Target-cell-specific bidirectional synaptic plasticity at the CA1-subiculum synapse

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Dedicated to the memory of

my grandmother Chana Shor (Shlamkovitch)
my grandfather Hirsha Leib Tzaphnat
my great aunt Chana Laskin (Chane)
and her brother Avraham Chane
and her cousin Ketty Poller (Lutchevsky)

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Es ist generell akzeptiert, dass der Hippocampus entscheidend an der Gedächtnisgenerierung von Säugetieren mitwirkt. Das Subiculum ist das Hauptziel von CA1-Pyramidenzellen und dient somit als ein Relais für abgehende Informationen des Hippocampus. Pyramidenzellen im Subiculum können anhand ihrer Aktivitätsrate als explosionsartig aktionspotentialbildend (burst-spiking) bzw. als regulär aktionspotentialbildend (regular-spiking) klassifiziert werden. In der gegenwärtigen Studie demonstrieren wir, dass burst-spiking- und regular-spiking-Zellen bei Ratten prinzipiell zu unterscheidende Formen von durch Niedrig-Frequenz induzierter synaptischer Plastizität zeigen. In burst-spiking-Zellen induziert Niedrig-Frequenz-Stimulation (bei 0,5-5Hz) eine frequenzabhängige Langzeitdepression (LTD) mit einem Maximum bei 1Hz. Diese LTD ist von der Aktivierung der NMDA-Rezeptoren abhängig und überlagert eine mGlu-Rezeptor-abhängige Langzeitpotenzierung(LTP). Im Gegensatz hierzu wird in regular-spiking-Zellen durch eine Niedrig-Frequenz-Stimulation eine mGlu-Rezeptor-abhängige LTP induziert, die eine NMDA-Rezeptor abhängige LTD überlagert. Beide Prozesse basieren auf einem postsynaptisch ablaufendem Ca^{2+}-Signal, da BAPTA die Induktion von synaptischer Plastizität in beiden Zelltypen unterbindet Folglich finden mGlu-Rezeptor-abhängige LTP und NMDA-Rezepor-abhängige LTD in CA1-Subiculum Zellen simultan statt. Die vorherrschende Ausrichtung synaptischer Plastizität hängt vom untersuchten Zelltyp ab. Unsere Daten geben einen neuen Mechanismus für das sliding-threshold-Modell synaptischer Plastizität zu erkennen, in welchem die LTP- und LTD-Induktion von dem relativen Aktivierungsstatus des NMDA- und des mGlu-Rezeptors angestoßen wird. Des Weiteren können wir belegen, dass die oben genannte bidirektionale Plastizität von der Koaktivierung muskarinerger Acetylcholin-Rezeptoren abhängt, da Scopalamin synaptische Plastizität beider Zelltypen blockiert. Zusätzlich demonstrieren wir, dass der L-Typ-Calcium-Kanalblocker Nifedipine in burst-spiking-Zellen LTD zu LTP, in regular-spiking-Zellen LTP zu LTD konvertiert und dass somit die Polarität synaptischer Plastizität von spannungsgesteuerten Calcium-Kanälen moduliert wird. In Zellen des Subiculums scheint die bidirektionale synaptische Plastizität somit von einem komplexen Signalordnungssystem reguliert zu werden, welches zellspezifische Rekrutierung sowohl von liganden- als auch von spannungsgesteuerten Ionen-Kanälen involviert. Diese komplexe Regulierung könnte an hippocampalen Datenausgangssynapsen zur Feineinstellung von synaptischer Wirkkraft notwendig sein. Unsere Beobachtung der Korrelation von der Ausrichtung synaptischer Plastizität mit den Entladungscharakteristiken von postsynaptischen Zellen enthüllen einen neuen und gleichzeitig verblüffenden Mechanismus der Zielgenauigkeit, der dazu dienen könnte, die Signifikanz

neuronaler Informationen zu justieren, indem man hippocampalen Datenausgang entweder auf burst-spiking- oder regular-spiking-Zellen des Subiculums lenkt.

It is commonly accepted that the hippocampus is critically involved in the explicit memory formation of mammals. The subiculum is the principal target of CA1 pyramidal cells and thus serves as the major relay station for the outgoing hippocampal information. Pyramidal cells in the subiculum can be classified according to their firing properties into burst-spiking and regular-spiking cells. In the present study we demonstrate that burst-spiking and regular-spiking cells show fundamentally different forms of low frequency- induced synaptic plasticity in rats. In burst-spiking cells, low-frequency stimulation (at 0.5-5 Hz) induces frequency-dependent long-term depression (LTD) with a maximum at 1 Hz. This LTD is dependent on the activation of NMDAR and masks an mGluR- dependent long-term potentiation (LTP). In contrast, in regular-spiking cells low-frequency stimulation induces an mGluR-dependent LTP that masks an NMDAR-dependent LTD. Both processes depend on postsynaptic Ca^{2+}-signaling as BAPTA prevents the induction of synaptic plasticity in both cell types. Thus, mGluR-dependent LTP and NMDAR-dependent LTD occur simultaneously at CA1-subiculum synapses and the predominant direction of synaptic plasticity relies on the cell type investigated. Our data indicate a novel mechanism for the sliding-threshold model of synaptic plasticity, in which induction of LTP and LTD seems to be driven by the relative activation state of NMDAR and mGluR. further more we give evidence that this bidirectional plasticity relies upon the co-activation of muscarinic acetylcholine receptors, as scopolamine blocks synaptic plasticity in both cell types. In addition, we demonstrate that the L-type calcium channel inhibitor nifedipine converts LTD to LTP in burst-spiking cells and LTP to LTD in regular-spiking cells, indicating that the polarity of synaptic plasticity is modulated by voltage-gated calcium channels. Bidirectional synaptic plasticity in subicular cells therefore appears to be governed by a complex signaling system, involving cell-specific recruitment of ligand and voltage-gated ion channels as well as metabotropic receptors. This complex regulation might be necessary for fine-tuning of synaptic efficacy at hippocampal output synapses. Our observation that the direction of synaptic plasticity correlates with the discharge properties of the postsynaptic cell reveals a novel and intriguing mechanism of target specificity that may serve in tuning the significance of neuronal information by trafficking hippocampal output onto either subicular burst-spiking or regular-spiking cells.

Memory as a concept

Early philosophy and later experimental psychology referred to memory as changes in the individual's behavior as a result of that individual's experience a terese typical example of such 'behavioral' definition is: 'lasting change in behavior resulting from previous experience' (Henry L. Roediger, 2007). This definition of memory was rejected primarily for the reason that some lasting experience-dependent changes in the individual's behaviour are the outcome of fatigue, intoxication, injury and/or disease. Secondly, it was realized that memory could not be defined based merely on performance at the time of a particular test. These reasons made the definition evolve into an outcome of experience-dependent changes in the potential to behave and no
t only the manifested behavior. This definition introduced 'knowledge' as a critical attribute of memory. This latter term can be more precisely stated as 'internal representation', which in the case of neuroscience would mean a neuronally encoded structured version of the world that could potentially guide behavior. The function of memory is, therefore, to retain over time experience-created or modified internal representations.

Learning

Learning is a mechanism for information acquisition, which may be measured as changes in performance, or in potential for performance. In terms of the underlying neuronal circuit, one may speak of the neural plasticity processes leading to the altered behavioral performance, where plasticity is the ability to undergo modification without immediate relaxation or disintegration. Plasticity is a general term that is used to describe neuronal changes that are associated with learning and memory. By changes in neuronal excitability, the phenomenon of plasticity has consequences in changes of the probability of a particular form of behaviour. Neural excitability may be regarded, in that context, as global when excitability of the whole neuron is altered or alternatively of a synapse with restricted area specificity. Restricted changes in excitability increase the potential information storage capabilities of the system.

Neural plasticity

Plasticity is the capacity of a neural activity triggered by experience of some sort to modify thoughts, feelings and behaviour via modification of the neural circuit function of a system. Specifically, plasticity is any mechanism that modifies the efficacy or excitability of synaptic transmission (Citri and Malenka, 2008). Plasticity can be induced by a particular synaptic input, by either a pre-synaptic or a post-synaptic mechanism, or a combination of the two. It can also be induced by hormonal and non-synaptic neurotransmitters. Plasticity is manifested by morphological changes in the neurons such as the distribution of ion channels in the membrane and microtubule-associated proteins, which are important for the structural rigidity of the cell and in addition signaling and kinesis proteins. In the developing nervous system, astrocytes and Schwann cells promote synapse formation and function. Data from cell specific mutant studies support the notion that learning and memory formation can start from one or few sites and initiate the downstream plasticity process.

Short term synaptic plasticity

Short-term synaptic plasticity has a temporal range of several milliseconds to several minutes and can be expressed in many ways . Short term adaptation to sensory inputs, short lasting alterations of behavior and short lasting memory are some of the important phenomena underlined by short term synaptic plasticity. Upon brief repeated activity, calcium is accumulated transiently at the pre-synaptic terminal, which in turn modifies the probability of exocytosis of synaptic vesicles (Katz and Miledi, 1968; Zucker and Regehr, 2002). Paired pulse facilitation and depression are common forms of short-term plasticity. In this phenomenon, a stimulus is followed by a second one, which is enhanced or depressed, respectively. At short inter-stimulus intervals, depression is observed at all synapses. This depression is manifested either by inactivation of voltage-gated calcium or sodium channels or by depletion of the pool of vesicles ready to be released. Facilitation may be observed at many synapses at longer inter-stimulus intervals. This kind of plasticity may be explained by the left over calcium that remains at the presynaptic site as a result of previous synaptic activation, thereby increasing the probability of release of synaptic vesicles upon a subsequent second stimulation.

Post-tetanic potentiation is observed after a high frequency train lasting between 200ms and 5s. This kind of potentiation is explained mainly by the elevated concentration of calcium caused by the stimulus train and hence affects the release probability of vesicles, directly or indirectly resulting in the alteration of functions of the pre-synaptic proteins (Citri and Malenka, 2008). At computational or signal processing level, the different forms of short-term synaptic plasticity function as filters. As an example, at higher probability of vesicular release, a synapse will depress upon high frequency stimulation, while a low frequency stimulation will be transmitted reliably (Abbott and Regehr, 2004).

Memory and plasticity

Memory, which is believed to be structurally built up by plasticity mechanisms, is usually divided into two domains; short-term memory, where no protein or mRNA synthesis is required by the mechanism, and long-term memory, where the mechanisms require both protein and mRNA synthesis. However, this classification also accommodates several intermediate phases of memory, which like long-term memory, apply protein synthesis but do not require mRNA synthesis. Each of these intermediate phases has its own signature molecular mechanism. Recent studies suggest different temporal domains of long-term memory with different expression mechanisms (Wainwright et al., 2002).

Criteria for Plasticity as a mechanism of memory

It all started in 1949, when Donald Hebb proposed for the first time, albeit theoretically, that "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." This became the basis of what is now known as Hebbian plasticity. Significantly, he predicted structural and/or metabolic changes in the neurons as the underlying basis of activity dependent long-term changes in the neurons. His convictions were experimentally buttressed by Bliss and colleagues (Bliss and Lomo, 1973; Bliss and Gardner-Medwin, 1973), when they reported long-term potentiation (LTP) in the rabbit hippocampus in vitro in response to synaptic stimulation. Since the experimental discovery of LTP, it has been demonstrated that a synapse that exhibits LTP is also able to exhibit long term depression (LTD) (Dudek and Bear, 1992), meaning the weakening of the synaptic strength in response to certain paradigms of synaptic activity. The fact that a synapse is modifiable in a bidirectional way has far reaching consequences on the computational abilities of the brain as a whole. It is probably worthwhile to state here that the phenomena of LTP and LTD that are expressed in different areas or even in the different synaptic connections in the same areas of the brain, differ considerably in terms of their underlying mechanisms (Citri and Malenka, 2008).

The Synaptic Plasticity and Memory hypothesis (SPM) states that learning is a phenomenon that is accompanied by patterns in neural activity that represents the occurrence of some kind of events (Martin and Morris, 2002b). This neural activity changes the synaptic connections per se in the brain and the memory will be expressed upon reactivation of the circuitry that was previously altered. Morris (Morris and Rugg, 2004) stated that 'Memory is a property of the entire organism, while plasticity is a property of the synapses'; this probably emphasizes the complexity of memory as a phenomenon on one hand, and the lack of a complete knowledge about how a change in the individual synapses goes about in creating a memory, on the other. Nonetheless, Bliss and Collingridge (Bliss and Collingridge, 1993) outlined the 3 basic properties of long term plasticity: associativity, input specificity and persistence over time. Note that persistence might be problematic

in defining memory mechanism, since while plasticity must last a reasonable length of time to be considered a memory mechanism, there is no reason why LTP or LTD should last indefinitely, as memory traces can be activated in an activity dependent manner. LTP induction must start by pre-synaptic activity and proceed to postsynaptic activation for potentiation to occur. On the other hand, back propagating dendritic action potential is important for alternating from LTP to LTD in the neo-cortex and hippocampus (Bi and Poo, 1998). There are four criteria according to Morris & Martin (Martin and Morris, 2002a) for the evaluation of the SPM hypothesis:

The detectability criterion, which postulates that behavioral memory must be associated with synaptic efficacy changes somewhere in the brain. Mimicry is an important test as to whether changes in synaptic strength are sufficient to memory formation. The test simply states that if we artificially induce changes in the synaptic weights, the animal should experience memory for an event that did not really happen. The anterograde alteration criterion states that upon preventing synaptic weight change during learning, the memory of the experience will be impaired, while, the retrograde alteration criterion states that a memory of an experience will be altered upon alteration of synaptic weights that were induced by learning experience. (Martin and Morris, 2002a).

A great deal of knowledge has been gathered in the last few years concerning the biochemical events underlying the induction and expression of LTP and LTD (Malinow et al., 2000; Nicoll, 2003). Increases in the postsynaptic calcium concentration after synaptic activation of NMDA receptors or voltage gated calcium channels are believed to be general mechanisms for LTP and LTD induction. This rise in calcium concentration triggers a variety of enzymatic reactions that lead to direct and indirect phosphorylation of proteins resulting in a change in receptor sensitivity and number (Malinow and Malenka, 2002).

Long Term Potentiation

The most prominent forms of Long Term Potentiation (LTP) are NMDA receptor dependent. NMDA receptor dependent LTP requires synaptic activation of NMDA receptors during postsynaptic depolarization, which can be achieved with different induction protocols (Bliss and Collingridge, 1993; Malenka and Nicoll, 1999). The calcium influx through NMDA receptors and the rise in calcium concentration at the dendrites is a necessary condition for LTP. Many signaling proteins and as a consequence, many intracellular cascades are found to be involved in inducing LTP. It seems that calcium/calmodulin-dependent protein kinase is required for NMDA receptor dependent LTP independent of the induction protocol used. These cascades alter the single channel conductance of synaptic AMPA receptors (AMPAR) and lead to incorporation of additional AMPAR to the postsynaptic density. In addition, the number of dendritic spines is increased. In parallel, there are changes in the synapse structure, like alterations of the size of the pre-synaptic active zone. The maintenance of these changes for a longer time period utilizes de novo transcription and local dendritic proteins (Citri and Malenka, 2008).

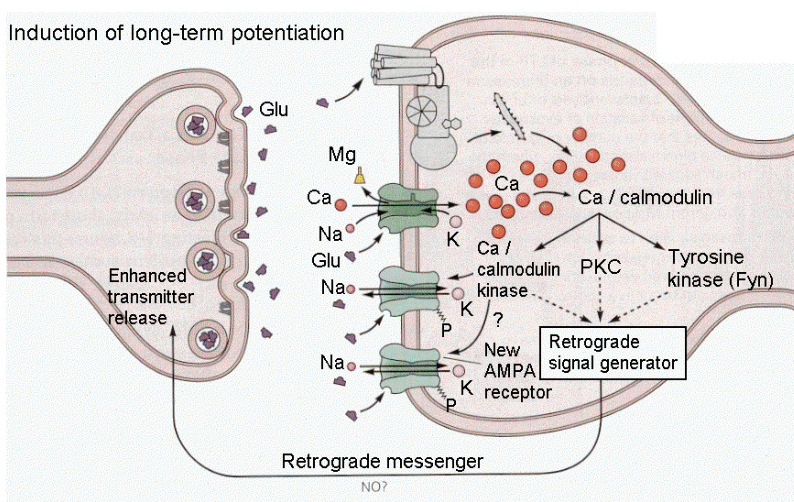


Illustration 1 | During the early phase of LTP, high-frequency stimulation opens non-NMDA glutamate channels leading to hypopolarization. This dislodges Mg++ from the NMDA glutamate channels, and Ca++ enters the cells. The calcium triggers the activity of Ca-dependent kinases, PKC and Ca-calmodulin, and tyrosine kinase. Ca-calmodulin kinase phosphorylates non-NMDA channels, increasing their sensitivity to glutamate and a messenger is sent retrogradely to the presynaptic terminal to increase the release of transmitter substance. Illustration taken from Principles of Neural Science, New York: McGraw-Hill.

Long Term Depression

Long Term Depression (LTD) is an activity-dependent decrease in synaptic transmission that can be expressed as heterosynaptic or homosynaptic forms. Homosynaptic LTD is input specific while by definition, heterosynaptic LTD is a reduction of synaptic efficiency at nonstimulated synapses. The first LTD reported was the heterosynaptic form which occurred at the CA1 region of the hippocampus through a non-tetanized pathway in parallel to LTP pathway expressed after tetanic stimulation (Lynch et al., 1977). Later on, it was found to occur at the dentate gyrus as well. Homosynaptic LTD was initially reported as a reversal phenomenon of LTP, when a low – frequency stimulation was applied (Barrionuevo et al., 1980). A non-LTP-dependent homosynaptic LTD was firstly seen at the CA1 area as a response to low frequency stimulation (Dudek and Bear, 1992;Mulkey and Malenka, 1992).

Since then, LTD has been reported in various other brain structures and was established as a principle plasticity phenomenon like LTP (Martin et al., 2000) or homeostatic plasticity (Turrigiano and Nelson, 2004). However, LTD seems to depend much more on the conditions during induction (Kemp and Bashir, 2001), like frequency and place of the stimulation (Braunewell and Manahan-Vaughan, 2001; Poschel et al., 2005).

Hippocampus anatomy

The hippocampal formation is constructed from several related brain regions, which together comprise a functional system. The CA3, CA2 and CA1 areas are subdivisions of the hippocampus proper, and the other components of the hippocampal formation are the dentate gyrus, subiculum, presubiculum, parasubiculum and entorhinal cortex (illustration 2). The reason for these five areas to be considered a single formation is the functional linkage between them.

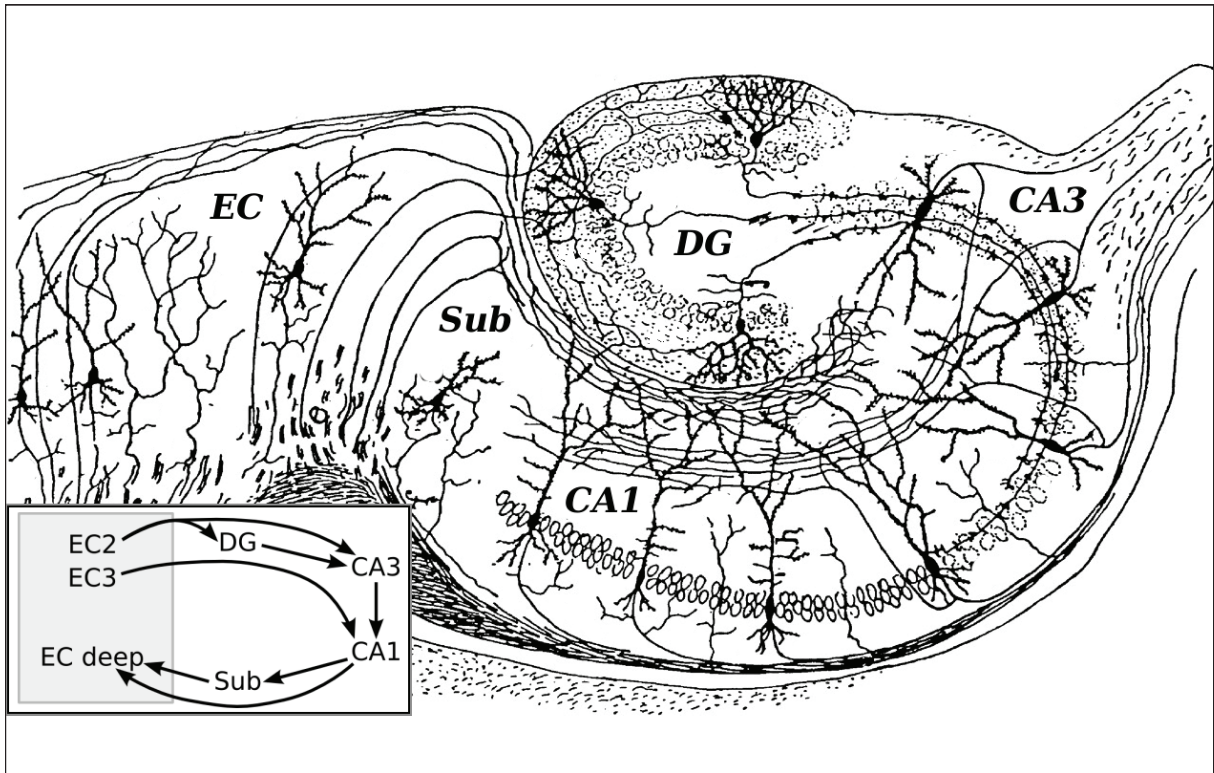


illustration 2: Basic circuit and anatomy of the hippocampus, shown using a modified drawing by Ramon y Cajal. DG: dentate gyrus. Sub: subiculum. EC: entorhinal cortex

Dentate gyrus

The dentate gyrus is a cortical v-shaped region which has a similar structure at all levels of the hippocampal formation. The supra-pyramidal blade is between the CA3 and the CA1 areas while the opposite blade is called infra-pyramidal blade; the connection between the blades is named the crest. The dentate gyrus has three different layers: the molecular layer, which is the cell-free layer; the principle cell layer that is filled with densely packed cells and is four to eight granule cells thick. The third layer is the polymorphic cell layer. At the principle layer, the main cellular constituent is the granule cell. Granule cells are closely apposed to each other and their dendritic tree is characterized by cone-shaped spiny

dendrites directed to the superficial molecular layer. The supra-pyramidal blade cells tend to be larger than those in the infra-pyramidal blade. There are approximately 1.2 million granule cells in the rat dentate gyrus, and although cell proliferation and neurogenesis is carried on even into adulthood, the number of cells is not altered. The septal granule cells are packed more densely then the temporal ones. The granule cell is the only type of cell in the dentate gyrus which has an axon that leaves the dentate gyrus and penetrates another field of the hippocampus, the area CA3. Another cell type, the mossy cell, projects from the dentate gyrus in one hemisphere to that in the other side. Other cell types in the dentate gyrus are interneurons. One well studied type of interneuron is the pyramidal basket cells, located in the deep granular cell layer. Their axons surround the cell bodies of the granule cells and form synapses with them. The molecular layer constitutes primarily of the dendrites of the granule, basket and polymorphic cells as well as of axons and terminal axonal arbors from the entorhinal cortex and other sources.

The dentate gyrus receives input mainly through the perforant pathway which projects from the entorhinal cortex. The mossy fibers are the only projections of the dentate gyrus to any brain area. These projections arise from the granule cells and end at the CA3 area. The mossy fibers end as complex en passant pre-synaptic terminals, which are called mossy fiber expansions that are unique for their large size (giving them a mossy look, hence the name). Another unique feature of the mossy fibers is that although each expansion is typically in contact only with one pyramidal cell, it makes many contacts with the dendritic tree of that cell. Each of the mossy fibers projects to approximately about 15 CA3 pyramidal cells that are distributed through out the tranverse axis of CA3.

Hippocampus proper

The hippocampus proper is divided into two regions: a region that is in proximity to the dentate gyrus and characterized by large cells and a region that follows from it, constituting of small cells. The large-celled region is further divided into two regions namely, CA3 and CA2, while the small-celled region is named CA1. The CA3 region receives input from the mossy fibers originating at the dentate gyrus, while CA1 receives input from the schaffer collaterals originating at CA3. CA2 region has, as mentioned above, large pyramidal cells but does not receive input from the mossy fibers. The pyramidal cell layer of the CA1 is packed tighter than that in the CA2 and CA3.

Layering of the hippocampus

The cell-free layer is located between the alveus and the stratum pyramidalis the pyramidal layer and is called stratum oriens. It contains the dendrites of the pyramidal cells and some interneurons. CA3 and CA2 receive input mainly from the axon collaterals of of neurons located within the same areas. CA1, on the other hand, receives input mainly from CA3. CA3 and CA2 receive input also from the entorhinal cortex through collaterals of the perforant pathway fibres that project to the dentate gyrus. While the CA1, in a place dependent manner, also receives projections from the different layers of the entorhinal cortex, it can project back to the same entorhinal cortex layers. All areas of the CA2 and CA3 project to CA1 and the area which they project to is dependent on the location of the CA3/CA2 cells. CA1 projects in a topographic manner to the subiculum, and to the deep layers of the entorhinal cortex. Projections from the CA1 to the SUB exhibit a transverse organization: projections from the proximal one third of the CA1 selectively terminate in the most distal third of the SUB. Conversely, projections that originate in the distal third of the CA1 selectively innervate the proximal third of the SUB (Amaral et al. 1991; Tamamaki and Nojyo 1990).

Subicular complex

The subiculum, parasubiculum and presubiculum are often called the "subicular complex". Each of them is considered as a distinct cortical area mainly because of their different neuroanatomical characteristics (see below). The principal cell layer of the subiculum is comprised of large pyramidal cells. This layer starts at the end of the distal region of the CA1. The cells extend their apical dendrites into the molecular layer and their basal dendrites into the deep pyramidal layer. The pyramidal cell population is divided into two distinct groups according to the cells' electrical properties. The first group has a characteristic regular firing pattern characterized by a single action potential or trains of single spikes upon stimulation or prolonged depolarization. The second group has a characteristic burst firing pattern upon stimulation or depolarization. These two groups are not morphologically distinct, but exhibit a differential distribution at the principal layer. According to some studies (Greene and Totterdell, 1997; Staff et al., 2000; Harris et al., 2001; Menendez et al., 2003) The regular spiking cells are more abundant at the superficial region of the principal layer, while the burst-firing cells are more common at the deeper layer, although this were questioned by Jarsky et al. (Jarsky et al., 2008). In this work, they found that the well-defined morphological boundaries between the hippocampal subregions CA1 and subiculum do not correspond to abrupt changes in electrophysiological properties. Rather, they observed that the percentage of bursting neurons is linearly correlated with position in the proximal-distal axis across the CA1 and the subiculum, the percentages of bursting neurons being 10% near the CA1-CA2 border, 24% at the CA1-subiculum border, and higher than 50% in the distal subiculum, Furthermore, Some bursting cells were antidromically activated by stimuli applied to the superficial or deep layers of presubiculum, but never by stimuli applied to deep layers of medial entorhinal cortex (dMEC). Some non-bursting subicular neurons were antidromically activated by stimuli applied to dMEC, but never by stimuli applied to presubiculum, indicating that subiculum burst- and regular-spiking cells project either to the presubiculum or to the EC, respectively (Stewart 1997). Deep cells (mostly intrinsically bursting [IB] class) had one or more ascending axon collaterals that typically remained within the region circumscribed by their apical dendrites. Superficial cells (mostly regular spiking [RS] class) tended to have axon collaterals that reached longer distances in the cell layer

The subiculum is one of the two main output structures of the hippocampus (Swanson and Cowan, 1975; Swanson et al., 1981). It projects on to the parasubiculum, the presubiculum and the entorhinal cortex. The connection to the presubiculum may be viewed in terms of the function of the subiculum as a last step of information processing through the hippocampal formation. The perforant path fibers are directed from the entorhinal cortex to the molecular layer of the subiculum and in a reciprocal manner the subiculum projects back to the entorhinal cortex. Some other cortical and subcortical areas the subiculum projects to are the perirhinal, retrosplenial, prefrontal, and the cingular cortex, mammillary bodies, hypothalamus, amygdala, septal complex, nucleus accumbens and thalamus (Amaral and Witter, 1995; O'Mara et al., 2001)

The entorhinal cortex is the main entry point for the sensory information processed by the hippocampal formation and more than that, functions as the main relay station for hippocampus processed information back to the neocortex. The hippocampus has a large unidirectional projection system that connects the different regions, which implies a serial flow of information first from the entorhinal cortex to the dentate gyrus through the CA3 and so on. This usually perceived notion has to be considered carefully under the known parallel connectivity of the hippocampus projections.

Subiculum and memory

It has been suggested that the hippocampus serves as storage for intermediate-term memory that finally may be stored as a long-term memory in the cortex. It has been shown that the projections from the hippocampus to the cortex are operating as rapidly as the modifiability within the hippocampus itself (Deadwyler and Hampson 2004). The immediate question that comes to mind is: what are the functions of the subiculum as a consequence of it being an interface between the hippocampus proper and cortical and subcortical structures? According to O’Mara (O’Mara, 2006), it is reasonable to suggest that the subiculum reverses the inhibitory functions of the dentate gyrus. The granule cells of the dentate gyrus fire infrequently and at low rates (Jung and McNaughton, 1993) thus serving as a filter for the hippocampus. The subiculum, on the other hand, may act as an output amplifier for those signals on their way to the cortical and subcortical

areas. This is due to the ability of the subicular burst firing cells to fire bursts of action potential in response to orthodromic stimulation. Further more, it has been suggested that the subiculum may have a functional segregation of its dorsal and ventral parts (O'Mara 2006). The dorsal part has been shown to have a role in processing space, movement and memory (Anderson, 2004; Deadwyler and Hampson 2004). On the other hand, the ventral part was shown to serve as an interface between the hippocampal formation and the hypothalamic-adrenal-pituitary (HPA) axis and mainly as an inhibtor of the HPA axis (Lowry, 2002; Mueller, 2004). The immediate implication of this hypothesis is that a number of CA1 place cells may converge upon a subicular pyramidal cell in addition to the input regarding self motion of the animal combining movement and place information at one subicular cell. The hypothesis suggests the dorsal subiculum to be a site of integration of hippocampal spatial information and body movement information. For the role of the subiculum in the HPA axis, O'Mara (O'Mara, 2006) underpins the ventral subiculum as an important component in regulation of the response to stress. This prediction is supported by experiments in ventral subiculum lesions (Maren, 1999). Commins et al. (Commins et al., 2001) investigated the effects of systemic stress induced by lipopolysaccharide (LPS; a potent endotoxin which induces HPA axis changes similar to those induced by behavioural stress) on synaptic transmission/synaptic plasticity in the CA1-subicular pathway. Similar to behavioural stress, LPS blocked LTP induction and reduced paired-pulse facilitation in the CA1-subicular pathway. Importantly, LPS did not affect baseline synaptic transmission in this pathway but did, however, reduce the magnitude of PPF; thus, the effects of LPS on synaptic transmission in this pathway depends on the frequency and length of stimulation. LPS inhibits hippocampal-dependent spatial learning in the watermaze. Thus, systemic stress induced by an LPS-induced primary immune response has similar consequences to behavioural stress on synaptic plasticity and learning in the CA1-subicular axis.

The two stage model of memory formation

The two stage model of hippocampus-dependent memory formation suggests that during explorative behaviour theta range oscillations (4–12 Hz) with superimposed, smaller amplitude gamma (γ) activity (30–100 Hz)(Bragin et al., 1995) constitute the spatial and temporal correlates

of memory trace formation within the hippocampus proper (Buzsaki, 1989; Buzsaki et al., 1983). Subsequently, during memory consolidation, previously stored information is transferred from the hippocampus to the cortical mantle during sharp wave-ripple complexes (SPW-Rs) characterized by a 60 ms slow wave field potential transient with superimposed ripple oscillations at 130 – 200 Hz (Buzsaki, 1986; Buzsaki, 1998). Memory consolidation is thought to be based on the replay of previously stored information within condensed time windows during SPW-Rs. These occur during slow wave sleep (SWS) and quiet wakefulness (Buzsaki, 1986) but also during periods of goal-related decision making (Diba and Buzsaki, 2007). On the other hand, working memory related theta-gamma network oscillations are thought to provide a temporal template, which facilitates coincidence detection, binding and thereby storage of information (Lisman and Idiart, 1995; Buzsaki, 1996; Bartos et al., 2007a). In hippocampal slices, γ-oscillations can be induced either pharmacologically (Buhl et al., 1998; Fisahn et al., 1998; Poschel et al., 2002) or by tetanic stimulation of area CA1 (Traub et al., 1996) (for review see (Bartos et al., 2007)). Gamma-frequency oscillations (30-100 Hz) result from synchronous neuronal network activity occurring in a variety of brain structures (Singer and Gray, 1995), most prominently in the hippocampus (Bragin et al., 1995), a region that plays a central role in declarative memory formation (Zola-Morgan and Squire, 1993; Morris et al., 1982). Hippocampal γ-oscillations have been proposed as a mechanism for coincidence detection and thus to promote the storage and retrieval of information (Bartos et al., 2007; Ritz and Sejnowski, 1997; Gray, 1994; Singer, 1993). Theta-gamma oscillation have been shown to exist in the subiculum (D'Antuono et al., 2001; Colling et al., 1998). Together with the place cells of the subiculum and their relation to environmental cues, the subiculum may play a role in working memory and memory consolidation as well as in coincidence detection and in storage and retrieval of information. Working memory is not a conclusive concept and is interpreted differentially according to the discipline examining it. For the cognitive neuroscientist, working memory is related to encoding and the formation of a new memory while for the experimental/ behavioural neuroscientist, it is related to the retrieval and use of memory (Henry L.Roediger, 2007) Complex thoughts require the manipulation of information, The manipulation of information, in turn, requires temporary storage. This temporary storage is thought to be provided by the framework of working memory. The overall assumption is that this system is limited in its capacity but flexible in its operation (Henry L.Roediger, 2007). The Hitch and Baddeley (Hitch and Baddeley,

1976) model studied the verbal suburst firingystem. This model proposed that a temporary storage system evolved from mechanisms of speech perception and production. The system is assumed to be crucial in the acquisition of language and for the temporary storage of language information and can be employed for the guidance of behavior (Baddeley, 2001). The visual equivalent of this system is able to combine visual information with information on spatial location and probably also motor information as evidenced by Smith and Jonides (Smith and Jonides, 1997). Working memory is supposedly controlled by habits and schemata that relay on environmental cues while performing routine and well learned actions. On the other hand, upon a novel behavior requirement, the supervisory attentional system (SAS) is assumed to operate; a different component that serves as a temporary storage system for the complex thoughts, that is accessible to conscious awareness (Baddeley and Jarrold, 2007).

The concept of consolidation means a progressive post encoding stabilization of the memory trace. During the consolidation phase memory is profoundly affected by amnesic agents. The role of consolidation is therefore in dispute with the question: is it really a stabilization of information or rather that the information is stored immediately and 'consolidation' refers to the accessibility of the information. Another question that remains open is whether the consolidation happens once, or is it activated and reconsolidated each time? Over the last 40 years, the idea of molecular memory consolidation has grown into a dogma in which when we learn or encode some experience, the synapses in the brain involved in learning or the encoding procedure are strengthened. This dogma brings us once again to the relevance of synaptic plasticity and the subiculum.

Synaptic plasticity at the CA1–subiculum synapse

Synaptic plasticity at the CA1 projection to the subiculum was first examined in an in vivo model (Commins et al., 1998b; Commins et al., 1998a; Commins et al., 1999). High frequency stimulation at the CA1-subiculum projection results in LTP. Plasticity in subiculum was reported through the use of standard induction protocols. A rapid potentiation, measured by field excitatory post synaptic potentials, was induced at the CA1-subiculum projections using high frequency stimulation

(HFS). 5 minutes post-HFS, LTP remained stable for 30 minutes which was resistant to further HFS (O'Mara et al., 2000). In a different study, fundamental differences between the two different pyramidal cells (bursting and regular firing) that constitute the subiculum region were found. Prolonged high-frequency stimulation induced NMDA receptor-dependent LTP in both cell types. While LTP relied on postsynaptic calcium in regular firing neurons, no increase in postsynaptic calcium was required in bursting cells. Furthermore, paired-pulse facilitation revealed that the site of LTP expression was postsynaptic in regular firing neurons, while presynaptic in burst firing neurons. Moreover, PPF revealed that the site of LTP expression was postsynaptic in the regular firing cells while presynaptic in the burst firing cells. Kokaia demonstrated that long-term potentiation (LTP) at CA1-subicular cell synapses can be readily induced by high-frequency stimulation (HFS) of the afferents, but not by pairing of low-frequency stimulation with depolarization of postsynaptic cells. This tetanus-induced LTP is input specific, insensitive to the N-methyl-D-aspartate (NMDA) receptor antagonist 3-[(R)-2Carboxipiperazin-4-yl]-propyl-1-phosphonic acid (R-CPP), and reduces PPF in potentiated synapses. Their data indicate that CA1-subicular cell synapses in mice exhibit LTP, which can be expressed presynaptically, and its induction does not require NMDA-receptor activation (Kokaia, 2000).

Both in vivo (Commins et al. 1998a) and in vitro studies (Kokaia 2000) found evidence for a presynaptic expression of LTP at CA1-SUB synapses that seems to be confined to burst-spiking cells (Wozny et al. 2008a; Wozny et al. 2008b). In various brain regions, it has been shown that synaptic plasticity may indeed rely on the activation of presynaptic NMDA-R (Casado et al. 2002; Duguid and Smart 2004; Humeau et al. 2003; Sjoestroem et al. 2003). It is feasible that the subunit composition of presynaptic NMDA-R differs from that of receptors expressed at the postsynaptic site. Indeed, in various cortical areas, presynaptic NMDA-R have been reported to contain NR2B subunits (Brasier and Feldman 2008; Sjoestroem et al. 2003; Woodhall et al. 2001). This might explain why LTP at CA1-SUB synapses has been found to be blocked by APV (Boeijinga and Boddeke 1996; Roberts and Greene 2003; Wozny et al. 2008b) but not by CPP (Kokaia 2000). APV and CPP are NMDA-R antagonists known to show differences in their NMDA-R subunit selectivity. For NMDA-R expressed in Xenopus oocytes, CPP has a nearly 7-fold higher affinity for NR2A than NR2B, whereas APV has nearly the same affinity for these two subunits (Feng et al. 2005). Indeed, Wozny’s et al. findings (Wozny et al. 2008b), indicate that high concentrations of

D-APV (100 μM) are required to block LTP in burst-spiking cells (Wozny et al. 2008b).

Li et al. (Li et al., 2005) demonstrated that the pairing protocols of low frequency stimulation (LFS) at 3 Hz and postsynaptic depolarization of -50 mV elicited a reliable LTD in the subiculum. This LTD induction protocol did not result in any significant changes of the paired-pulse ratio of the EPSC. Furthermore, it did not depend on either NMDA receptors or voltage-gated calcium channels (VGCCs). Bath application of the G-protein coupled muscarinic acetylcholine receptors (mAChRs) antagonists, atropine or scopolamine, blocked the LTD, suggesting that mAChRs are involved in the LTD. It was also completely blocked by either the Ca^{2+} chelator BAPTA or the G-protein inhibitor GDP-ß-S in the intracellular solution

Previous work of the group

My group's past findings show that the subiculum receives strong glutamatergic input from CA1 (Behr et al., 1998) and is, thus, part of the polysynaptic hippocampal loop. The entorhinal cortex also projects via monosynaptic excitatory perforant path projection from layer III to the subiculum (Behr et al., 1998) which forms a bypass for the trisynaptic hippocampal loop. Further more, my group has shown that synaptic terminals that arise from CA1 pyramidal cells do not function as a single compartment, but rather show specialized synaptic plasticity onto subicular pyramidal cells dependent on the type of electrical characteristic of the cell targeted upon (Wozny et al., 2008). Tetanic stimulation of CA1 axons targeting burst firing cells showed much more pronounced LTP than in the regular firing cells. In regular firing cells the induction of LTP is reminiscent of LTP in area CA1. It is NMDA receptor dependent and relies on the postsynaptic depolarization and subsequent postsynaptic Ca^{2+} signalling. Though LTP in burst firing neurons is likewise NMDA receptor dependent, postsynaptic depolarization and Ca^{2+} signalling are not required for the induction of LTP. While LTP in regular firing cells shows no change in PPF, in bursting neurons LTP is associated with a decrease of PPF (Wozny et al., 2008). Furthermore, in a recent study by Wozny et al., elevation of cAMP either by activation of adenylyl cyclase or by a cAMP analog caused a long-lasting increase in EPSC amplitudes in bursting neurons and

occluded tetanus-induced LTP. Moreover, LTP in bursting cells was inhibited by the PKA inhibitors Rp-8-CPT-cAMP and H-89. In sharp contrast, LTP in regular firing cells could still be induced in the presence of forskolin. Additionally, the forskolin-induced enhancement of EPSC amplitudes in bursting neurons caused a change in PPF. The degree of EPSC blockade by the low-affinity competitive AMPA receptor-antagonist -DGG suggests an increase of glutamate concentration in the synaptic cleft in burst firing cells after the application of forskolin.(Wozny et al., 2008). Overall, these data show a target-cell specific expression of presynaptic and postsynaptic mechanism of LTP in the subiculum which as noted above is a major hippocampal output region.

Goals

The goal of this thesis is to investigate the cell specific and input specific LTD and LTP in subicular regular-spiking and burst-spiking cells using low-frequency stimulation protocols in hippocampal slices.

Hypothesis

1. We will demonstrate that subicular pyramidal cells show a cell specific induction, transduction and expression mechanisms of LTP and LTD following LFS.
2. We will determine pre- and postsynaptic induction mechanisms of LFS-induced LTD and LTP, and will characterize the underlining molecular mechanisms.
3. We will provide evidence that subicular pyramidal cells show a cell specific threshold for LTD and LTP. We will study the cell specific bi-directional plasticity of subicular pyramidal cells in each cell type.

Slice preparation

Animal procedures were conducted in accordance with the guidelines of the European Community Council and approved by the Regional Berlin Animal Ethics Committee (G0328/98,G0024/04). Wistar rats (4-6 weeks) of both sexes were decapitated under deep ether anaesthesia and their brains were quickly removed. Horizontal slices, 400 μm thick, containing the hippocampus and the entorhinal cortex were prepared with a vibratome (Campden Instruments, Loughborough, UK). For sharp microelectrode recordings, the preparation was performed in ice-cold, oxygenated (95% O2, 5% CO2) artificial cerebrospinal fluid (ACSF) and the slices were transferred for storage to an interface recording chamber continuously perfused (1.5-2 ml/min) with oxygenated and pre-warmed (34° C) ACSF. The composition of ACSF was as follows (in mM): NaCl 129, Na2PO4 1.25, NaHCO3 26, KCl 3, CaCl2 1.6, MgSO4 1.8, glucose 10 at a pH of 7.4. For patch-clamp recordings, slices were prepared in ice-cold, saccharose-based ACSF (in mM): NaCl 87, Na2PO4 1.25, NaHCO3 26, KCl 2.5, CaCl2 0.5, MgCl2 7, saccharose 75, glucose 25 at a pH of 7.4. After preparation, slices were kept under submerged conditions at 35° C for ~ 30 min and then transferred to a physiological ACSF solution at room temperature for further storage.

Electrophysiology

Single cell recordings in the pyramidal cell layer of the subiculum were performed at near physiological temperatures (32°-34° C) with sharp microelectrodes (40-100 Mμ) filled with 2.5 M potassium acetate or with patch-clamp electrodes (4-6 Mμ). Patch-clamp electrodes were filled with (in mM): K-gluconate 135, KCl 20, HEPES 10, phosphocreatine 7, Mg-ATP 2, Na-GTP 0.3, EGTA 0.2 and adjusted with KOH to a pH of 7.2. Access resistance did not exceed 20 Mμ and varied less than 20% in the course of the experiment. No series resistance compensation was used.

All experiments were performed in the presence of bicuculline (5μM) or SR-95531 (gabazine, 1μM) to block GABAA receptor-mediated responses. In order to prevent polysynaptic responses, concentrations of MgSO4 and CaCl2 were elevated to 4 mM each.

For characterization of intrinsic discharge and membrane properties, hyper- and depolarizing current steps (200 ms, 0.1 nA) were used. Action potential amplitude was defined as the peak amplitude relative to the peak of the fast after-hyperpolarization. Action potential width was measured at half-maximal amplitude. Sag ratio was calculated by dividing the steady state voltage during a hyperpolarizing current pulse of 300 pA by the peak voltage during hyperpolarization. Overshoot ratio was calculated by dividing the steady state voltage during a hyperpolarizing current pulse of 300 pA by the peak overshoot voltage after the end of the hyperpolarizing current pulse.

Excitatory postsynaptic potentials/currents (EPSPs/EPSCs) were evoked by alveus stimulation. The stimulus intensity was between 1.5-4 V after adjusting the EPSP/EPSC amplitudes to 40-60% of the maximum response. In a subset of experiments, an incision of the alveus was made between the subiculum and the stimulation electrode in order to exclude antidromic activation of subicular pyramidal cells. Baseline responses were recorded at 0.033 Hz for at least 10 min. For induction of synaptic plasticity, paired-pulse low-frequency stimulation (15 minutes, 50 ms inter-stimulus interval) at 0.5, 1, 3 and 5 Hz in voltage-clamp, current-clamp or bridge mode was used (for details, see Results section). Changes in synaptic strength were measured for a period of 30 minutes after induction.

Signals were low-pass filtered at 3 kHz, sampled at 10 kHz by an ITC-16 interface (Instrutech Corp., Great Neck, NY, USA) and processed by TIDA software (HEKA GmbH, Lambrecht, Germany). Normalized EPSPs/EPSCs were averaged for the last five minutes of baseline recordings. LTP and LTD were calculated as percentage values of the normalized baseline EPSP/EPSC amplitude between 20 to 25 min after induction. Statistical analysis was performed by applying Student's t-test or by analysis of variance (ANOVA) for comparison of means between groups (SPSS, SPSS Inc., USA). Statistical significance was set to $P < 0.05$.

Drugs

The following drugs were used: nifedipine, 20μM; scopolamine, 30 mM; D-2-amino-5-phosphonovaleric acid (D-APV), 100μM; 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA), 30 mM; (RS)-a-methyl-4-carboxyphenylglycine (MCPG), 500μM, bicuculline, 5μM; SR 95531 hydrobromide (gabazine), 1μM. Drugs were purchased from Sigma-Aldrich, Germany and Tocris, UK. All drugs were applied throughout the entire course of the experiments and at least 10 min prior to recording. Application of D-APV and/or MCPG did not affect baseline transmission. Intracellular loading with the calcium chelator BAPTA did not alter the spiking behaviour of subicular pyramidal cells. As an indicator for effective BAPTA loading in burst-spiking cells, we observed a block of the slow after-hyperpolarization after the burst discharge (data not shown). Though some authors report BAPTA-mediated rundown of postsynaptic responses, in our study stable baseline responses could be obtained in most of the recordings with BAPTA. Cells that showed a rundown of responses (~15%) were not included.

Electrical characterization of subicular pyramidal cells

In order to characterize electrically the type (regular or bursting firing) of the recorded cells, hyper-and depolarizing current steps were applied under current clamp mode (fig 1). Each current step was of 200ms duration and a magnitude of 0.1nA. The main criterion for the classification of the cells as burst firing was the appearance of burst discharges, while the criteria for classifying a cell as regular firing was the absence of such bursts. Subicular burst firing cells (47.2% of 249 recorded cells) had typical bursts of 2-4 action potentials with a frequency of 180Hz followed by single spikes without frequency-adaptation. Regular spiking cells (46.11% of 249 cells), however, had characteristic single spike trains with an initial frequency of about 120Hz and displayed frequency adaptation (fig 2a,b; fig 3a,b). In addition, a subset of cells that were classified as high-threshold burst firing cells (4.45%) were not included in the study and had typical single spikes upon weak depolarization but showed initial burst discharge when the depolarization current was increased. Cells which had a high frequency firing pattern with a frequency >200Hz were classified as interneurons (2.22%) and were likewise not included in this study.

Burst firing cells showed lower input resistance than regular firing cells in accordance with previous studies (O'Mara et al., 2001;Menendez et al., 2003). Moreover, sag and over-shoot potentials were more pronounced in burst firing cells (See P values of t-test in table 1) in the regular firing cells (fig 1), consistent with a higher expression of Ih in burst firing cells (van Welie et al., 2006)

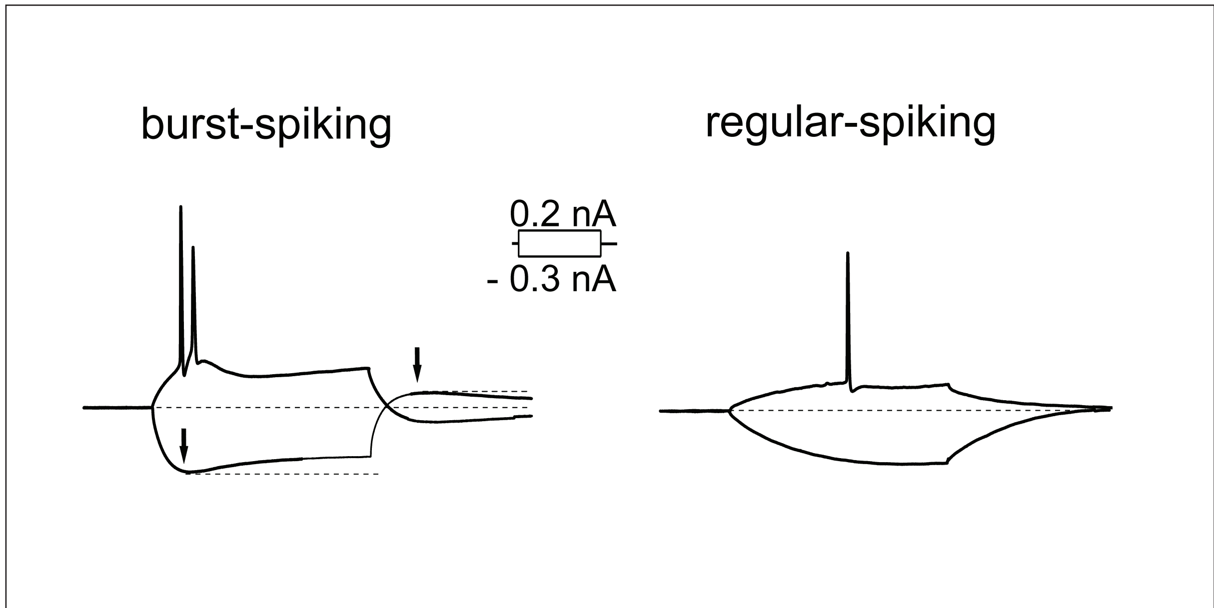


Figure 1 | Voltage responses to depolorizing and hyperpolarizing current steps of subicular burst-spiking and regular-spiking cells. Burst spiking cells display sag and overshoot potentials (indicated by arrows). Figure adapted from (Fidzinski et al., 2008)

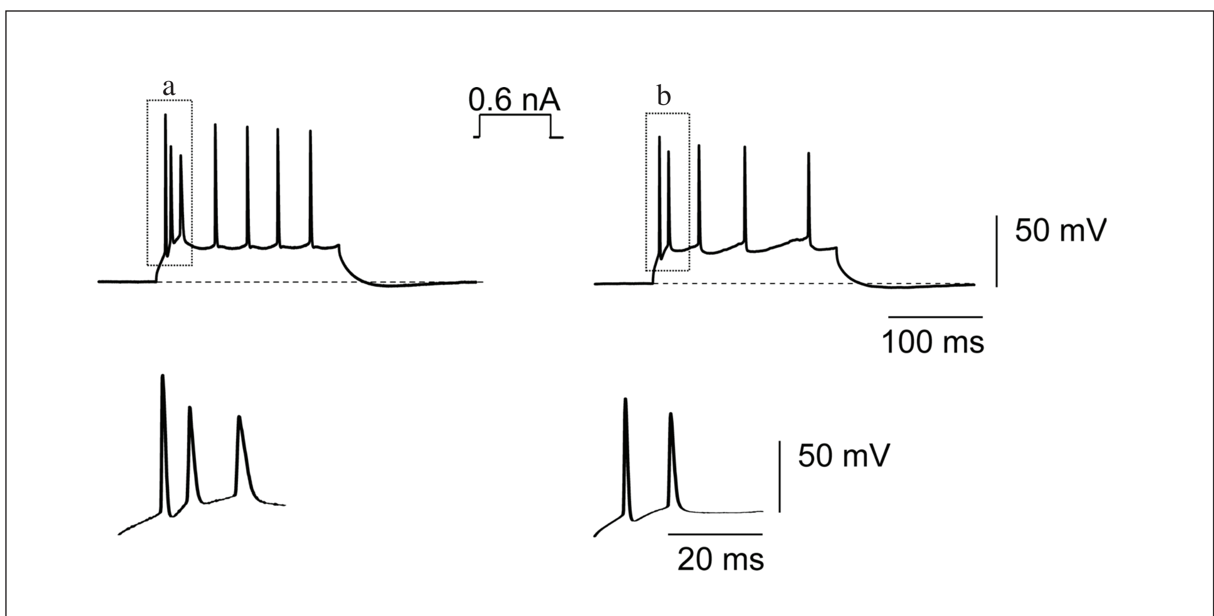


Figure 2 | Illustration of the frequency adaptation in regular and Burst spiking cells (a) and different initial spike frequencies in each cell type (b). Figure adapted from (Fidzinski et al., 2008)

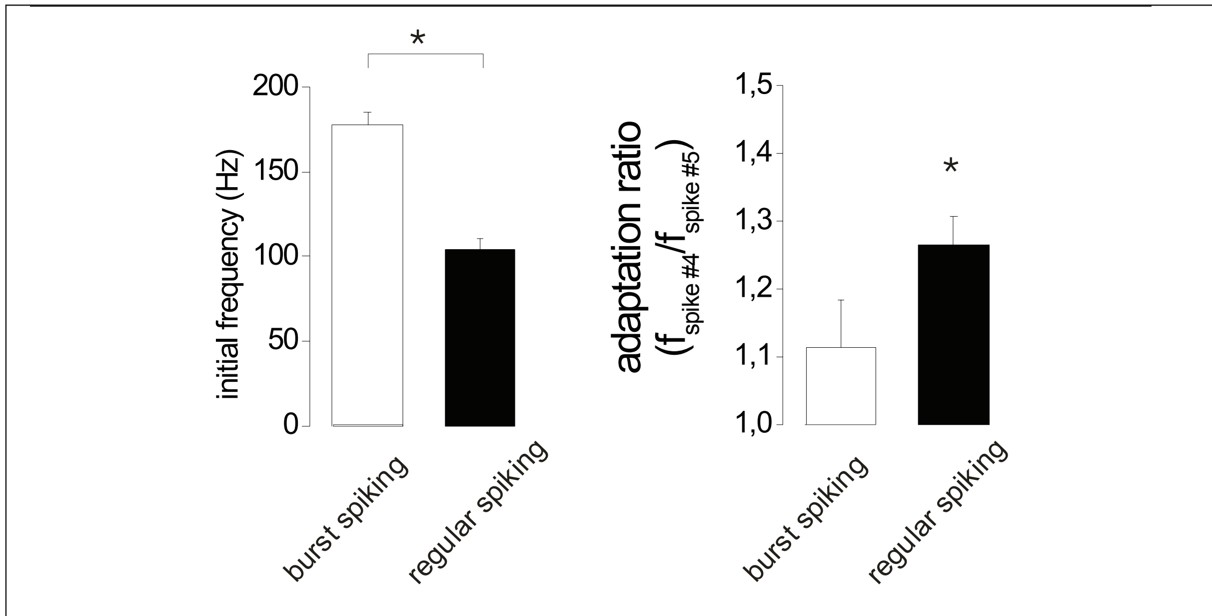


Figure 3 | Initial frequency (left plot) and adaptation ratio(right plot). Upon o.6 nA current step the time between 1st and 2nd spikes was evaluated and the reciprocal of that time was taken for the frequency (left plot) P<0.05. Frequency adaptation was calculated as the mean of the reciprocal of the time between the 3rd and the 4th spike divided by the 4th and the 5th spike P<0.05. Figure adapted from (Fidzinski et al., 2008).

	BS cells	RS cells	P-value
Number	55	49	
RMP (mV)	−58.6 ± 0.5	−57.9 ± 0.8	0.41
Rinput (MΩ)	84.0 ± 5.7	120.1 ± 14.3*	0.02
Sag ratio	0.81 ± 0.01	0.85 ± 0.01*	0.03
Overshoot ratio	6.0 ± 0.5	7.6 ± 0.4*	0.01
Spike height (mV)	83.4 ± 3.5	78.6 ± 2.9	0.31
Spike half-width (ms)	0.92 ± 0.06	0.97 ± 0.07	0.61

Data are given as means ± SEM. *P < 0.05, comparing BS and RS cells.

Table 1 | Membrane characteristics of subicular cells according to their electric properties *P-value refers to t-test comparison between the values of burst firing and regular firing neurons

Low-frequency stimulation results in LTD in burst firing cells and late-onset LTP in regular firing cells

CA1 efferents were stimulated at 4 different low frequencies (0.5, 1, 3 and 5Hz) in order to investigate frequency dependence of synaptic plasticity in both types of subicular pyramidal cells. Sharp microelectrode recording was used in order to leave the intracellular composition of the cells unchanged. 0.5Hz frequency stimulation resulted in LTD in the burst firing cells (85.7±8.2% of baseline, n=9, P<0.01) but failed to induce synaptic plasticity in the regular firing cells (108.9±11.5% of baseline n=7) (fig 4).

1Hz frequency stimulation induced LTD in the burst firing cells (67.0±7.2% of baseline n=12 P<0.001) while regular firing cells responded with late onset LTP (122.9±11.9% of baseline n=9 P<0.001) (fig 5).

3Hz frequency stimulation did not show any difference compared to 1Hz stimulation in both cell types, LTD in burst firing cells (82.3±12.5% of baseline n=9 P<0.01) and late onset LTP in the regular firing cells (129.1±18.8% of baseline, n=6 P<0.01) (fig 6).

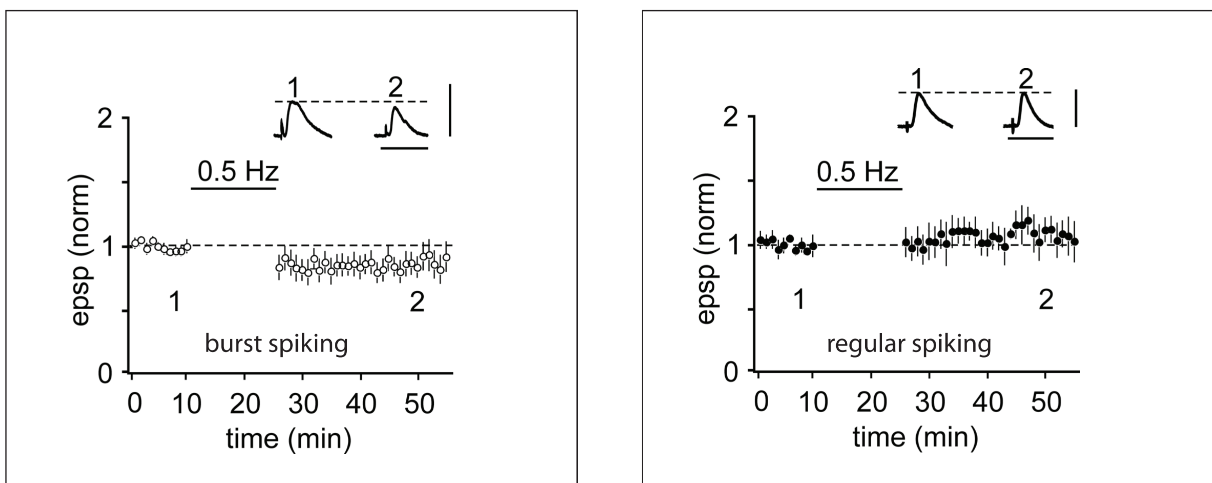


Figure 4 | 0.5Hz LFS induced LTD in burst spiking cells (left plot) and no plasticity at the regular spiking cells (right plot). The recorded EPSPs are before (1) and 25 min after (2) the stimulation . Scale bars 5 mV and 50 ms. Figure adapted from (Fidzinski et al., 2008)

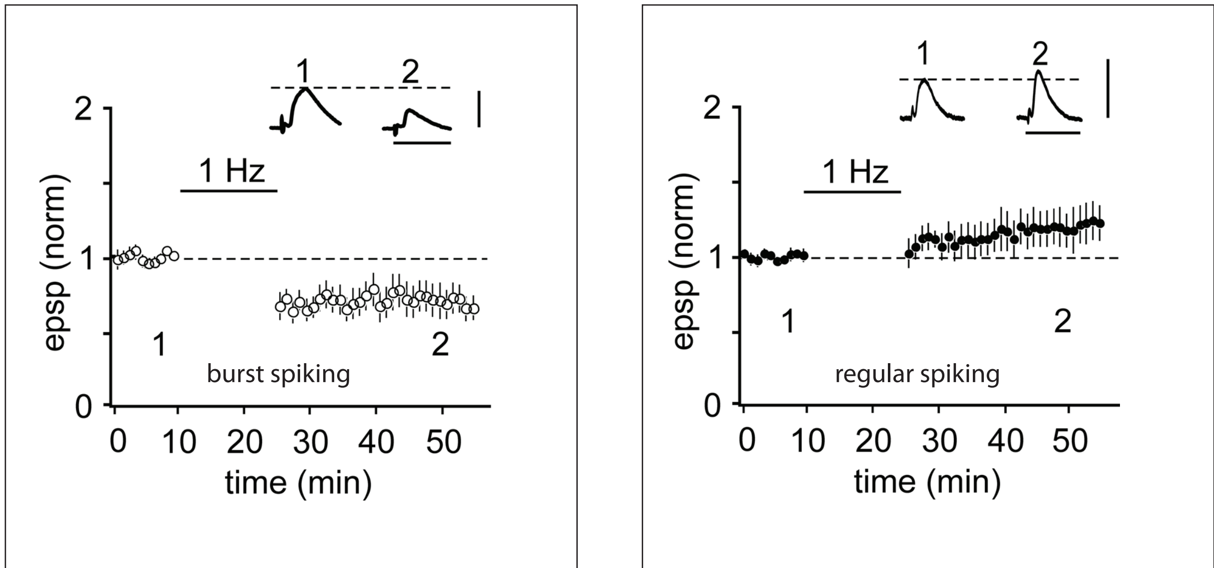


Figure 5 | 3Hz LFS induced plasticity at the burst spiking cells (left plot) and no plasticity (right plot). The recorded epsp´s are before and 25 min after the stimulation . Scale bars 5 mV and 50 ms. Figure adapted from (Fidzinski et al., 2008)

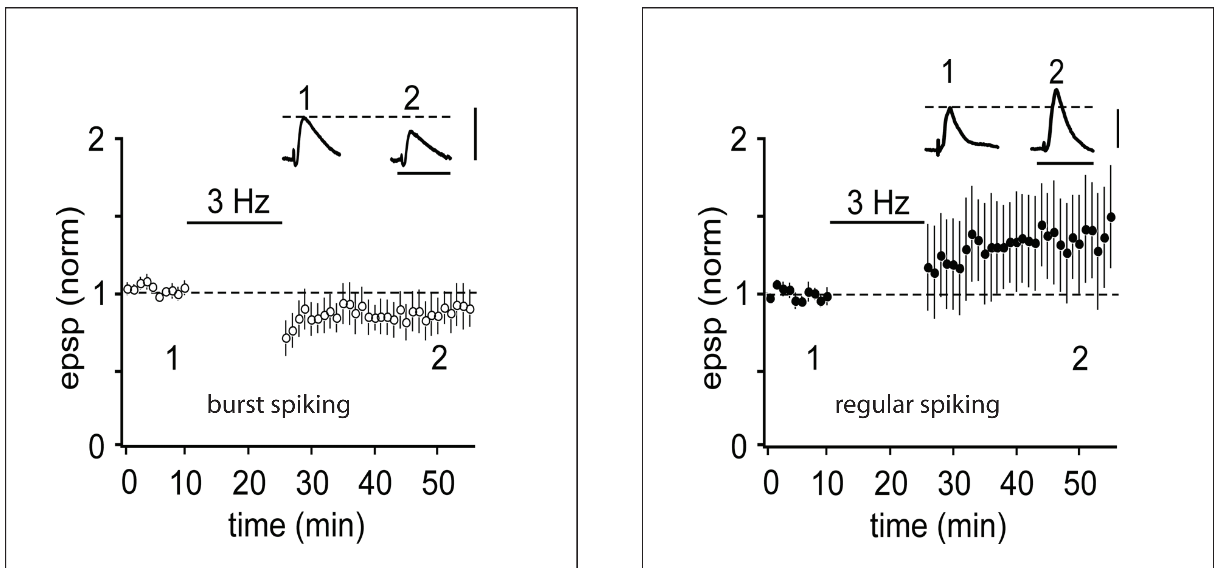


Figure 6 | 3Hz LFS induced plasticity at the burst spiking cells (left plot) and no plasticity (right plot). The recorded epsp´s are before and 25 min after the stimulation . Scale bars 5 mV and 50 ms. Figure adapted from (Fidzinski et al., 2008)

5Hz frequency stimulation did not cause any synaptic plasticity in the burst firing cells (115.9±14.4% of baseline n=7) while in the regular firing cells a robust LTP was expressed (135.5±18.3% of baseline n=6 P<0.01) (fig 7)

The overall frequency dependence in burst firing cells showed clear LTD which was inversely correlated with stimulation frequency (maximum of the depression at 1Hz and no plasticity at the frequency of 5Hz). The, regular cells showed potentaition which increased in magnitude as a function of frequency (fig 8).

Overall, summarized over all frequencies investigated , LTD was observed in61.1% of the investigated Burst firing cells, and LTP was observed in 22.2% of the Burst firing cells. The opposite ratio was found for Regular firing cells, with 62.9% showing LTP and 14.8% of the cells exhibiting LTD. This high variability of synaptic strength outcomes is illustrated in fig 9. Variance analysis showed that on average burst firing cells responded to LFS at 0.5–5 Hz with a significantly lower synaptic gain than regular firing cell(P<0.01). Moreover a linear correlation was found between synaptic strength and stimulation frequency in burst firing cells (r=0.377±0.342 n=36 P<0.05) as well as in regular firing cells (r=0.407±0.378 n=28 P<0.05) (fig 9).

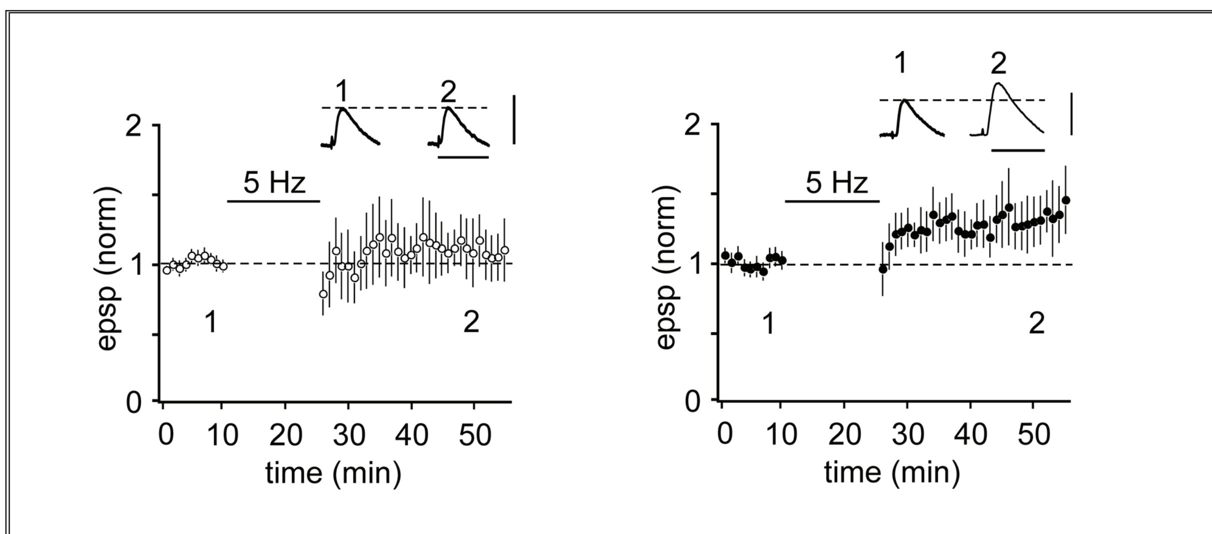


Figure 7 | 5Hz LFS induced plasticity at the burst spiking cells (left plot) and no plasticity (right plot). The recorded EPSPs are before and 25 min after the stimulation . Scale bars 5 mV and 50 ms. Figure adapted from (Fidzinski et al., 2008)

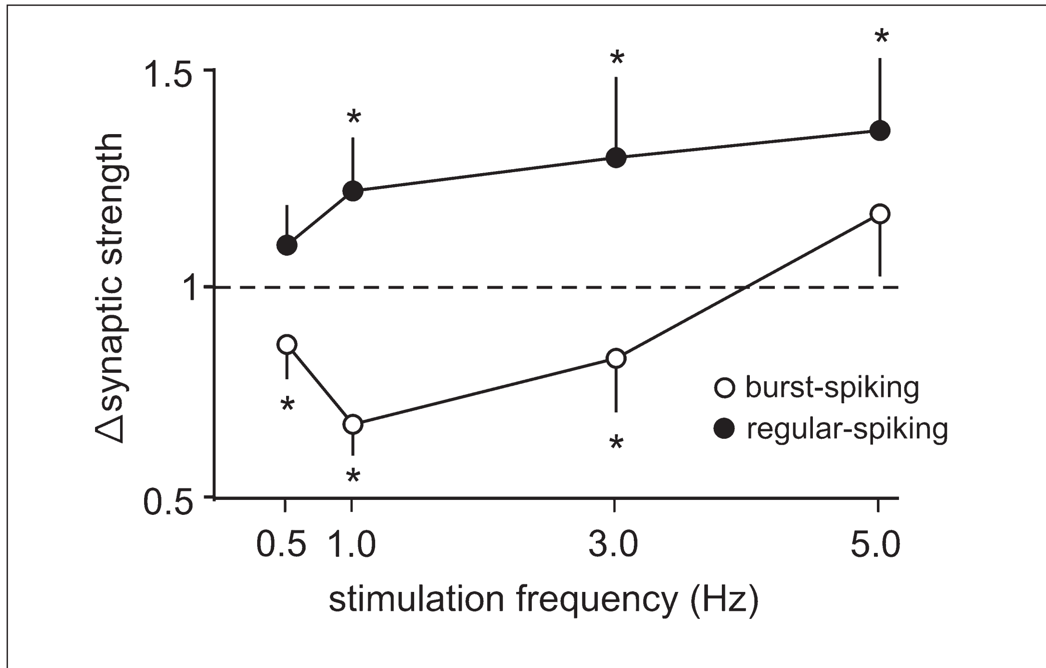


Figure 8 | Overview of stimulation frequency dependence of the plasticity at both cell types. Figure adapted from (Fidzinski et al., 2008)

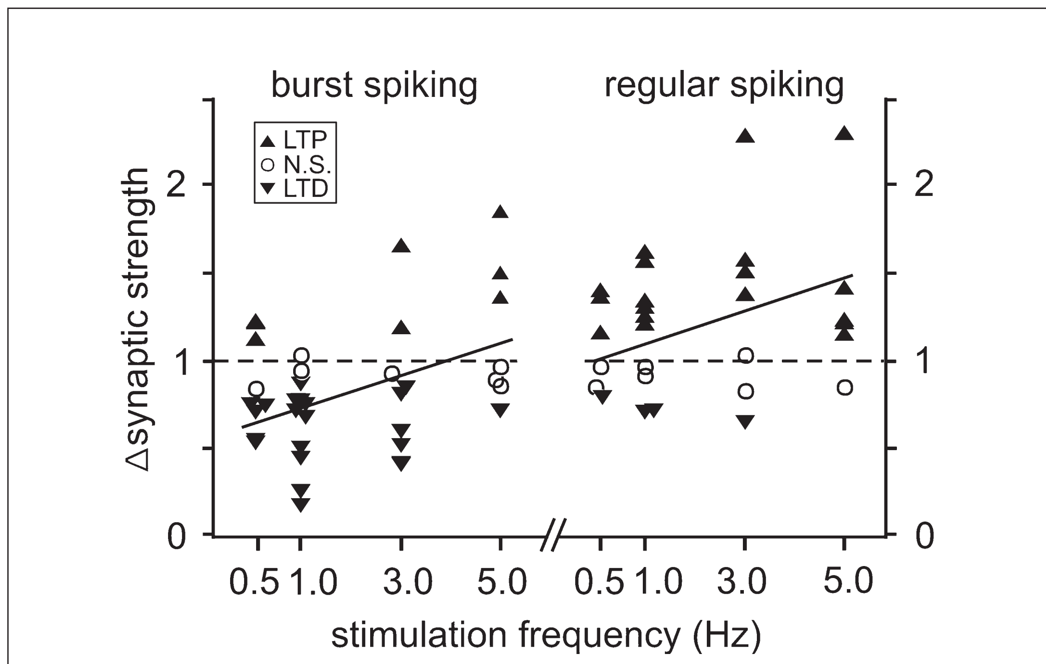


Figure 9 | Summary of synaptic plasticity induced at each cell. Symbols indicate LTD (upside down triangle), LTP (triangle) no plasticity (circle) (at $P < 0.05$). Linear regression line indicates positive correlation between stimulation frequency and change of synaptic strength. Figure adapted from (Fidzinski et al., 2008)

Plasticity does not depend on the membrane potential at the soma

In order to reveal the underlying mechanisms of the long term plasticity phenomena in our preparation, sharp-electrodes recordings are insufficient. Therefore, we conducted induction protocols in whole-cell patch recordings. To validate our LFS-protocol, we examined two different LFS induction protocols with 1Hz stimulation. In the first protocol, performed in voltage clamp mode at holding potential of -70 mV, we stimulated at 1 Hz for 15 min. The second protocol was performed in current clamp condition. 1Hz stimulation was performed for 15 minutes. Both induction protocols displayed similar results (P>0.1 in all comparisons, student's t-test): Burst firing cells displayed LTD (72.2±4.9% of baseline n=8 P<0.01 in voltage clamp mode, fig 10; and 71.3±5.6% of baseline n=10 P<0.001 in current clamp mode, fig 11), while regular firing cells showed LTP (133.3±12.4% of baseline n=14 P<0.05 in voltage clamp, fig 12 and 131.4±9.2% of baseline n=9 P<0.01) (fig 13).

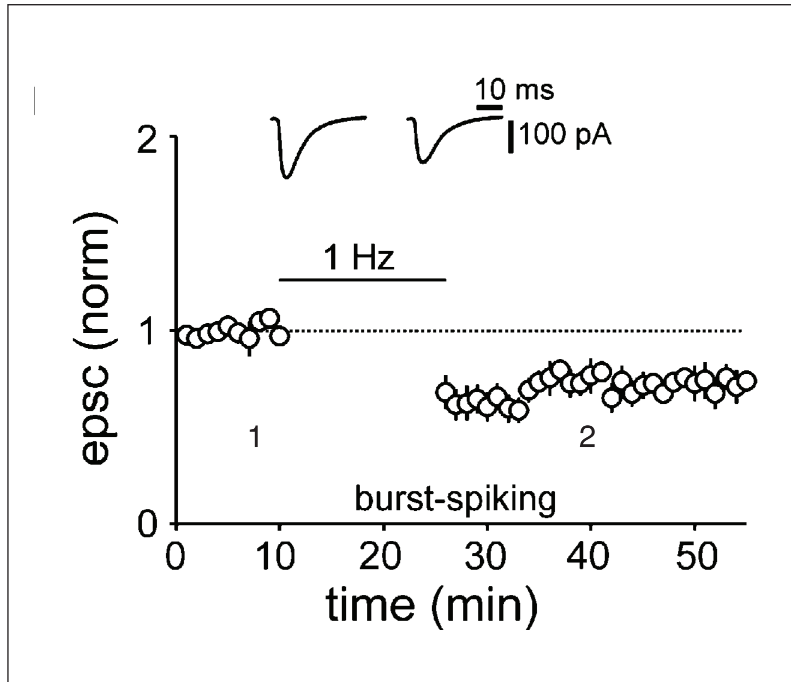


Figure 10 | LFS at 1Hz in voltage clamp mode at -70mV induces LTD in burst spiking cells. Averaged time courses of evoked EPSC amplitudes before and after induction with LFS at voltage clamp mode at -70mV are shown. Superimposed traces are averages from 10 single responses each and were recorded during baseline (left-1) and 25 min after induction (right-2). Figure adapted from (Shor et al., 2009)

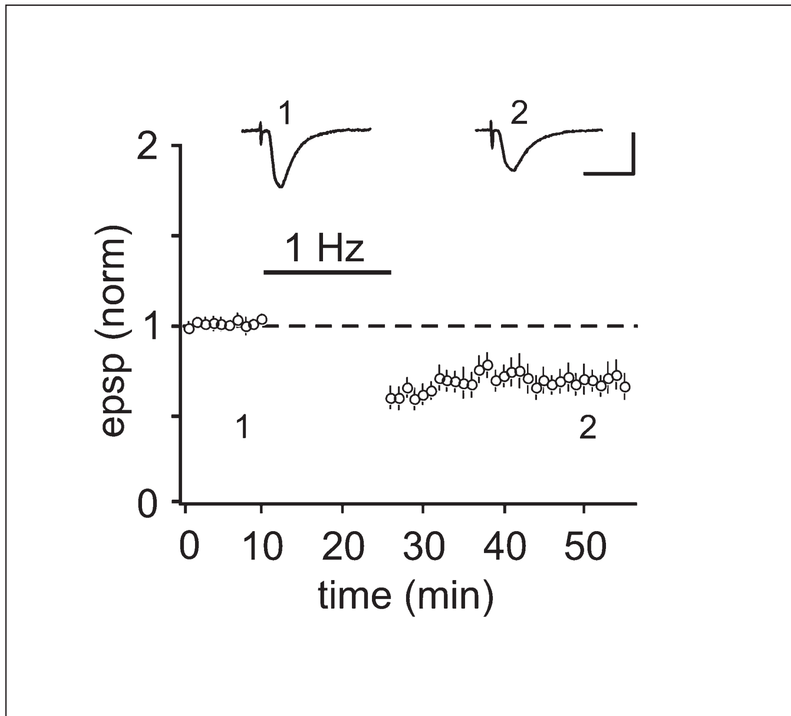


Figure 11 | LFS at 1Hz at bridge mode at the intrinsic membrane potential induces LTD in burst spiking cells. Averaged time courses of evoked EPSC amplitudes before and after induction with LFS at bridge mode are shown. Superimposed traces are averages from 10 single responses each and were recorded during baseline (left-1) and 25 min after induction (right-2). Scale bars 100pA 10ms. Figure adapted from (Fidzinski et al., 2008)

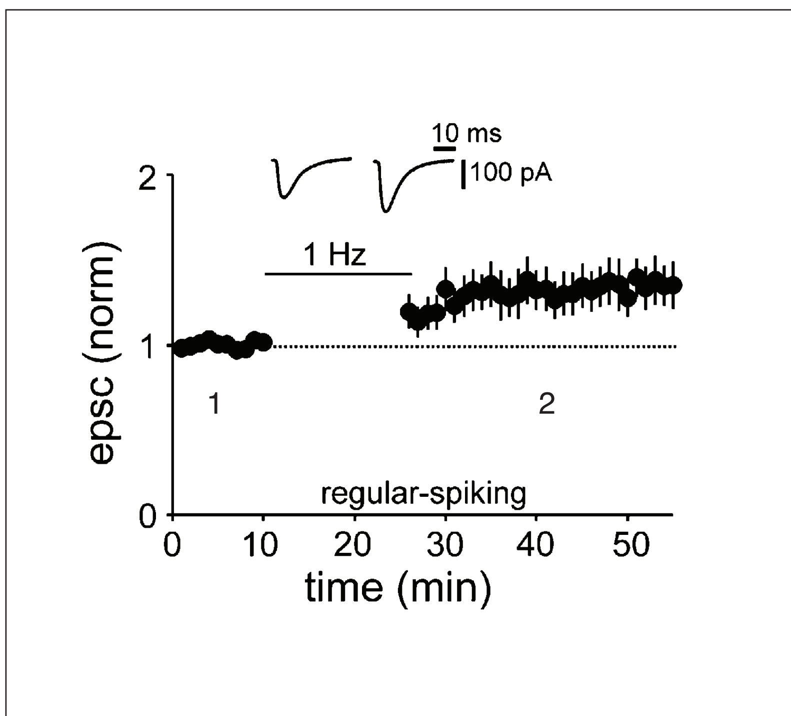


Figure 12 | LFS at 1Hz at voltage clamp mode at -70mV induces LTP in regular spiking cells. Averaged time courses of evoked EPSC amplitudes before and after induction with LFS at voltage clamp mode at -70mV are shown. Superimposed traces are averages from 10 single responses each and were recorded during baseline (left-1) and 25 min after induction (right-2). Figure adapted from (Shor et al., 2009)

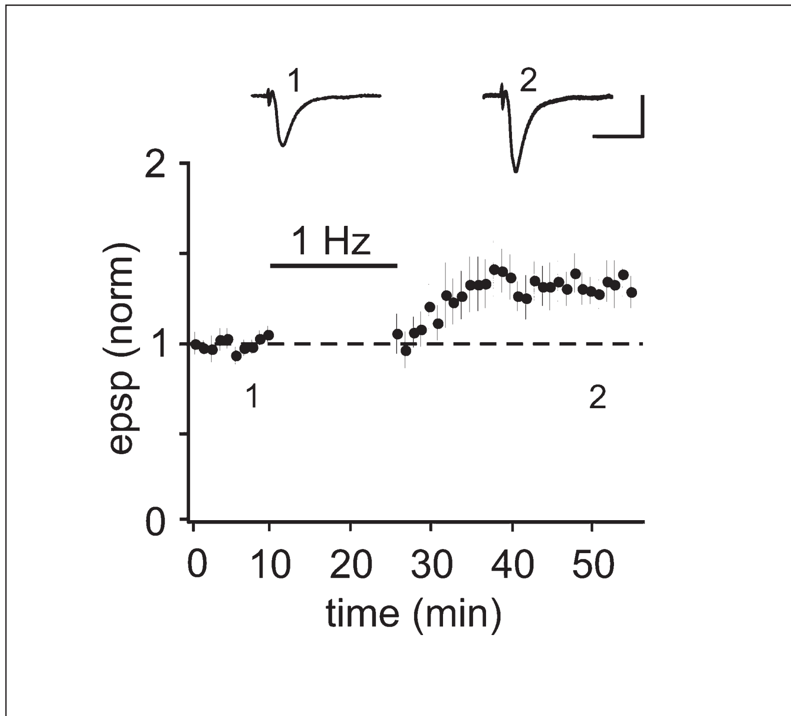


Figure 13 | LFS at 1Hz at bridge mode at the intrinsic membrane potential induces LTP in regular spiking cells. Averaged time courses of evoked EPSC amplitudes before and after induction with LFS at bridge mode are shown. Superimposed traces are averages from 10 single responses each and were recorded during baseline (left-1) and 25 min after induction (right-2). Scale bars 100pA 10ms. Figure adapted from (Fidzinski et al., 2008)

LFS-induced LTP and LTD are co-expressed in subicular burst firing and regular firing cells

We further studied the mechanism of synaptic plasticity induced by LFS-protocol in current clamp. In various brain regions, activation of postsynaptic NMDA receptors is important for the induction of synaptic plasticity. LTD in burst firing cells was blocked by the NMDA receptor antagonist D-APV and revealed a masked late onset LTP (123.2±9.2% of baseline n=11 P<0.05) (fig 14).

These results suggest an NMDA-receptor-dependent LTD that masks an NMDA-receptor-independent late-onset LTP.

This form of late-onset LTP was reported previously by O'Leary and O'Connor (O'Leary, D.M. & O'Connor, 1998) to depend on the activation of metabotropic glutamate receptors (mGluR). In accordance with that, co-application of D-APV and the type I/II mGluR antagonist MCPG, resulted in complete abolishment of synaptic plasticity following the same LFS induction protocol in burst firing cells (fig 15).

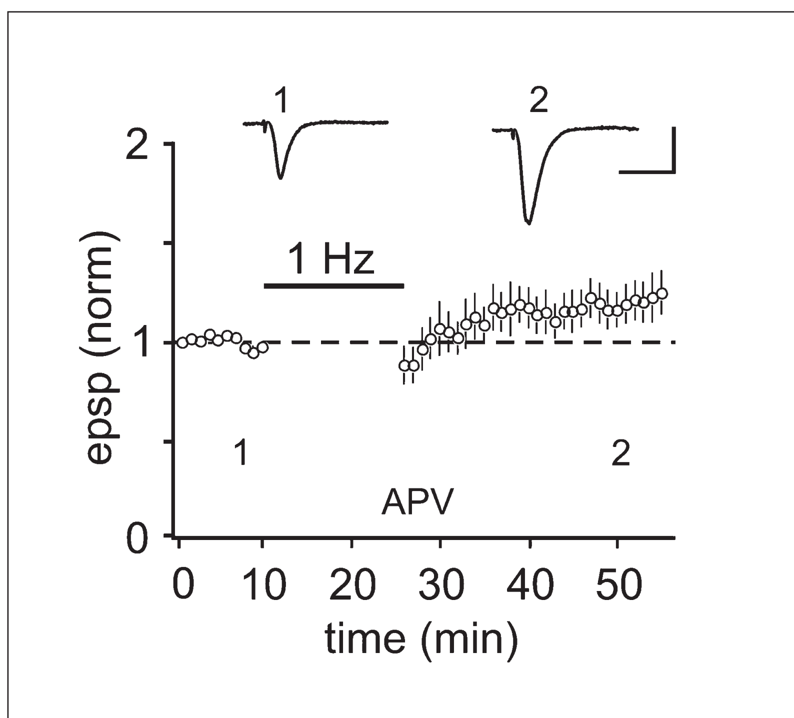


Figure 14 | LFS at 1Hz at bridge mode at the intrinsic membrane potential and the usage of NMDA-receptors antagonist D-APV unmask LTP in burst spiking cells. Averaged time courses of evoked EPSC amplitudes before and after induction with LFS at bridge mode are shown. Superimposed traces are averages from 10 single responses each and were recorded during baseline (left-1) and 25 min after induction (right-2). Scale bars 100pA 25ms. Figure adapted from (Fidzinski et al., 2008)

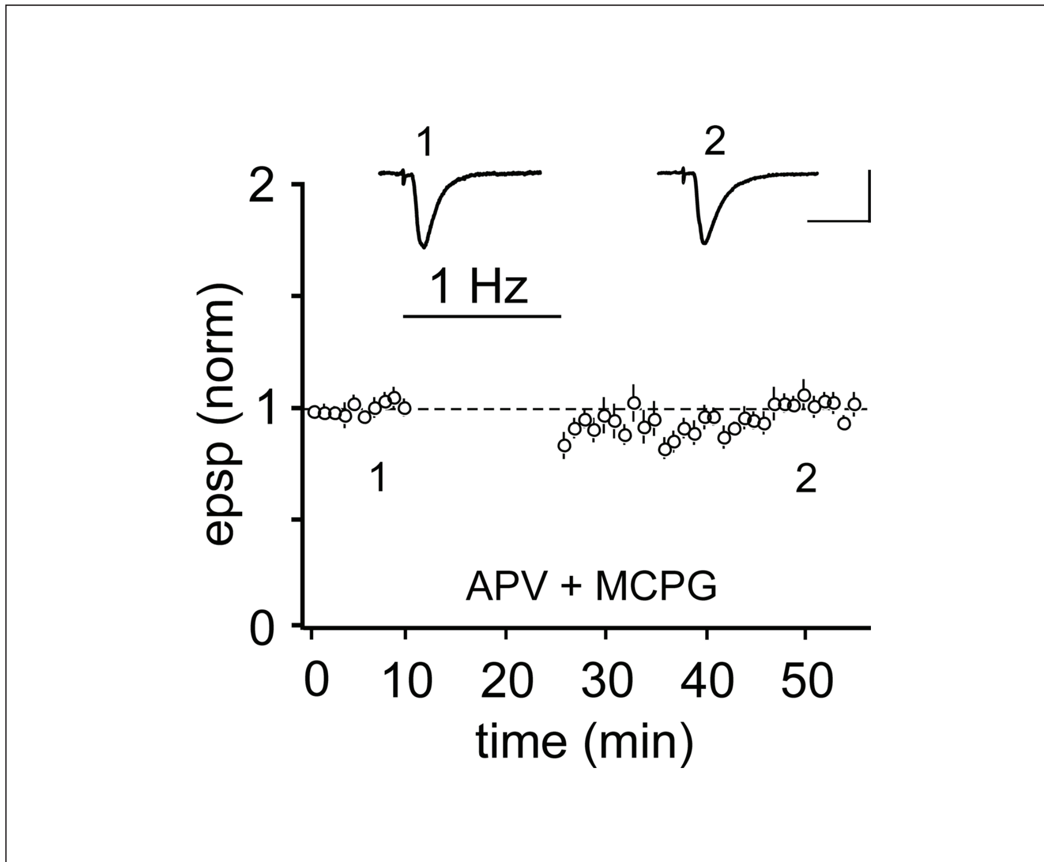


Figure 15 | LFS at 1Hz at bridge mode at the intrinsic membrane potential and the co-application of NMDA-receptors and mGluR antagonists D-APV and MCPG, respectively blocked both LTD and LTP in burst spiking cells. Averaged time courses of evoked EPSC amplitudes before and after induction with LFS at bridge mode are shown. Superimposed traces are averages from 10 single responses each and were recorded during baseline (left-1) and 25 min after induction (right-2). Scale bars 100pA 10ms. Figure adapted from (Fidzinski et al., 2008)

In regular firing cells, in accordance to the above mentioned study, the late-onset LTP was not blocked by the NMDA receptor antagonist D-APV (153.4±9.4% of baseline n=9 P<0.001) (fig 16), but application of MCPG blocked the NMDA-receptor-independent late-onset LTP, revealing an LTD (76.2±7.0% of baseline n=8 P<0.05) (fig 17).

Co-application of D-APV and MCPG prevented synaptic plasticity in regular firing cells (97.4±4.9% of baseline n=6) (fig18).

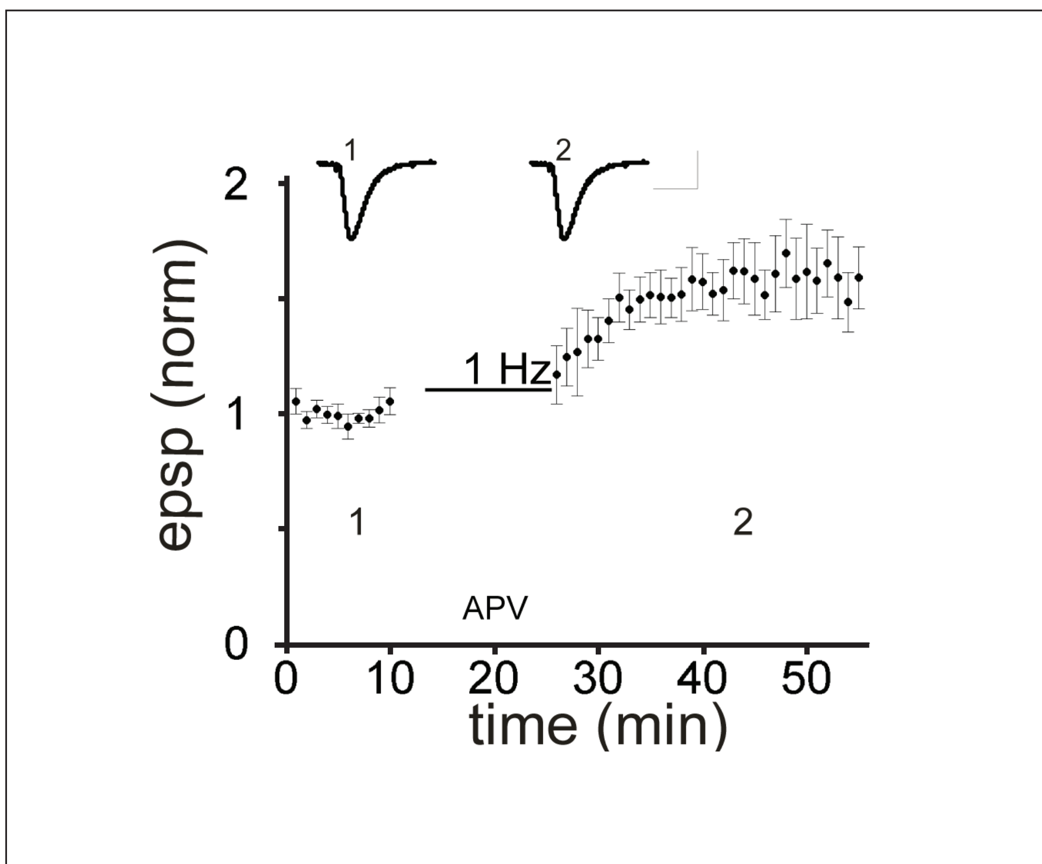


Figure 16 | LFS at 1Hz in bridge mode at the intrinsic membrane potential and the usage of NMDA-receptors antagonist D-APV did not block LTP in regular spiking cells. Averaged time courses of evoked EPSC amplitudes before and after induction with LFS at bridge mode are shown. Superimposed traces are averages from 10 single responses each and were recorded during baseline (left-1) and 25 min after induction (right-2). Scale bars 100pA 25ms

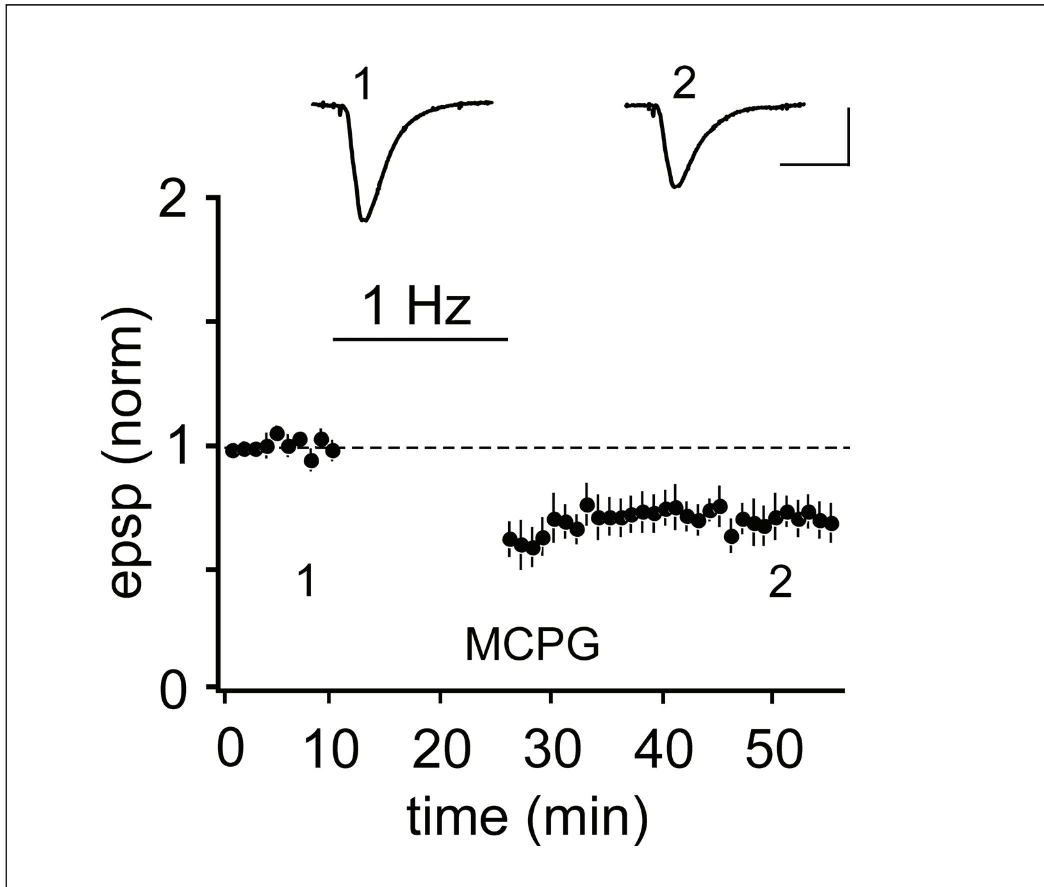


Figure 17 | LFS at 1Hz at bridge mode at the intrinsic membrane potential and the usage of mGluR antagonist MCPG blocked LTP in regular spiking cells and unmasked LTD. Averaged time courses of evoked EPSC amplitudes before and after induction with LFS at bridge mode are shown. Superimposed traces are averages from 10 single responses each and were recorded during baseline (left-1) and 25 min after induction (right-2). Scale bars 100pA 25ms. Figure adapted from (Fidzinski et al., 2008)

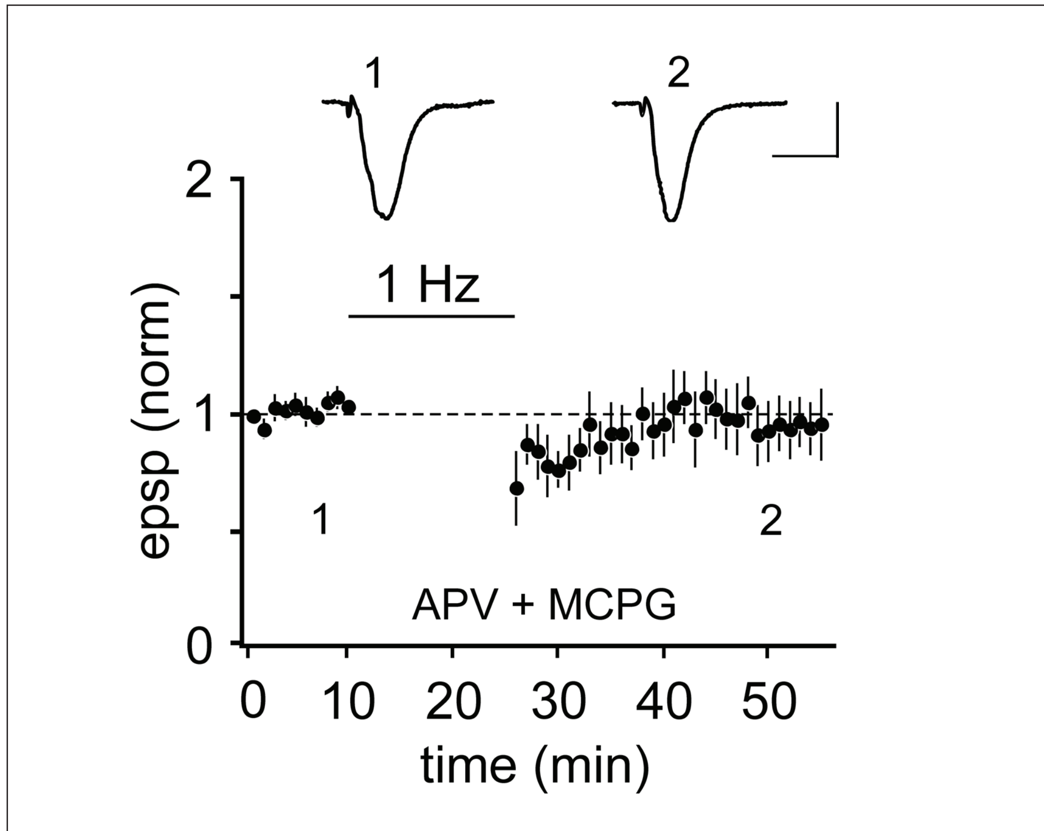


Figure 18 | L LFS at 1Hz at bridge mode at the intrinsic membrane potential and the co-application of NMDA-receptors and mGluR antagonists D-APV and MCPG respectively blocked both LTD and LTP in regular spiking cells. Averaged time courses of evoked EPSC amplitudes before and after induction with LFS at bridge mode are shown. Superimposed traces are averages from 10 single responses each and were recorded during baseline (left-1) and 25 min after induction (right-2). Scale bars 100pA 10ms. Figure adapted from (Fidzinski et al., 2008).

LFS-induced LTP and LTD in subicular burst firing and regular firing cells depends on postsynaptic calcium

When loading the postsynaptic cells with the calcium chelator BAPTA, both cell types did not express any synaptic plasticity (burst firing cells: 97.2±7.2% of baseline n=8; regular firing cells: 96.7±4.6%of baseline n=8) (fig 19), indicating that LFS-induced LTD and LTP rely on postsynaptic calcium signaling.

Notably, during LFS, a transient facilitation of EPSPs in both cell types was oburst firingerved (fig 20). However, differences in their values (table 2) suggest that different mechanisms might be involved. The facilitation in burst firing cells at frequencies between 0.5-3Hz, which induced LTD, was significantly stronger than in the regular firing cells. In addition, a negative correlation was found between the time-amplitude curve integral of EPSP during stimulation and the change in synaptic strength for both cell types (burst firing cells: r=0.38±0.32 n=36 P<0.05; regular firing cells: r=0.55±0.33 n=25 P<0.01) (fig 21).
Recall that LTD in burst firing cells and regular spiking cells was inhibited either by the blockade of NMDAR or by loading the cells with the Ca^{2+} chelator BAPTA. Taken together, these data suggest that the lower induction threshold for NMDAR-dependent LTD in burst firing cells might be mediated by enhanced NMDAR-driven Ca^{2+} currents in burst firing compared with regular firing cells.

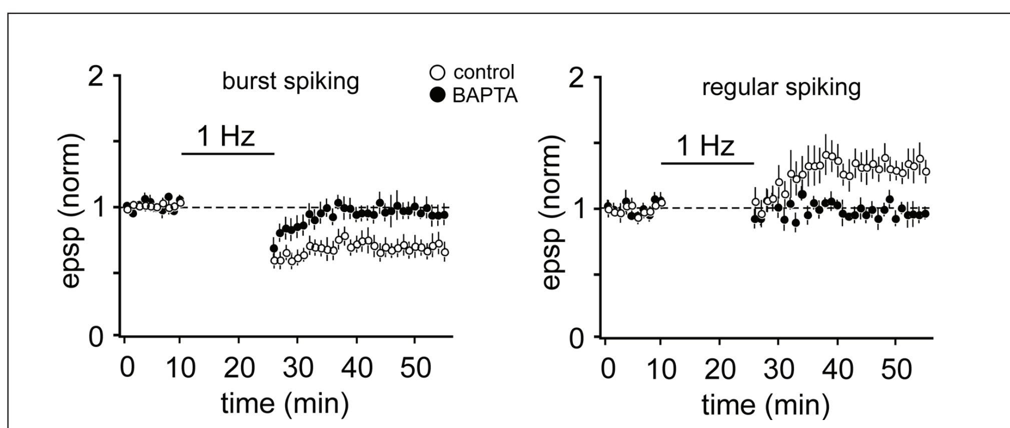
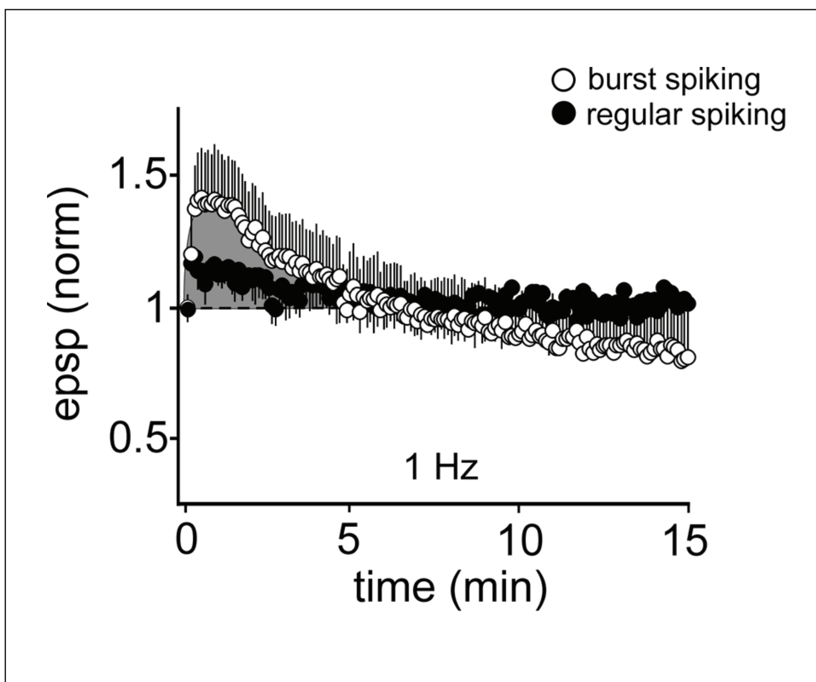


Figure 19 | Postsynaptic loading of BAPTA prevents any synaptic plasticity phenomena in both cell types. Figure adapted from (Fidzinski et al., 2008).



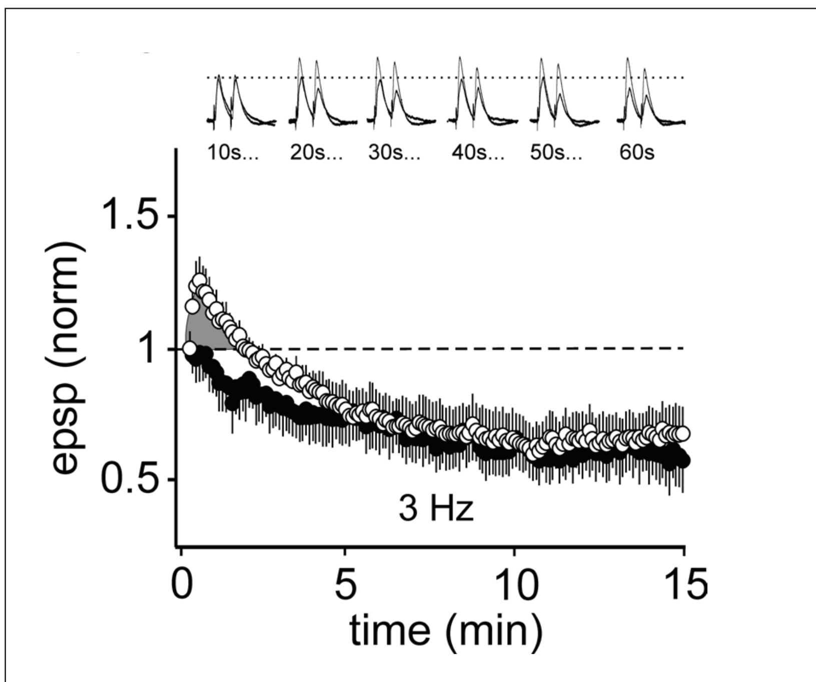


Figure 20 | EPSP amplitudes in regular and burst spiking cells during the LFS at 1Hz and 3Hz. Grey area depicts the integrals of the facilitation of EPSP amplitudes to time for burst spiking cells. Figure adapted from (Fidzinski et al., 2008)

	Frequency (Hz)	Maximal ΔEPSP (mV)	Total duration of increase (min)	Integral (mV*s)
burst spiking	0.5	5.6 ± 0.4	2.3 ± 0.2	**148.1 ± 16.8**
	1.0	6.8 ± 0.4	**2.9 ± 0.4**	**209.9 ± 33.0**
	3.0	**6.1 ± 0.5**	**2.0 ± 0.3**	**120.1 ± 27.2**
	5.0	5.8 ± 1.3	0.6 ± 0.2	19.8 ± 11.3
regular spiking	0.5	6.7 ± 0.9	2.0 ± 0.1	101.4 ± 25.0
	1.0	6.5 ± 0.9	2.2 ± 0.2	117.5 ± 17.9
	3.0	4.4 ± 0.7	0.9 ± 0.1	21.1 ± 7.9
	5.0	3.8 ± 0.4	0.7± 0.3	15.5 ± 9.6

Table 2 | Peak amplitude, duration and time-amplitude integral of the facilitation of EPSP amplitudes for each stimulation frequency and cell type. Figure adapted from (Fidzinski et al., 2008)

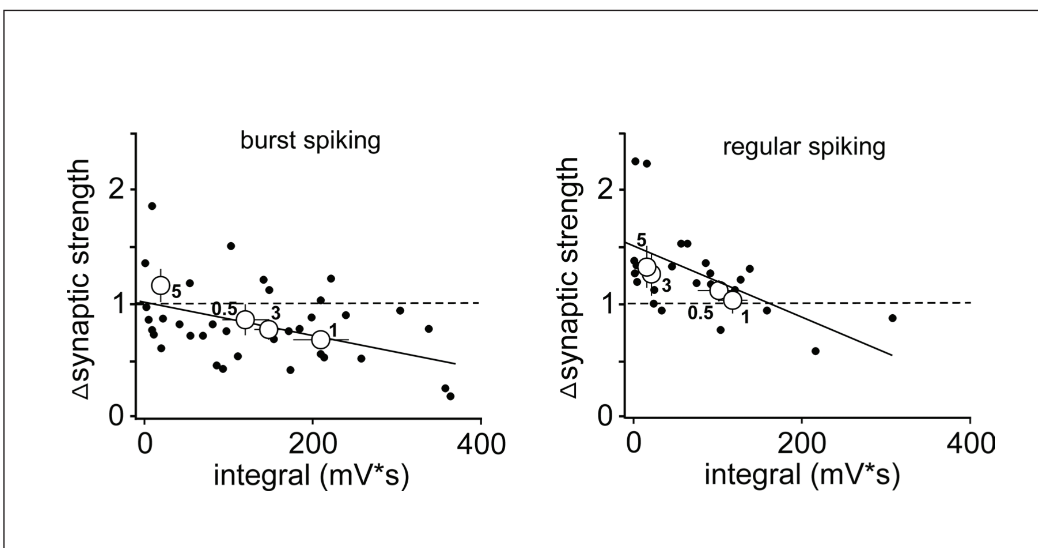


Figure 21 | Synaptic strength changes plotted against time-amplitude integral of EPSP facilitation for each cell (filled circles) at each cell type and the means of each stimulation frequency (open circles). Regression lines show negative correlation between integral and synaptic strength for both cell types

LFS induced synaptic plasticity is dependent on muscarinic acetylcholine receptors

Li et al (Li et al., 2005) demonstrated that subicular LTD induced by a pairing protocol depends on the activation of mAChR. We therefore tested the effect of the muscarinic receptor antagonist scopolamine on LFS-induced bidirectional synaptic plasticity. Scopolamine application had no effect on baseline transmission and did not change the paired pulse ratio in either cell type (burst firing cells: 1.60±0.08, n=10, P=0.261Vs. control; regular firing cells: 1.44±0.07, n=11, P=0.9 vs. control)

Scopolamine blocked LTD in burst firing cells and LTP in regular firing cells (burst firing cells 96.9±4.7% of baseline n=10 P=0.067; regular firing cells: 95.2±4.0% of baseline n-=11 P=0.14) (fig 22-23) indicating that the co-activation of mAChR is necessary for both forms of synaptic plasticity.

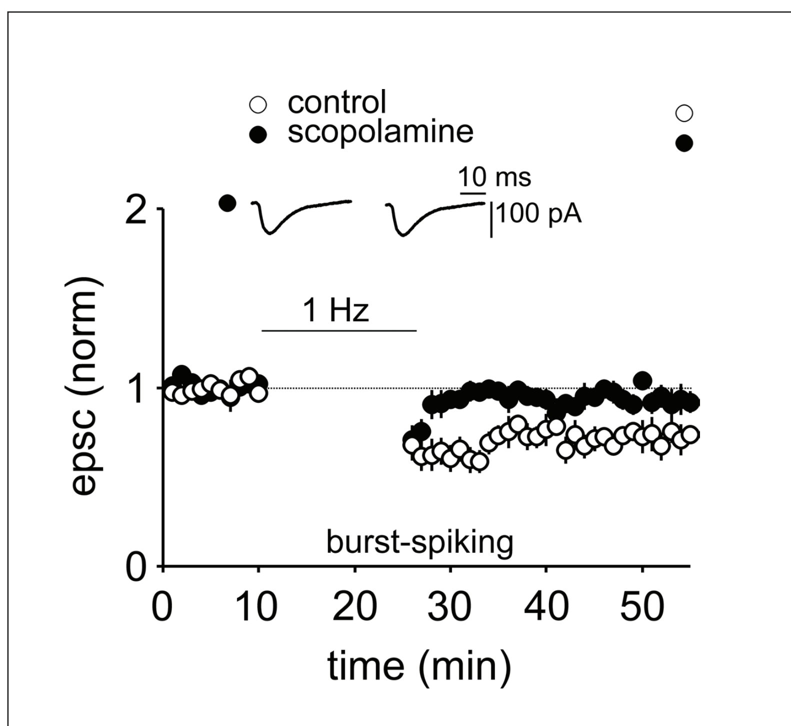


Figure 22 | Involvement of muscarinic neurotransmission in subicular bidirectional plasticity. LFS at 1Hz at bridge mode at the intrinsic membrane potential and application of scopolamine blocked LTD in burst spiking cells. Superimposed traces are averages from 10 single responses each and were recorded during baseline (left) and 25 min after induction (right). Figure adapted from (Shor et al., 2009)

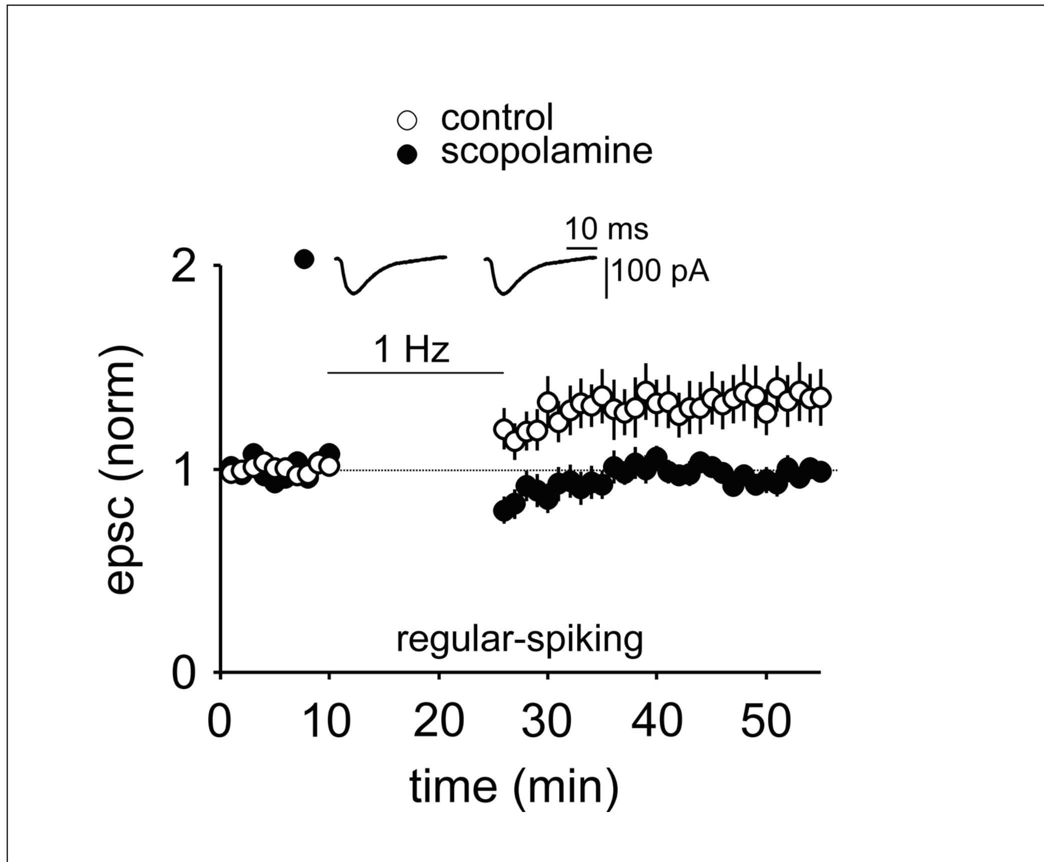


Figure 23 | Involvement of muscarinergic neurotransmission in subicular bidirectional plasticity. LFS at 1Hz at bridge mode at the intrinsic membrane potential and application of scopolamine blocked LTD in regular spiking cells. . Superimposed traces are averages from 10 single responses each and were recorded during baseline (left) and 25 min after induction (right). Figure adapted from (Shor et al., 2009)

Voltage gated calcium channels play a modulatory function in the bi-directional synaptic plasticity

Although glutamatergic receptors are the main source of calcium signals that play a role in synaptic plasticity, Voltage gated calcium channels (VGCCs) may play a modulatory role as well. In order to test that, we applied nifedipine, an L-type calcium channel blocker. Upon induction with LFS-protocol, we oburst firingerved the prevention of LTD in burst firing cells and the expression of late onset LTP (129.4±11.3% of baseline n=10 P<0.05) (fig 24). In regular firing cells the late onset LTP was abolished upon application of nifedipine, revealing LTD (86.5±4.0% of baseline n=9 P<0.05) (fig 25). The overall effects of muscarinic acetylcholine receptor and VGCC blockage are summarized in Figure 26.

The application of nifedipine had no effect on the pair-pulse ratio (burst firing cells: 1.73±0.1 n=10 P=0.3 Vs. control; regular firing cells: 1.31±0.1 n=9 P=0.8 Vs. control), eliminating pre-synaptic mechanisms. These results suggest that VGCC modulate intracellular calcium signals in subicular burst firing and regular firing cells, and determine the polarity of synaptic plasticity in a cell-specific manner.

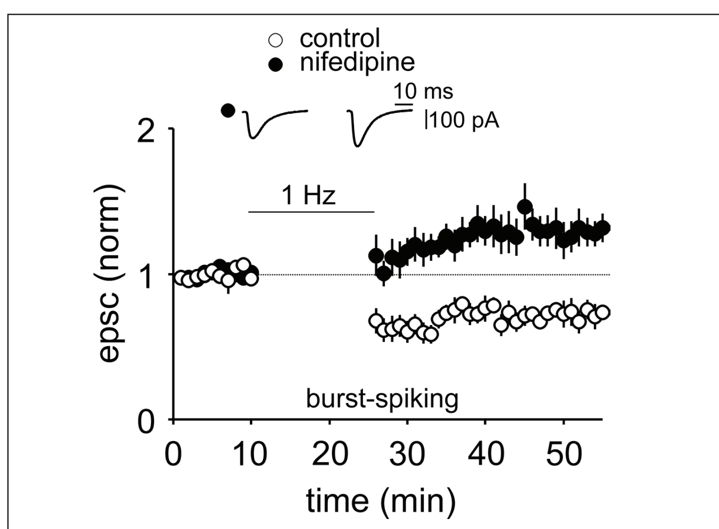


Figure 24 | Involvement of voltage gated calcium channels in subicular bidirectional plasticity. LFS at 1Hz in bridge mode at the intrinsic membrane potential and application of nifedipine reversed the polarity of synaptic plasticity from LTD to LTP in burst spiking cells. Superimposed traces are averages from 10 single responses each and were recorded during baseline (left) and 25 min after induction (right). Figure adapted from (Shor et al., 2009

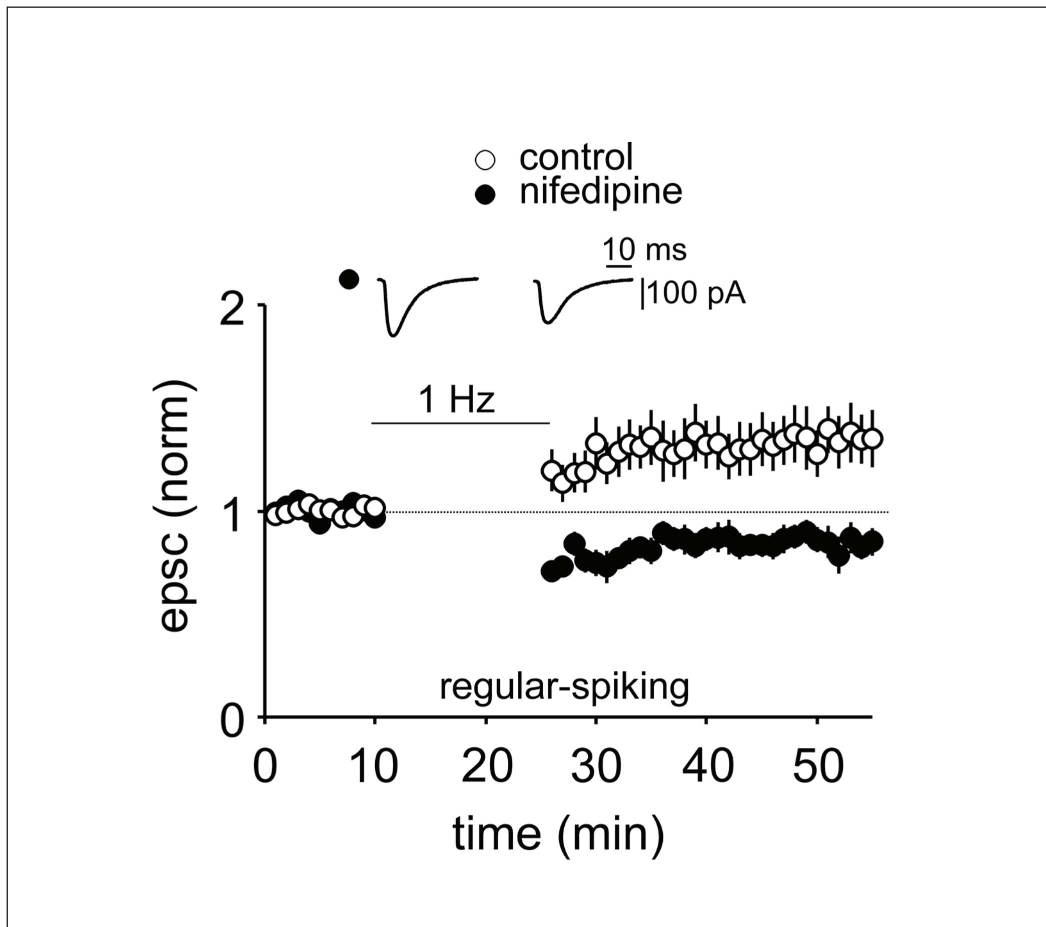


Figure 25 |Involvement of voltage-gated calcium channels in subicular bidirectional plasticity. LFS at 1Hz at bridge mode at the intrinsic membrane potential and application of nifedipine reversed the polarity of synaptic plasticity from LTP to LTD in regular spiking cells. Superimposed traces are averages from 10 single responses each and were recorded during baseline (left) and 25 min after induction (right). Figure adapted from (Shor et al., 2009)

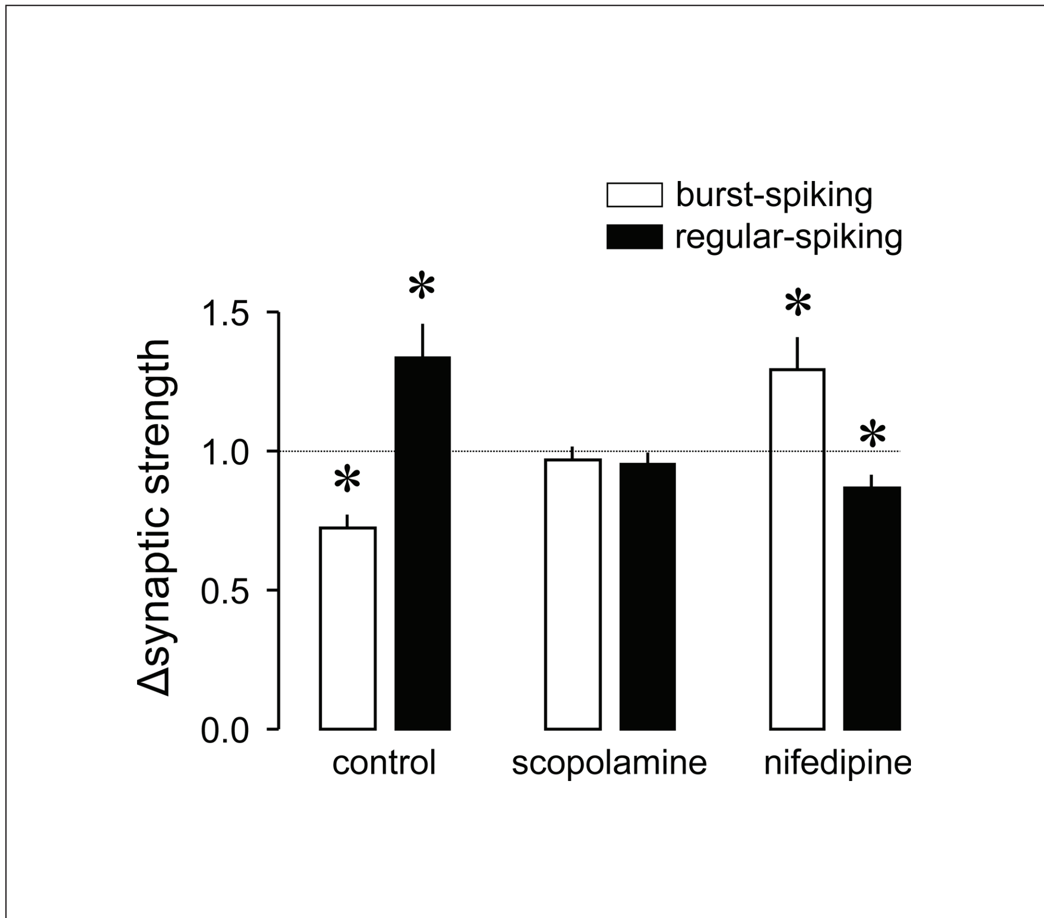


Figure 26 | Summary of changes in synaptic strength for each cell type

Our study provides evidence that LTP and LTD occur simultaneously at CA1-subiculum synapses. We demonstrate that LFS induces an NMDAR-dependent LTD and an mGluR-dependent LTP in subicular pyramidal cells. The predominant direction of synaptic plasticity is dependent on the cell type investigated. In burst firing cells, LFS induces LTD that masks a simultaneously occurring LTP, whereas in regular firing cells LFS results in LTP that masks LTD. Taken together, these results suggest that NMDAR-dependent LTD and mGluR-dependent LTP are co-expressed in subicular neurons, but different induction thresholds determine distinct directions of synaptic plasticity in each cell type. Furthermore, we show that LFS-induced LTD in burst firing cells and LTP in regular firing cells require activation of mAChR. In addition, we demonstrate that the polarity of synaptic plasticity is modulated by VGCC, as the L-type calcium channel inhibitor nifedipine converts LTD to LTP in burst firing cells and LTP to LTD in regular firing cells. Bidirectional synaptic plasticity in subicular cells therefore appears to be governed by a complex signaling system, involving cell-specific recruitment of ligand and voltage-gated ion channels, as well as metabotropic receptors. This complex regulation might be necessary for fine-tuning of synaptic efficacy at hippocampal output synapses.

Previous studies that investigated LFS-induced synaptic plasticity using field potential recording in vivo and in vitro reported no synaptic plasticity at 1Hz or LTP at 3 and 5Hz (Huang and Kandel, 2005) or a late onset LTP (Anderson et al., 2000). However, in the studies did not differentiate between the different subicular cell types.

Our data indicate that only burst firing cells express LTD. In field potential recording, LTD might be masked by LTP expressed in regular firing cells.

There have been many computational models for neural plasticity and their roles in activity driven development of neural circuits and storage of memories (Hopfield and Tank, 1986). The Hebbian synapse was implemented in most physiological studies (Sejnowski, 1981;Rumelhart, 1986;Kohonen, 1984), assigning LTP to co-activated connected neurons. However, an almost immediate problem these models had to face was the saturation of the synaptic connections and consequent the loss of stored information. This problem was initially approached by incorporating a mechanism for LTD induction. However, the resulting networks were such that half of the

connections were saturated and half were depressed to the minima. Bienenstock, Cooper and Munro (Bienenstock et al., 1982) overcame this problem by introducing activity dependence for induction of both LTP and LTD. In this model, termed 'sliding threshold model', the threshold for inducing synaptic plasticity was dynamically determined as a function of the current synaptic strength. According to this model, high levels of synaptic activation reduced the probability of LTP and increased LTD likelihood. At low levels of synaptic activation, the threshold of LTP induction is lowered, increasing its probability and LTD becomes less likely. Consequently, the connection strengths within the network remain linear, thus maximizing the storage capacity of information in the network.

Experimental data supporting the notion that LTD and LTP are dynamically regulated was seen already as Huang and colleagues (Huang et al., 1992) reported that application of brief high frequency stimulation that resulted in short term potentiation did suppress later induction of LTP at the Schaffer collateral-CA1 synapse. Other studies showed that similar treatment resulted in an enhancement of LTD evoked by LFS in area CA1(Wexler and Stanton, 1993).

Our results, describing different polarity of overall apparent plasticity in the two different subicular cell types are in line with this model. Illustration 3 depicts the described plasticity in the subicular neurons in light of the sliding threshold model. In burst spiking neurons the higher activity causes a larger LTP activation threshold. Therefore, at the activity level dictated by LFS, the suprathreshold regular firing cell activity yields LTP, while the subthreshold activity of the burst firing cell yields LTD. The sliding threshold is implemented by the relative activation of mGLUR , NMDAR and VGCCs (Artola and Singer, 1993;Stanton, 1996).

Taken at face value, the translation of the sliding threshold model to our results predicts that burst firing cells exhibit a higher expression of active NMDA receptors. Indeed, this notion is supported by the higher amplitude and longer duration of the transient EPSP facilitation during stimulation in burst firing cells.

It has previously been shown that the threshold for LTD formation in CA1 region can be dynamic and dependent on behavior. Exposure to novel environments causes a leftward shift in

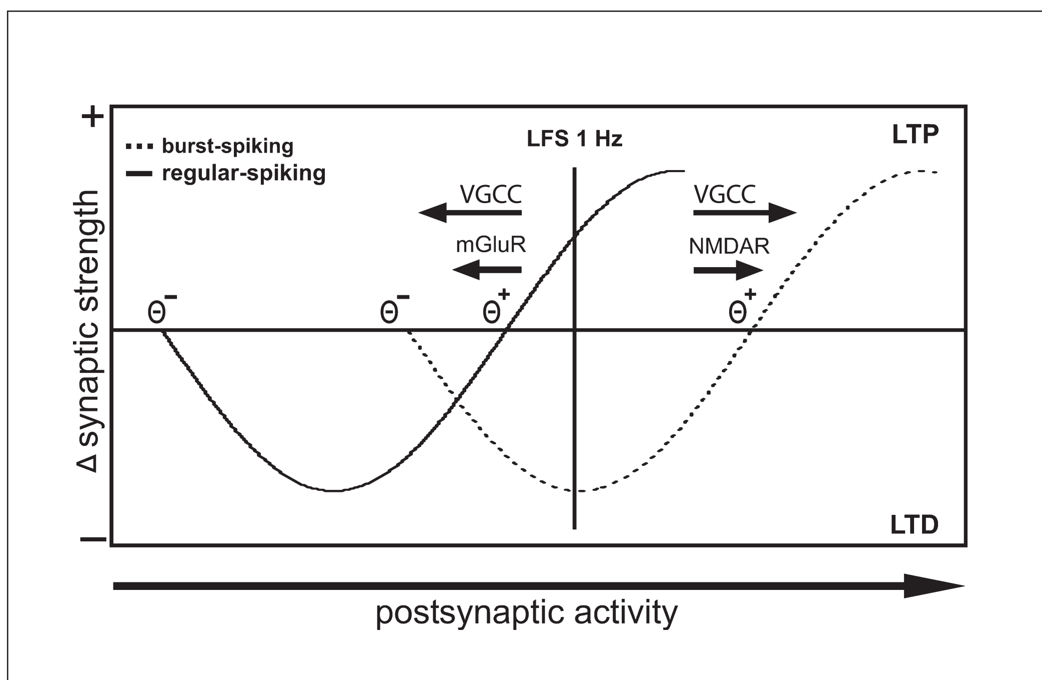


Illustration 3 | Model for synaptic plasticity in pyramidal cells of the subiculum. Activity that is above the basal level and surpasses a modification threshold known as Θ- leads to expression of LTD. Postsynaptic activity that is higher than a modification threshold known as Θ+ leads to expression of LTP. In burst firing cells (dashed line), predominant activation of NMDAR and VGCCs shifts modification threshold Θ+ to the right leading to the expression of LTD. In regular firing cells (solid line), predominant activation of mGluR and VGCCs shifts modification threshold Θ+ to the left such that LFS at 1 Hz leads to expression of LTP. Thus, the sliding thresholds for induction of LTP and LTD will be driven by the relative activation state of NMDAR, mGluR (arrows) and VGCC.

the threshold (Manahan-Vaughan and Braunewell, 1999), providing experimental support for the sliding threshold theory.

Our results show dependence of LTD in both burst firing cells and regular firing cells on NMDA receptors .

Inhibition of NMDARs blocks LTD (Dudek and Bear, 1992; Mulkey and Malenka, 1992), and activation of NMDARs induces it (Cummings et al. 1996, Kamal et al. 1999), Kandler et al. 1998, and Li et al. 2004). Moreover, it is reported that modulations in the expression, trafficking and gating of NMDA receptors are key mechanisms in the experience-dependent modification of neural circuitry in the developing brain, as well as in the mature brain (Stephens and Weidmann, 1989;

Cull-Candy et al., 2001). For example, experience-dependent changes in the subunit composition of NMDA receptors have been reported in the visual cortex (Snyder et al., 2001; Philpot et al., 2001). The directionality of the plasticity as well as the tuning has been shown to rely on the relative activation of the NMDA-receptors (Bear and Kirkwood, 1993). The mechanism by which NMDARs gate LTD induction is through its permeability to Ca^{2+} into the postsynaptic neuron. Indeed, buffering a rise in Ca^{2+} prevents LTD (Mulkey and Malenka, 1992). Moreover, photolytic intracellular uncaging of Ca^{2+} induces LTD (Yang et al., 1999). Depolarization paired with LFS, produces plasticity, the polarity of which dependends on post-synaptic calcium concentration in the perirhinal cortex (Cho et al., 2000). Furthermore, the different plasticity outcomes are reliably predicted according to intracellular calcium transients in the dendrites. Finally, the calcium wave to the soma displays different characteristics according to the cell propensity to undergo LTD or LTP (Ismailov et al., 2004). Thus, the simple model emerged that Ca^{2+} entering the postsynaptic dendritic spine through the NMDAR is the trigger for LTD.

LTD and LTP are timing dependent processes. Spike-timing-dependent plasticity (STDP) is a general term for functional changes in neurons and at synapses that are sensitive to the timing of action potentials in connected neurons. The phrase 'STDP' typically refers to increases or decreases in the efficacy of synaptic transmission (Bi and Poo, 1998; Markram and Sackmann, 1997)

The phenomenon (STDP) is believed to arise from a nonlinear process that leads either to large calcium transients and thus to LTP (by pre-synaptic action potential that precedes the post-synaptic action potential), or smaller calcium transients and thus LTD (due to post-synaptic action potential preceding the pre-synaptic action potential). STDP in the entorhinal cortex is known to be NMDA-receptor-dependent (REF). However, calcium concentration in the postsynaptic neurons is affected by other factors as well. First, depolarization causes the opening of voltage dependent calcium channels. Moreover, some AMPA receptors are permeable to calcium. Finally, intracellular calcium stores can be opened as a result of signaling cascades. Thus, it has been shown that calcium transients of large magnitude, elicited by broadening of action potentials, can enhance timing-dependent synaptic depression in the entorhinal cortex (Zhou et al., 2005). Finally, NMDA receptor activation is dependent on depolarization. In the subiculum, EPSPs consist of AMPAR-mediated sodium currents, enabling the activation of NMDA receptors, and thus allowing calcium currents

through them (Behr et al., 1998). Thus, the dynamic interplay between the calcium concentration at the dendritic sites of the synapse and NMDA receptors is quite diverse and the kinetics of these processes may determine the direction of synaptic plasticity.

In our studies, we showed that LTP in regular firing cells is MGluR dependent, since it was reversed in these cells upon application of the mGluR antagonist MCPG.

Early studies proposed a role for group I mGluR in the induction of LTP. Recent studies showed that mGluRs have a crucial role in coincidence detection as well as in regulation of synaptic plasticity (Nevian and Sakmann, 2006;Lu et al., 2007). The mGluR agonist, ACPD, enhanced LTP induced by HFS in CA1 (McGuinness et al., 1991) while MCPG blocked LTP induced by HFS at the dentate gyrus and CA1 (Bashir et al., 1993;Breakwell et al., 1996). Application of group I mGluR agonist 1 S,3 R-APCD induced a long-lasting potentiation of EPSPs and EPSCs in CA1 region of the hippocampus in vivo and in vitro (Bortolotto and Collingridge, 1993;Bortolotto and Collingridge, 1995;Chinestra et al., 1993). This form of APCD-induced LTP was shown to have common maintenance mechanisms with tetanus induced LTP. It requires high afferent activity or depolarization of the postsynaptic site and is dependent on PKC and the release of calcium from the intracellular stores. The role of mGluR in NMDA-receptor-independent LTP induction was shown in the CA3 region by stimulation of the mossy fibers (Haruta et al., 1994;Ito and Sugiyama, 1991a;Zalutsky and Nicoll, 1990). Moreover, mossy-fiber-CA3 LTP is inhibited by MCPG and the mGluR antagonist D,L-2-amino-3-phosphono-propionate(D,L-AP3) (Ito and Sugiyama, 1991b;Bashir and Collingridge, 1994). LTP in the dorsolateral septal nucleus is induced by 1S,3R ACPD and the application of the Ca^{2+} chelator BAPTA and non-hydrolysable GTP to the postsynaptic site blocked LTP confirming the requirement of G-protein coupled signalling pathways and increase in postsynaptic calcium concentration. The studies (Chinestra et al., 1993;Izumi and Zorumski, 1994) which failed to show dependence of LTP on mGluR activity may suggest that mGluR activation is only crucial for certain aspects of LTP induction, for example to lower the threshold for induction of LTP.

The simultaneous plasticity at one cell type reported in our study correlates to other studies which revealed similar phenomena. Simultaneous LTP of non-NMDA and LTD of NMDA-receptor-

mediated responses were reported previously in nucleus accumbens where both forms of plasticity were observed in the same cell and similar change in post-synaptic calcium concentration was seen to have opposite effects on the synaptic responses (Kombian and Malenka, 1994). Another study by Bernard and Wheal in an experimental model of epilepsy (kainic acid model) showed a simultaneous expression of an NMDAR-dependent LTD and an AMPAR-dependent LTP (Bernard and Wheal, 1995). Further supporting evidence for the bidirectional plasticity we found in the subiculum may come from the somatosensory cortex. There, pyramidal neurons form synapses with two types of interneurons, fast spiking and low-threshold spiking. Both synapse types undergo long term plasticity. However, while mGluR were reported to be crucial in the induction of LTD in both types of interneurons, NMDA receptors are a crucial component for inducing LTP only in low threshold spiking interneurons. Simultaneous LTD and LTP was described also at CA3-CA1 synapses and the direction of the plasticity was reported to be mediated by kinases for LTP and phosphatases for LTD in the same cell (O'Connor et al., 2005). Studies in the neocortex layer 5 showed simultaneously occurring LTD and LTP induced by pairing high frequency pre- and postsynaptic firing. In this study, the LTP induction was mediated by both presynaptic and postsynaptic mechanisms. On the other hand, LTD is mediated by endocannabinoids released from the postsynaptic site, serving as a retrograde messanger, thereby inducing presynaptic changes (Sjostrom, 2007).

In our studies, all forms of synaptic plasticity in both types of neuron are abolished upon application of the muscarinic acetylcholine receptor scopolamine. Pharmacological studies in humans demonstrated that blockage of muscarinic cholinergic receptors by drugs like scopolamine impairs encoding of new memories, while the retrieval of stored memories stayed intact (Atri et al., 2004;Hasselmo and McGaughy, 2004). Infusions of scopolamine into the CA3 region of the rat hippocampus results in selective impairments of encoding but not retrieval in Hebb-Williams maze (Rogers and Kesner, 2003). Thus the involvement of cholinergic neurotransmission through muscarinic receptors in memory consolidation and cognition is widely recognized. Physiologically, the most prominent effects of ACh are slow depolarization and decrease in both voltage-dependent and voltage insensitive components of membrane conductance. All of these effects on membrane properties depend upon interaction with muscarinic receptors (Benardo and Prince, 1982). Two major types of muscarinic acetylcholine receptors are known. The M1 receptor subtype, the predominant

receptor type in the hippocampus (Volpicelli and Levey, 2004), activates the phospholipase C pathway resulting in production of inositol trisphosphate and diacylglycerol. M2/M4 receptor subtype, on the other hand, is negatively coupled to cAMP production (Caulfield et al., 1993).

One of the effects of mAChR activation in the hippocampus is potentiation of NMDA receptor currents. Studies indicated that M1 receptor subtype co-localizes with the NMDA receptors on CA1 pyramidal cell bodies and dendrites. Indeed, treatment with mAChR agonist carbachol causes an increase in NMDA induced current, while pretreatment of hippocampal slices with the antagonist for M1, M1 receptor subtype-toxin, blocks the carbachol-induced potentiation of NMDA currents (Marino et al., 1998).

The two stage model of memory formation requires very different dynamics during each stage. Activity in the hippocampal network is highly dependent on the activity state of the animal. Thus, during active waking, activity in area CA1 is predominantly determined by inputs from the entorhinal cortex . In contrast, during quiet wakefulness and slow wave sleep, the opposite pattern may be seen, where outputs from CA3 affect the CA1 and the entorhinal cortex. This difference has been attributed to differential acetylcholine modulation between these two states). It has been shown that during active waking states there is a high release of acetylcholine, which might contribute to theta rhythm, as acetylcholine correlates with the amplitude of theta oscillation (Marrosu et al., 1995;Monmaur et al., 1997) and cholinergic blockage reduces theta oscillation amplitude. Experiments with brain slices demonstrate that acetylcholine suppresses transmission at excitatory recurrent collaterals in the CA3 region of the hippocampus and suppresses transmission at the Schaffer collaterals connecting CA3 and CA1 and at the CA1 to subiculum synapse (Hounsgaard, 1978;Valentino and Dingledine, 1981;Hasselmo et al., 1995;Dutar and Nicoll, 1988;Sheridan and Sutor, 1990). In quiet waking state, measurements of acetylcholine levels in the hippocampus show a decrease relative to the active waking state (Marrosu et al., 1995), which would lead to a release of the glutamatergic synapses from cholinergic suppression. This notion is consistent with the much larger synaptic potentials in the CA1 region and entorhinal cortex during quiet waking (Winson and Abzug, 1978) and sharp wave generation in the area CA3 (Buzsaki, 1986e). Lower acetylcholine levels indicate stronger feedback between CA3 and CA1 and from CA1 to the entorhinal cortex (Buzsaki, 1986d). A striking decrease in acetylcholine concentrations in the hippocampus was

observed during slow wave sleep which would further release glutamatergic synapses from suppression, resulting in stronger excitatory feedback from CA3 to CA1 and entorhinal cortex (Buzsaki, 1986b).

The various modulations of the hippocampal circuitry can be regulated by effects of mAChR on cytoplasmic calcium signals, and interactions with ionotropic and metabotropic glutamate receptors (Bashir, 2003). As reported by Nash et al. in 2004 (Nash et al., 2004), mAChR-stimulated inositol 1,4,5-trisphosphate production, which, in turn, facilitates calcium release from the internal stores in hippocampal neurons. mAChR stimulation reduces NMDA responses in CA3 hippocampal pyramidal cells via Ca^{2+}-dependent activation of tyrosine phosphatase (Grishin et al., 2005). Taken together with our current study, these reports support the notion that mAChR are fundamental to plasticity in the hippocampus and, as our study shows, are critically involved in the bidirectional cell specific synaptic plasticity in the subiculum. Recall that we showed calcium dependence of plasticity in both the burst firing cells and regular firing cells in the subicilum, although the induction mechanisms are qualitatively different: LFS causes LTD in the burst firing cells in an NMDA receptor dependent manner, and LTP induction in in regular firing cells is mGluR-dependent,. It might be that mAChR modulates the calcium levels upon activation and thus calcium levels may directly influence the mGluR and NMDA receptors. Alternatively, mAChR might induce calcium release from internal stores, thereby mediating and modulating the direction of the synaptic plasticity, in a concentration and cell type dependent manner.

In hippocampal pyramidal cells, voltage and calcium gated ion channels located in the dendritic spines open as a consequence of glutamate receptor activation and act within a complex loop that feeds back to regulate synaptic signals(Bloodgood and Sabatini, 2008). The role of Cav1.2 voltage gated calcium channels in induction of long term synaptic plasticity in the hippocampus was also demonstrated to be NMDA receptors independent in hippocampal synaptic plasticity (Moosmang et al., 2005). In this study the function of Cav1.2 calcium channels was evaluated in spatial learning, synaptic plasticity and triggering of learning-associated biochemical processes. The study used a mouse model with inactive Cav1.2 calcium channel gene in the hippocampus and neocortex. Using this model, they showed a loss of a late-phase LTP, which is protein synthesis dependent and NMDA-R-independent, at the Schaffer collateral\CA1, in addition to severe

impairment in hippoocampal dependent spatial memory. Another study showed that VGCCs express a different form of LTP in CA1 than the NMDA receptor form of LTP(Morgan and Teyler, 1999). Further, upon altering a β3 subunit of the calcium channel, an NMDA receptor dependent LTP was reported (Jeon et al., 2008). VGCCs show differential effects on synaptic plasticity depending upon the structure and pattern of their activation. In CA1 and entorhinal cortex, the VGCC mediated calcium transients are required for NMDA receptor dependent LTD (Zhou et al., 2005;Christie et al., 1997). Inhibition of the VGCCs enhances the LFS-induced LTD and blocks LTP in CA1 ((Raymond and Redman, 2006;Udagawa et al., 2006). According to our study in subiculum, the NMDA-receptor dependent LTD in burst firing cells and the mGluR-dependent LTP in the regular firing cells require co-activation of VGCCs (Shor et al 2008). The distinct nature of the synaptic plasticity in the two types of cells in subiculum is therefore modulated by VGCCs. Given that the amplitude of calcium transients and synaptic potentials in dendrites depend on activation of VGCCs and mAChR, the polarity of synaptic plasticity might be fine-tuned by these factors to control the output of the hippocampus to other cortical and sub-cortical regions .

The spatial distribution of burst firing cells and regular firing cells in the proximo-distal and deep-superficial axes of the subiculum (Greene and Totterdell, 1997; Staff et al., 2000; Harris and Stewart, 2001; Harris and Stewart, 2001) and the topography of subicular efferent fibres (Naber and Witter, 1998; Ishizuka, 2001) suggest that burst firing cells and regular firing cells may target different brain structures. These findings may be relevant in supporting the hypothesis of O'Mara (O'Mara, 2006b), where a segregation of function within the subiculum was proposed. In this model, which is primarily based on lesion studies, the dorsal part of the subiculum is principally concerned with processing information about space, movement and memory, while the ventral part of the subiculum is an interface between the hippocampus and the HPA axis, functioning mainly in the inhibition of the HPA axis.

Stress is defined as high excitability or arousal, a perception of aversiveness and lack of control over outcomes (Kim and Diamond, 2002). Stress response is controlled by the HPA axis, which, in turn, is controlled and regulated by the hippocampus. Behavioral and systemic stress results in the release of corticotropin-releasing hormone (CRH) from the hypothalamus into the portal circulation of the anterior pituitary, which then releases adrenocorticotropin-releasing

hormone (ACTH) into the blood stream. This pathway results in corticosterone release from adrenal cortex. Behaviorally, ACTH initiates the so called ”fight or flight” response, mobilizes energy stores, decreases reflex threshold, increases respiratory rate, muscle tension and gastric motility, and are behaviorally adaptive. Simulating such stress responses inhibits LTP, causes hippocampal atrophy, and impairs hippocampus dependent learning. The subiculum in general, regulates the HPA-axis stress response and the CA1-subiculum synapses are affected by behavioral and systemic stress (Commins, 2000). Behavioral stress accompanied with LTD induction protocols produces LTD. Consistent with Lowry (Lowry, 2002b; Herman, 1995; Herman, 1998; Mueller, 2004), the ventral subiculum plays a dynamic and inhibitory role in the HPA axis and therefore regulates the stress response. A study (Maren, 1999) that used lesions of the ventral subiculum showed, in accordance with the proposed role of the ventral subiculum, the attenuation of the HPA response to systemic and behavioral stressors.

Experiments on freely moving animals tested during exploration of objects or in mazes and in open field environments show that subicular cells, unlike the CA1 cells, tend to fire throughout the environment and show several peaks of activity. These data imply that subicular place-fields have a lower resolution and comprise of much larger areas of comparable environments than those of area CA1 (O'Mara et al., 2000; Sharp and Green, 1994; Barnes et al., 1990). The perirhinal cortex, responsive to novelty or familiarity of objects, projects to the subiculum. These findings can be explained by the convergence of multiple CA1 place cells onto a single subicular cell, thus resulting in the convergence of movement information in the subiculum. The multiple activity peaks probably reflect separate place cell inputs. Movement information is derived through the CA1 projections to the subiculum and other cortical areas converging onto the entorhinal cortex. Overall, the dorsal part of the subiculum, according to the above hypothesis, is an area of integration between spatial information of the hippocampus and general whole-body movement information which is mainly cortical in origin. Moreover, there is evidence for the combination of spatial and working memory information (Deadwyler and Hampson, 2004) and combination of spatial and object information(Anderson and O'Mara, 2003;Anderson and O'Mara, 2004). Sharp (Sharp, 1997) has shown that the subicular place fields are extremely stable in two distinctively different environments, namely, cylindrical and square open fields. Moreover, subicular place cells anticipate future location faster than the CA1 place cells (Sharp, 1999a). In spatial delayed nonmatch-to-sample

tasks, these differences are sustained (Deadwyler and Hampson, 2004). Task relevant information is encoded by the subiculum for a short period of time while the CA1 cells are progressively engaged in retrieval processes. Upon entering a new environment, the place fields evolve through entorhinal cortex to the CA1 and the subiculum (Brun et al., 2002). Moreover, upon exposure to this new environment, place cells of the subiculum emerge immediately, while the CA1 cells need to be exposed to the environment two or three times in order for them to develop their place fields (Wills et al., 2005).

The spatial distribution and topography of the subicular efferents (Greene and Totterdell, 1997;Ishizuka, 2001) suggest that the two types of subicular pyramidal cells may target different subcortical structures. Regular firing cells seem to project to the entorhinal cortex, while the burst firing cells seem to project to the presubiculum (Stewart, 1997). The inputs to these two types of cells originate mainly from the CA1, entorhinal cortex and the thalamic reuniens nucleus (Witter et al., 1989a;Witter et al., 1989b). These findings are not in contradiction with the differential outputs of the cells for the reason that although distinct inputs seem to be distributed in a topographic manner across the subiculum, they may have a different synaptic integration in the different cell types as our results clearly show.

These findings and hypotheses relate directly to different but related concepts of memory. Firstly, by linking the environmental cues to the significance of the subiculum in encoding and processing them, the subiculum should be related to the working memory and memory consolidation concepts.

We have shown in our study that the subiculum utilizes cellular and synaptic mechanisms independently to provide differential processing of sensory information from the hippocampus to other cortical and sub cortical brain regions. Hence the distinct forms of synaptic plasticity in burst firing cells and regular firing cells may reflect the vast dynamic capacity for information storage by the subiculum and underpin its unique role in information processing in the hippocampal-cortical axis.

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