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Title: Place cells and long-term potentiation in the hippocampus

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Abstract

Place cells show location-specific firing patterns according to an animal’s position in an environment and are thought to contribute to the spatial representation required for self-navigation. Decades of study have extensively characterized the properties of place cells and suggested the involvement of long-term potentiation (LTP), a long-lasting synaptic strengthening, in place cell activity. Here, we review the basic characteristics of place cell activity and the findings that support the idea that LTP contributes to the formation, maintenance, and plasticity of place cell activity.

Keywords

Hippocampus, place cell, cognitive map, synaptic plasticity, long-term potentiation (LTP), NMDA receptors

Abbreviations

LTP (Long-term potentiation), CA1 (Cornu Ammonis area 1), CA3 (Cornu Ammonis area 3), NMDA receptor (N-methyl-D-aspartate receptor), CaMKIIα (Calcium/calmodulin-dependent protein kinase type II alpha), cAMP (Cyclic adenosine monophosphate), PKA (Protein kinase A), ERK (extracellular-signal-regulated kinases), CREB (cAMP response element-binding protein), PKMζ (protein kinase Mζ), AMPA receptor (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor), NR1 (glutamate ionotropic receptor NMDA type subunit 1), GluR1 (glutamate ionotropic receptor AMPA type subunit 1), CPP ((±)-3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid), ZIP (Zeta Inhibitory Polypeptide)

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Our brains are able to provide us with a sense of our own position in an environment, a process that is thought to be mediated by the hippocampus. O'Keefe and Dostrovsky (1971), using a set of implanted microelectrodes in rat brains, discovered that neurons in the hippocampus exhibit firing in some specific locations but not in others within an environment. These neurons with location-specific firing patterns were later named “place cells” (O'Keefe, 1979). Place cells have been most extensively studied in rats and mice, but they are also found in the human hippocampus (Ekstrom et al., 2003).

**Place cells in the hippocampus**

Place cells are hippocampal neurons that fire at a high frequency when an animal visits a specific region of an environment (Figure 1A). Each place cell has its own preferred firing region, commonly known as a place field. Different place cells have place fields in different regions of an environment. Some place cells fire in response to the combination of an animal's position and certain other factors (e.g., sensory stimuli, behavior), whereas others appear to fire based solely on an animal's position, regardless of other factors (Kubie et al., 1990; O'Keefe, 1976). Place cells have been identified in all hippocampal subregions, the CA1-3 areas (O'Keefe and Dostrovsky, 1971; McNaughton et al., 1989; Leutgeb et al., 2004; Park et al., 2011; Lu et al., 2015; Mankin et al., 2015) and the dentate gyrus (Jung and McNaughton, 1993; Alme et al., 2010; Neunuebel et al., 2012). In the CA1 area, pyramidal cells have been shown to exhibit place cell activity (Bittner et al., 2015; Dombbeck et al., 2010; Fox and Ranck, 1981; Fox and Ranck, 1975; Henze et al., 2000). However, the identity of place cells in the dentate gyrus has been controversial. Different cell types, such as granule cells, young granule cells and mossy cells, have been proposed to exhibit place cell activity (Jung and McNaughton, 1993; Alme et al., 2010; Neunuebel et al., 2012; Danielson et al., 2016). In addition to these place cells in the hippocampus, other cell types in brain regions outside the hippocampus have been reported to show spatially modulated firing patterns, such as head direction cells in the presubiculum and other areas (Taube, 2007) and grid cells in the medial entorhinal cortex (Rowland et al., 2016).

**Characteristics of place cell activity**

*Visual information exerts strong control over the position of place fields*

Studies have shown that place cell activity is strongly controlled by the visual information that animals receive from the surrounding environment. Muller and Kubie (1987) tested whether place cell activity is influenced by the position of a visual landmark in the form of a white cue card placed on the wall of a cylindrical enclosure. They found that when the cue card was moved by 90° steps along the cylindrical wall, the positions of the place fields followed the rotation of the cue card position (Figure 1B). Similarly, O'Keefe and Speakman (1987) moved all spatial cues in an experimental room together by 90° steps around the center of a four-arm maze and found that the place fields of many place cells followed the rotation of the spatial
cues. Thus, visual information plays a critical role in determining the positions of place fields.

*Place fields are determined by multiple types of sensory information*

Although visual information was found to play a critical role under some conditions, as described above, Quirk et al. (1990) showed that place cells are able to maintain constant spatial firing patterns even in the dark. Furthermore, a study using blind rats indicated the existence of place fields in the complete absence of visual input (Save et al., 1998), suggesting that place cells do not rely solely on visual information to generate their location-specific firing patterns. Olfactory (Zhang and Manahan-Vaughan, 2015) and tactile inputs (Gener et al., 2013) have also been shown to help determine the locations of place fields. Thus, the location specificity of place cell firing is not determined by any specific sensory modality but by the integration of multiple types of sensory information, although a particular sensory modality can be dominant depending on the nature of the surrounding environment.

*Remapping between environments*

When an animal is exposed to two different environments, what happens to place cells? Studies have shown that some place cells with place fields in one environment had no place field in another environment (Muller and Kubie, 1987; O'Keefe and Conway, 1978; Thompson and Best, 1989). Other place cells were found to be active in multiple environments; however, the positions of their place fields seemed to change randomly between environments (Muller and Kubie, 1987) (Figure 1C). Place cells whose place fields are at the center of one environment had place fields either at the center or in the periphery of another environment. Thus, different groups of place cells become active in different environments, and the positions of their place fields do not show any predictable relationship between environments. Muller and Kubie termed this phenomenon “remapping”.

*Task demands affect place cell activity*

When an animal explores an open field to forage randomly scattered food, place fields are typically non-directional, i.e., place cells fire in their place fields independently of the direction from which the animal approaches the place field (Muller et al., 1994). In contrast, when an animal moves back and forth between fixed feeding positions along a restricted path, such as a linear track or the narrow arms of a radial maze, the firing of the majority of place cells depends on the direction in which the animal moves (McNaughton et al., 1983). Some place cells have their place fields only when the animal moves through the path in one direction, but not in the other direction. Other place cells have their place fields when the animal moves through the path in both directions. In this case, the locations of place fields can be the same or different when the animal moves in different directions. To examine what determines the directionality of a place field, Markus et al. (1995) trained rats in two different tasks on the same platform. In one task, the rats were trained to forage food that was randomly scattered across the platform. Under this condition, the majority of place cells had non-directional place fields. In the second task, the rats were trained to sequentially collect food at four fixed points on the
platform. Under this latter condition, the place fields of many place cells were
directional; place cells had their place fields in different positions depending on
whether food was collected from the four points in a clockwise or counterclockwise
direction. Moreover, approximately one third of place cells altered the positions of
their place fields between the two tasks. These results suggest that the position and
directionality of place fields can change depending on the tasks the animal is
performing, even if the animal performs the tasks in the same location.

**Function of place cell activity**

*Place cells, as a group, can signal which position in which environment an animal is located*

In a given environment, place cells selectively fire in their place fields. When a
particular place cell fires, we can infer that the animal is in the place field of this
place cell. However, if we consider the fact that a single place cell can have place
fields in multiple environments, the situation becomes complex. For example, Figure
2A illustrates a situation in which Neuron 1 has a place field in both environments A
and B (but not in C). In this case, even if we find that Neuron 1 fires at a particular
moment, we do not know whether the animal is in the place field in environment A or
in the other place field in environment B. Thus, a place cell cannot unambiguously
signal where the animal is located unless some other signals specify the
environment in which the animal is located.

This problem can be solved if we consider the firing of a group of place cells
simultaneously (Kubie and Muller, 1991). Figure 2B illustrates a situation in which, in
addition to Neuron 1, Neuron 2 is also considered. Neuron 2 has place fields in
environments B and C, but not in A. In this case, if we find that both place cells are
firing, we can infer that the animal is in the place field (overlapping between Neuron
1 and 2) in environment B. If Neuron 1 is firing but Neuron 2 is not, we can infer that
the animal is located in the place field of Neuron 1 in environment A. Of course, with
only two place cells, many other positions in the three environments cannot be
represented because neither cell has place fields and does not fire in those positions.
However, thousands of place cells together would be able to represent the total area
of many different environments.

**Self-navigation in an environment**

Soon after the discovery of place cells, O'Keefe and Nadel (1978) proposed that
place cells in the hippocampus could form the neural substrate of a "cognitive map"
(Tolman, 1948). A cognitive map is a mental process that represents an environment,
allowing an animal to navigate across the environment. Evidence supporting this
view came from studies that correlated place cell activity with behavior in spatial
navigation tasks. O'Keefe and Speakman (1987) trained rats to perform spatial
memory tasks on a four-arm radial maze (Figure 1D). The rats were trained to find a
food reward in a goal arm, which was always at the same position relative to spatial
cues in the experimental room. They found that, in successful trials, the majority of
place cells had place fields at stable locations relative to the goal arm ("perceptual"
in Figure 1D). In some trials, all spatial cues were removed before the rats were introduced into the maze. In these trials, the rats’ performance in choosing the goal arm assigned by the experimenter was no better than chance. Place cell activity was still observed, but the place fields were not at the same locations relative to the goal arm as observed in the trials with spatial cues (“experimenter’s goal” in Figure 1D). However, if they analyzed the positions of place fields relative to the arm that the rats chose (or “thought was the goal arm”) in the individual trials, the positions of the place fields were at the same location relative to the goal arm as found in the trials with spatial cues (“rat’s goal” in Figure 1D). Similar correspondence between the successful choice of a goal location and stable spatial firing patterns relative to the goal location was found in a spatial alteration task using a three-arm maze (Lenck-Santini et al., 2001). These findings suggest that spatial firing patterns of place cells are closely related to the rat’s perception of goal location.

Place cell activity reflects spatial memory

Studies have also indicated that place cells may have a role in holding information for spatial memory. In the studies by O’Keefe and Speakman (1987) described above, the authors performed another experiment in which they first introduced rats into the four-arm radial maze and then removed all the spatial cues. After 30-60 sec, the rats were allowed to choose the goal arm for a food reward. In this task, the rats showed good performance in choosing the correct goal arm. During the successful trials (“Memory” in Figure 1D), the place cells showed place fields in the same locations as in the task with visible spatial cues. To successfully perform this task without visual cues, the rats needed to retain information about (or memorize) the spatial relationships among the maze and the cues in the experimental room. The stable place fields observed after cue removal indicate that place cell activity is somehow related to the memory required for successful performance, potentially as a mechanism to retain spatial information. Similarly, stable place fields have been observed after the loss of visible spatial cues when the experimental room was darkened while animals were exploring the environment (Quirk et al., 1990).

Formation of place cell activity in a novel environment

In most of the studies mentioned so far, place cell activity was monitored in environments to which the animals were well familiarized beforehand. However, when an animal encounters an environment for the first time, what happens to place cell activity? This question has been addressed by recording place cell activity when an animal was introduced into a novel environment to which the animal had never been exposed.

Hill (1978) monitored place cell activity in a novel T-maze and found that 10 out of 12 recorded place cells started location-specific firing even from the first time the rats passed specific locations in the T-maze. This location-specific place cell activity seemed to be fully established from the beginning, as their firing rates did not show systematic changes during subsequent visits. The other two cells were inactive during the first visits but started location-specific firing after several visits.
A similar experiment was performed by Frank et al. (2004). They first familiarized rats to a T-maze consisting of three arms of a radial maze, after which they replaced one of the arms with a novel arm that the rats had never explored before. The authors found that place cell activity was highly unstable during the first few minutes in this novel arm; i.e., some place cells started showing location-specific firing, while others decreased their firing rate and field size. This initial period of instability may reflect the development of a spatial representation of the novel arm. Thereafter, place cell activity in the novel arm stabilized.

Wilson and McNaughton (1993) first familiarized rats to a square enclosure and then removed one wall of the enclosure to allow the rats to explore another square arena that was novel to the rats. Once the rats started exploring the novel arena, place fields rapidly developed and became stable after 6-10 min of exploration. Thus, place cell activity was not fully established upon first entry into a novel environment but developed rapidly with experience.

Kitanishi et al. (2015) first familiarized rats to a square enclosure in one room and then made the rats explore another square enclosure in a different room, to which the rats had never been exposed. They found that the firing rate of place cells increased gradually over 10-min sessions and that their firing patterns became stabilized. These studies differ somewhat in the details of their findings, possibly due to differences in the nature of novelty in the environments. However, overall, these studies indicate that place cell activity rapidly develops within a few minutes of the first exposure to a novel environment before stable spatial firing patterns appear.

**Stability and plasticity of place cell activity**

As described above, the spatial firing patterns of place cells stabilized after the first few minutes of instability/development. A number of studies observed stable place fields that lasted days (Kentros et al., 1998; Kitanishi et al., 2015; Muller and Kubie, 1987), and, in an extreme case, a study described highly stable place cell activity that lasted for several months (Thompson and Best, 1990). A recent study by Ziv et al. (2013) used a miniaturized microendoscope in freely moving mice and showed that spatial firing patterns of individual place cells are generally stable over months. However, they also found that many place cells that were active on one day were inactive on other days, with only 15-25% of place cells active on both days. Thus, active subsets of place cells may change between days with a relatively low overlap. However, one caveat is whether the Ca^{2+} events measured in this study always reflect the electrical spike firing measured in conventional place cell studies.

Even though place cell firing can be stable over long periods of time, place cell activity in a familiar environment can has also been reported to show plasticity reflecting the animal’s experience. Mehta et al. (1997) showed experience-dependent changes within a recording session that lasted for dozens of minutes. Firing rate and place field size increased with multiple passages through place fields, and the centers of place fields shifted backwards relative to the animal’s movement
direction on linear tracks. Lever et al. (2002) showed long-term divergence of place cell activity inside two enclosures with different shapes. They exposed rats to square and circular enclosures multiple times over a month and found that the spatial firing patterns of place cells, which were initially similar, gradually and incrementally diverged between the two enclosures.

**Long-term potentiation**

A few years after the discovery of place cells, Bliss and Lømo (1973) discovered an increase in the efficiency of synaptic transmission following repeated stimulation, which lasted for hours, days or longer. This experimentally induced phenomenon is known as long-term potentiation (LTP) and is often regarded as the cellular basis of memory (Martin et al., 2000). Learning in a memory task has been shown to physiologically induce LTP-like synaptic plasticity in the CA1 area (Whitlock et al., 2006). Furthermore, a large number of studies have shown that the blockade of LTP leads to memory impairment in spatial navigation tasks (Morris et al., 1986; Martin et al., 2000). Because place cell activity has been suggested to be the basis for spatial memory at the neuronal firing level, the mechanistic relationship between LTP and place cell activity has attracted the interest of many researchers in the field. In the following sections, we review the studies that have shown evidence that LTP is involved in place cell activity.

**LTP induction modifies place cell activity**

A simple test for evaluating the involvement of LTP in place cell activity is to examine whether LTP induction alters any aspect of place cell activity. Dragoi et al. (2003) conducted such an experiment by inducing LTP in the CA1 (and CA3) area via electrical stimulation using electrodes placed in the contralateral ventral hippocampal commissure. They found that LTP induction altered the spatial firing patterns of place cells in a familiar environment in a similar way to remapping; i.e., the place field locations of individual place cells changed, but other firing properties, such as firing rate and place field size, were not affected. These results suggest that LTP-like plasticity modifies place cell activity and may be a mechanism mediating remapping.

**NMDA receptor dependent form of LTP in the CA1 area**

Another approach to test the involvement of LTP in place cell activity is to examine the effects of blocking or otherwise manipulating LTP. Investigations of the molecular mechanisms of LTP have revealed genes/proteins involved in LTP and have provided many different approaches for experimental manipulation. Among the different types of LTP, the most extensively studied is the NMDA receptor-dependent form of LTP in the synapses between Schaffer collaterals and pyramidal cells in the CA1 area. Most studies testing the involvement of LTP in place cell activity have been performed by targeting this form of LTP in the CA1 area. Therefore, here, we
briefly review the molecular mechanism underlying the NMDA receptor-dependent form of LTP in the CA1 area (Herring and Nicoll, 2016).

NMDA receptors are ionotropic glutamate receptors that are permeable to cations, including Ca$^{2+}$. The binding of glutamate released from presynaptic terminals, together with the removal of Mg$^{2+}$ caused by postsynaptic depolarization, allows Ca$^{2+}$ ions to enter postsynaptic neurons. The influx of Ca$^{2+}$ leads to the activation of downstream signaling pathways mediated by different types of protein kinases. One such kinase is Ca$^{2+}$/calmodulin-dependent kinase IIα (CaMKIIα) (Lisman et al., 2012), which is known to be the most abundant protein in the brain (Erondu and Kennedy, 1985). Upon Ca$^{2+}$ influx through NMDA receptors, CaMKIIα phosphorylates AMPA receptors, another glutamate receptor subtype that mediates a major part of postsynaptic depolarization. This phosphorylation induces an increase in the number of AMPA receptors (Barria et al., 1997; Tan et al., 1994) in the postsynaptic membrane and enhances synaptic strength (Liao et al., 1995; Malinow et al., 1989; Waxhamll, 1989). These processes are supported by existing proteins and do not require new transcription or protein synthesis.

LTP induction also triggers different signaling cascades, involving cyclic AMP (cAMP), protein kinase A (PKA) (Abel et al., 1997; Frey et al., 1993), extracellular signal-regulated kinase (ERK) (Selcher et al., 2003), and cAMP response element-binding protein (CREB) (Barco et al., 2002), which lead to the ‘late phase’ of LTP. The late phase of LTP requires transcription and protein synthesis to maintain increased synaptic strength over a longer period. New protein synthesis occurs either from pre-existing mRNA or new mRNA synthesized in the nucleus. The latter includes a class of genes called immediate early genes, which are expressed rapidly and transiently in response to LTP. Some immediate early genes, such as c-fos and zif268 (Worley et al., 1993), encode transcription factors that regulate gene expression, whereas others such as Arc (Plath et al., 2006) are effectors that mediate functional and structural changes underlying LTP. LTP induction has also been suggested to lead to the translation and persistent activation of protein kinase Mζ (PKMζ) (Ling et al., 2002), which is essential for the long-term maintenance of synaptic strength potentiated by LTP.

Roles of NMDA receptors in place cell activity in the CA1 area

As a key molecule in initiating the signaling cascade, the NMDA receptor has been the focus of many studies. First, McHugh et al. (1996) examined the role of NMDA receptors in the CA1 area. Using cre/loxP technology in transgenic mice, they removed the NR1 gene (also known as GluN1) selectively in the CA1 area (Tsien et al., 1996). The regional specificity of this gene removal is age dependent (Fukaya et al., 2003). By 1.5 months of age, the NR1 mRNA and protein levels are reduced specifically in the CA1 area and subiculum, whereas by 2 months of age, the NR1 gene is absent from much wider areas in the forebrain. Thus, within a short time window around the age of 1.5 months, the NR1 gene knockout in these mice is specific to the CA1 area and subiculum. In these conditional knockout mice, NMDA
receptor-mediated synaptic currents were completely inhibited in CA1 pyramidal neurons, and LTP was impaired in Schaffer collateral-pyramidal cell synapses.

McHugh et al. (1996) examined place cell activity in the CA1 area of these mice at 4 months of age or older. They observed place cells with normal firing rates and whose spatial firing patterns were stable among multiple sessions distributed over hours. However, place cell firing was more diffuse over an environment (Figure 4A). These findings suggest that the NMDA receptor is not required for the generation of stable place cell activity but is essential for the normal location specificity of place cell firing. Cabral et al. (2014) analyzed firing location in individual passages through a place field and found that the diffuse firing patterns in the NR1 knockout mice were caused by variations in the locations of place fields between individual passages, whereas the size of place fields in individual passages were comparable to wild-type mice.

Other studies used a pharmacological method to block the NMDA receptor. By performing systemic injections of (±)-3-(2-carboxypiperazin-4-yl) propyl-1-phosphonic acid (CPP), a competitive NMDA receptor antagonist, Kentros et al. (1998) examined the role of NMDA receptors in the longer-term stability of place cell activity over days (Figure 4B). CPP injection did not affect the stability of place cell activity in a familiar environment, which was established during previous exposure to the familiar environment. Next, CPP was administered to the rats immediately before exposure to a novel environment. Spatial firing patterns were generated normally, and similar patterns re-appeared when the rats were re-exposed to the novel environment after a few hours. These findings are consistent with those of McHugh et al. (1996). However, when the rats were re-exposed to the novel environment on the following day, the place cells showed new spatial firing patterns that were similar to remapping. These findings suggest that NMDA receptors are required either for the formation of long-lasting, new spatial firing patterns and/or for the conversion of unstable, newly formed firing patterns to stable ones.

As mentioned earlier, place cells normally show an increase in the firing rate and size of their place fields and shift their place fields backwards relative to an animal’s movement after repeated passages through their place fields in a familiar linear track environment (Mehta et al., 1997). Ekstrom et al. (2001) found that these changes were abolished after the systemic injection of CPP, suggesting that NMDA receptors are also involved in experience-dependent plasticity of place cell activity in familiar environments.

Although there are discrepancies in details, overall, these studies indicate that NMDA receptors contribute to place cell activity. One potential explanation for the differences is that genetic ablation achieves a more complete blockade of NMDA receptor function compared with NMDA receptor antagonists. An alternative explanation is that genetic ablation results in chronic blockade of NMDA receptor signaling and that the accumulated effects of this genetic ablation may disrupt the basic firing properties of place cells in familiar environments. In contrast, transient inhibition of NMDA receptor function may specifically affect the formation and plasticity of place cell activity while keeping other basic properties intact.
Manipulation of LTP mechanisms downstream of the NMDA receptor impairs place cell activity in the CA1 area

Studies have also shown the involvement of signaling pathways downstream of the NMDA receptor in place cell activity.

CaMKIIα
Rotenberg et al. (1996) used transgenic mice carrying a mutant CaMKIIα gene with a Thr -> Asp substitution at codon 286. This single-codon mutation mimics autophosphorylation and renders CaMKIIα constitutively active even in the absence of Ca^{2+} ions (Fong et al., 1989). In these transgenic mice, LTP induction through high-frequency stimulation (100 Hz) was intact. However, a type of LTP, which is normally induced by low-frequency stimulation (5-10 Hz), was impaired (Mayford et al., 1995). Although place cells were detected in the CA1 area of these transgenic mice, these place cells were found less frequently, and their firing patterns were less precise and stable. Other studies using inhibitory mutants of CaMKIIα found similar impairment in the location specificity and stability of spatial firing patterns (Cho et al., 1998; Cho et al., 2012; Caccucci et al., 2007). Taken together with the work of Rotenberg et al. (1996), these results suggest that the proper regulation of CaMKIIα activity is essential for generating intact place cell activity.

Synaptic delivery of the AMPA receptor
Kitanishi et al. (2015) examined a role of synaptic delivery of the AMPA receptor in place cell activity. Using a viral vector, they expressed a dominant-negative mutant of GluR1 (an AMPA receptor subunit, also known as GluA1) to interfere with the synaptic delivery of GluR1-containing AMPA receptors and blocked LTP in a small portion of the CA1 area. In a novel environment, the rapid appearance of place fields within the first few minutes was compromised. Furthermore, the size of the place fields was larger during the first few 10-min sessions in the novel environment. In contrast, no impairment was observed in a familiar environment. These results indicate that synaptic delivery of GluR1-containing AMPA receptors is essential for the rapid formation of place fields with high location specificity in novel environments.

PKA
The involvement of the late phase of LTP, which requires PKA and protein synthesis, has also been investigated in the generation of place cell activity. Rotenberg et al. (2000) used transgenic mice expressing a dominant negative mutant of PKA, R(AB), in the large area of the forebrain that includes the hippocampus. These R(AB) transgenic mice were found to have reduced PKA activity in the hippocampus, displaying normal early-phase LTP but impaired late-phase LTP (Abel et al., 1997). Rotenberg et al. (2000) recorded place cell activity when these transgenic mice explored a novel environment for the first time (0 h), as well as after 24 h and 25 h. Although the spatial firing patterns of place cells appeared to be intact at each time point, comparing the patterns at 0 and 24 h revealed that the stability of the place fields was impaired in R(AB) transgenic mice. In contrast, the stability from 24 to 25 h was not compromised in these mice. These results suggest that the long-term stability of place cells in a novel environment requires intact PKA activity and intact late-phase LTP.

Protein synthesis
Agnihotri et al. (2004) examined the role of protein synthesis in the stability of place cell activity in the CA1 area. They recorded place cell activity in novel and familiar environments in sessions before and after the injection of a protein synthesis inhibitor, anisomycin, and evaluated the stability of spatial firing patterns. In a novel environment, stability up to 1 h was not affected by anisomycin. However, when anisomycin-injected mice were exposed to the same novel environment again at 6 h, spatial firing patterns differed substantially from those at 1 h, indicating that the stability of place cell activity was compromised. Such impairment in place cell activity was not observed in a familiar environment. These results suggest that new protein synthesis is not necessary for previously formed/stabilized place cell activity or for the short-term stability of newly-formed place cell activity. However, new protein synthesis is essential for the long-term maintenance of newly formed firing patterns in a novel environment.

**PKM zeta**

Barry et al. (2012) examined the role of PKMζ in place cell activity using the pseudosubstrate Zeta Inhibitory Polypeptide (ZIP), which has been shown to de potentiate previously established LTP (Ling et al., 2002) and is suggested to erase previously formed memory (Pastalkova et al., 2006). Bilateral injection of ZIP into the dorsal hippocampus resulted in immediate disruption of otherwise-stable spatial firing patterns in a familiar environment. Spatial firing patterns re-appeared after a 2-6-h recovery period. However, these post-injection firing patterns differed from those before ZIP injection in ways that mimicked remapping. These results suggest that PKMζ, and presumable the maintenance of LTP, is essential for maintaining stable spatial firing patterns of place cells.

**Where does LTP-like plasticity occur to establish place cell activity in the CA1 area?**

Most of the above studies manipulated LTP over large brain areas, thereby obscuring the precise areas where LTP-like plasticity is required to generate place cell activity in the CA1 area. By applying cre/loxP conditional knockout technology to the CA1 area, McHugh et al. (1996) observed diffused spatial firing patterns in the place cells of the CA1 area, suggesting that fine spatial representation of place cells requires NMDA receptor-dependent LTP in the excitatory neurons of the CA1 area itself. However, in addition to the issue of age-dependent region specificity mentioned above (Fukaya et al., 2003), impaired learning in these mice obscures the existence of such cell-autonomous regulation. With these potential caveats in mind, Kitanishi et al. (2015) used viral vectors to achieve local genetic manipulation of LTP through the inhibition of AMPA receptor trafficking, affecting only a minor portion of the CA1 area. With this local genetic manipulation, place cells exhibited diffuse spatial firing patterns in the LTP-manipulated area, but not in other non-affected areas in the same animal, during the exploration of a novel environment. This finding supports the role of LTP-like plasticity in mediating precise spatial representation by place cells in the CA1 in a cell-autonomous manner. Kitanishi et al. (2015) also found that LTP blockade in the CA1 area does not affect the stability of place cell
activity over one day. Considering that other widespread manipulations of LTP (Kentros et al., 1998; Agnihotri et al., 2004) did affect the stability of place cell activity, LTP-like plasticity outside the CA1 area should be responsible for the stability of place cell activity in the CA1 area. Thus, LTP-like plasticity in the CA1 area seems to be essential for the establishment of fine place fields in CA1 place cells, whereas the stability of place cell activity in the CA1 area may be determined by LTP-like plasticity outside the CA1 area.

Concluding remarks

As we reviewed above, studies have largely demonstrated that electrophysiological, genetic or pharmacological manipulations of NMDA receptor-dependent LTP in the CA1 area, result in the impairment of place cell activity. These results support the idea that LTP-like synaptic plasticity plays an essential role in place cell activity; synaptic strengthening facilitates the establishment of new, stable spatial firing patterns of place cells in novel environments, and the maintenance of potentiated synaptic strength sustains established patterns in familiar environments. However, many important questions remain unanswered. For example, what is the role of different types of LTP in place cell activity? Do they mediate different aspects of place cell activity? How do other genes/proteins underlying LTP contribute to place cell activity? Further investigation is required to fully understand the role of LTP-like synaptic plasticity in place cell activity.

Figure legends

Figure 1. Characteristics of place cell activity. (A) A schematic illustrating a typical experiment in a square enclosure. The top image shows the animal's trajectory as a gray line, while the bottom image uses red dots to represent the locations of place cell firing. (B) Color-coded rate maps for a place cell recorded while a rat explored a cylindrical enclosure with a cue card placed on its wall (shown by a curved line). Three recording sessions were performed when the cue card was placed at the 3 o’clock (left), 12 o’clock (middle) and then 3 o’clock (right) positions. Between each session, the cue card was moved while the rat was taken out of the enclosure. Note that the movement of the place field followed the movement of the cue card. In B and C, blueish colors represent high firing rates, while yellowish colors indicate low rates. Adapted from Muller et al. (1987). (C) Color-coded rate maps show the “remapping” of three place cells (cell 1, 2, and 3). These cells were recorded in a cylindrical enclosure with either a white (left side) or black (right side) cue card. Note that the location of the place fields changed depending on which card was placed. In the case of cell 2, the place field disappeared when the black card was used. Reproduced with permission from Bostock et al. (1991). (D) The contour plots show the firing rate of a place cell recorded from a rat in a four-arm maze. In this experiment, the rat was trained to navigate to a goal arm position relative to
visual spatial cues. The four panels show rate maps recorded under different conditions. “Perceptual” indicates recording sessions in the presence of visual spatial cues. “Memory” indicates recording sessions after the visual cues were removed in the presence of the rat in the maze. Note that spatial firing patterns are similar between the perceptual and memory periods. “Control” indicates recording sessions where the rat was introduced into the maze in the absence of visual cues. “Experimenter’s goal” shows a rate map formed relative to a goal arm arbitrarily chosen by the experimenter, while “rat’s goal” represents a rate map formed relative to a goal arm chosen by the rat. Note that the “rat’s goal” map shows a similar pattern to the perceptual and memory maps. Adapted from O’Keefe et al. (1987).

**Figure 2.** A group of place cells can signal which position in which environment an animal is located.

**Figure 3.** Molecular mechanism underlying NMDA receptor-dependent LTP in the CA1 area. NMDA receptor activation leads to a postsynaptic increase in Ca2+. The increased Ca2+ activates multiple types of protein kinases, including the Ca2+/calmodulin-dependent kinase IIα (CaMKIIα), protein kinase A (PKA), and protein kinase Mζ (PKMζ). The activation of CaMKIIα results in the phosphorylation of AMPA receptors, increasing the number of AMPA receptors in the postsynaptic membrane and strengthening the postsynaptic response. PKA activates gene expression via cyclic AMP response element-binding protein (CREB), increasing the transcription of downstream genes, including immediate early genes (e.g., activity-regulated cytoskeleton-associated protein (Arc)). The maintenance of established LTP requires persistent PKMζ activation.

**Figure 4.** Genetic or pharmacological blockade of LTP results in impaired place cell activity. (A) Color-coded rate maps of place cells in the CA1 area of wildtype (control) and forebrain-specific NR1 knockout (CA1-KO) mice. The color code represents the normalized firing rate, as shown in the right bar. The maps from three recording sessions (Run 1, 3, 5) are shown. Note that although the place fields are stable among sessions in both mice, the sizes of place fields are larger in NR1 knockout mice. Reproduced from McHugh et al. (1996) (B) Rate maps of four place cells in the CA1 area of a rat injected with CPP. Two recording sessions (D1W1 and D1W2) were performed in a novel environment within a few hours after CPP injection. On the next day, two additional sessions (D2W1 and D2W2) were recorded in the same environment. Note that stable place fields were observed in Cells 1 and 4 on the first day but that different spatial firing patterns appeared on the second day. Reproduced from Kentros et al. (1998) (C) Color-coded rate maps of 2 place cells each from GFP- (control) and GluR1-c-tail-expressing portions of the CA1 area. Rate maps in the first session in a novel environment (B1) represent 2-min periods divided from 10-min sessions. Maps for the fourth session in the same environment are shown as a reference. The right graph shows that firing rates inside the place fields gradually increased over 10 min in place cells from both control and GluR1-c-tail-expressing CA1 area. Note that the firing rate in the first 2 min was significantly lower in place cells in the GluR1-c-tail-expressing CA1 area. Adapted from Kitanishi et al. (2015).
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Figure 1

Figure 2
Figure 3
Figure 4