CA3-hilar region feedback in the hippocampus.
Science 265:1722-1724

Sik A., Penttonen M., Ylinen A. and Buzsa´ki G. (1995)
Hippocampal CA1 interneurons: an in vivo intracellular labeling study.
J. Neurosci. 15:6651-6665

Sik A., Penttonen M. and Buzsa´ki G. (1997)
Interneurons in the hippocampal dentate gyrus: an in vivo intracellular study.
Eur. J. Neurosci. 9:573-588

Silva A.J., Paylor R., Wehner J.M. and Tonegawa S. (1992a)
Impaired spatial learning in alphacalcium-calmodulin kinase II mutant mice.

Science 257(5067):206-211

Silva A.J., Stevens C.F., Tonegawa S. and Wang Y. (1992b).
Deficient hippocampal long-term potentiation in alpha-calcium-calmodulin kinase II mutant mice. *Science* 257(5067): 201-206

Silva A.J., Kogan J.H., Frankland P.W. and Kida S. (1998)
CREB and memory.
Annu. Rev. Neurosci. 21:127-148

Skinner B.F. (1935)
Two types of conditionated reflex and a pseudo type.
*J. Gen. Psychol.*12:66-77

Slotnick S.D. (2004)
Visual memory and visual perception recruit common neural substrates.
Behav. Cogn. Neurosci. Rev. 3(4):207-221 Review

Smith E.E. (2008)
The case for implicit category learning.
Cogn. Affect. Behav. Neurosci. 8(1):3-16 Review

Smith L.B. (1989)
From global similarity to kinds of similarity: the construction of dimensions in development.
In *Similarity and Analogical Reasoning,* ed. S Vosniadu, A Ortony, pp. 146-178.
Cambridge: Cambridge Univ. Press

Sorra K.E. & Harris K.M. (2000)
Overview on the structure, composition, function, development, and plasticity of hippocampal dendritic spines.
Hippocampus 10:501-511

Sowden P.T. & Schyns P.G. (2006)
Channel surfing in the visual brain.
Trends Cogn. Sci. 10(12):538-545

Speekenbrink M., Channon S. and Shanks D.R. (2008)
Learning strategies in amnesia.
Neurosci. Biobehav. Rev. 32(2):292-310 Review

Squire L.R. & Zola-Morgan S. (1983)
The neurology of memory: the case for correspondence between the findings for human and nonhuman primate.
In J. A. Deutsch (Ed.), *The physiological basis of memory* (pp. 199-268)
New York: Academic Press

Squire L.R., Zola-Morgan S. and Chen K. (1988)
Human amnesia and animal models of amnesia: performance of amnesic patients on tests designed for the monkey.
Behav. Neurosci. 11:210-221

Squire L.R. (1992)
Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans.
Psychol. Rev. 99:195-231

Squire L.R., & Alvarez P. (1995)
Retrograde amnesia and memory consolidation: a neurobiological perspective.
Curr. Opin. Neurobiol. 5:169-177

Squire L.R.& Zola S.M. (1996)
Structure and function of declarative and nondeclarative memory systems.
Proc. Natl. Acad. Sci. U.S.A. 93(24):13515-22 Review

Squire L.R., Clark R.E. and Knowlton B.J. (2001)
Retrograde amnesia.
Hippocampus 11:50-55

Squire L.R., Stark C.E. and Clark R.E. (2004)
The medial temporal lobe.
Annu. Rev. Neurosci. 27:279-306

Stäubli U., **Scafidi J**. **and Chun D**. (1999)
GABAB receptor antagonism: facilitatory effects on memory parallel those on LTP induced by TBS but not HFS.
J. Neurosci. 19(11):4609-4615

Stent G. (1973)
A physiological mechanism for Hebb's postulate of learning.
Proc. Natl. Acad. Sci. U.S.A. 70:997-1001

Struble R.G., Desmond N.L. and Levy W.B. (1978)
Anatomical evidence for interlamellar inhibition in the fascia dentata.
Brain Res. 152:580-585

Sutherland R.J., Dringenberg H.C. and Hoesing J.M. (1993)
Induction of long-term potentiation at perforant-path dentate synapses does not affect place learning or memory.
Hippocampus 3:141-148

Sutherland R.J., Weisend M.P., Mumby D., Astur R.S., Hanlon F.M., Koerner A., Thomas M.J., Wu Y., Moses S.N., Cole C., Hamilton D.A. and Hoesing J.M. (2001)
Retrograde amnesia after hippocampal damage: recent vs. remote memories in two tasks.
Hippocampus 11:27-42

Taha S. & Stryker M.P. (2002)
Rapid ocular dominance plasticity requires cortical but not geniculate protein synthesis.
Neuron 34:425-436

Takashima A., Petersson K.M., Rutters F., Tendolkar I., Jensen O., Zwarts M.J., McNaughton B.L. and Fernández G. (2006)
Declarative memory consolidation in humans: a prospective functional magnetic resonance imaging study.
Proc. Natl. Acad. Sci. U. S. A. 103:756-761

Thomas G.M. & Richard L.H. (2004)
Mapk cascade signalling and synaptic plasticity
Nat. Rev. Neurosci. 5(3):173-183

Tomizawa K., **Iga N**., **Lu Y.F**., **Moriwaki A**., **Matsushita M**., **Li S.T**., **Miyamoto O**., **Itano T**. **and Matsui H**. (2003)
Oxytocin improves long-lasting spatial memory during motherhood through MAP kinase cascade.
Nat. Neurosci. 6(4):384-390

Tsien J.Z. (2000)
Linking Hebb's coincidence-detection to memory formation.
Curr. Opin. Neurobiol. 10:266-273

Tsumoto T. (1992)
Long-term potentiation and long-term depression in the neocortex.
Prog. Neurobiol. 39**:**209-228

Tulving E. (1983)
Elements of episodic memory.
Cambridge: Oxford University Press.

Tulving E. & Markowitsch H.J. (1997)
Memory beyond the hippocampus.
Curr. Opin. Neurobiol. 7(2):209-216 Review

Vogels R. & Orban G.A. (1985)
The effect of practice on the oblique effect in line orientation judgments.
Vision Res. 25(11):1679-1687

Vogels R. & Orban G.A. (1986)
Decision processes in visual discrimination of line orientation.
J. Exp.Psychol. Hum. Percept.

Walters E.T., Carew T.J. and Kandel E.R. (1979)
Classical conditioning in Aplysia californica.
Proc. Natl. Acad. Sci. U.S.A. 76(12):6675-6679

Warrington E.K. & Weiskrantz L. (1968)
New method of testing long-term retention with special reference to amnesic patients.
Nature 217(5132):972-974

Weiler I. J., Hawrylak N. and Greenough W.T. (1995)
Morphogenesis in memory formation: synaptic and cellular mechanisms.
Behav. Brain Res. 66:1–6

Wheeler M.E., Petersen S.E., and Buckner R.L. (2000)
Memory's echo: vivid remembering reactivates sensory-specific cortex.
Proc. Natl. Acad. Sci. U. S. A. 97:11125-11129

Whitlock J.R., Heinen A.J., Shuler M.G. and Bear M. F. (2006)
Learning induces Long-Term Potentiation in the hippocampus.
Science. 313:1093–1097

Wittenberg G.M. & Tsien J.Z. (2002)
An emerging molecular and cellular framework for memory processing by the hippocampus.
Trends Neurosci. 25:501-505

Wolfe J.M. (1998)
Visual memory: what do you know about what you saw?
Curr. Biol. 8(9):R303-304 Review

Yun S.H., Huh K. and Jung M.W. (2000)
Selective enhancement of non-NMDA receptor-mediated responses following induction of long-term potentiation in entorhinal cortex.
Synapse 35(1):1-7

Yust R. & Bonhoeffer T. (2001)
Morphological changes in dendritic spines associated with long-term synaptic plasticity.
Annu. Rev. Neurosci. 24:1071-1089

Zalutsky R.A. & Nicoll R.A. (1990)
Comparison of two forms of long-term potentiation in single hippocampal neurons.
Science 248:1619-1624

Zhao M.G., **Toyoda H**., **Lee Y.S**., **Wu L.J**., **Ko S.W**., **Zhang X.H.**, **Jia Y**., **Shum F**., **Xu H**., **Li B.M**., **Kaang B.K**. **and Zhuo M**. (2005)
Roles of NMDA NR2B subtype receptor in prefrontal long-term potentiation and contextual fear memory.
Neuron 47(6):859-872

Zola-Morgan S.M. & Squire L.R. (1990)
The primate hippocampal formation: evidence for a time-limited role in memory storage.

Science 250:288-290