Molecular, Cellular, and Neuroanatomical Substrates of Place Learning

Alcino J. Silva, Karl Peter Giese, Nikolai B. Fedorov, Paul W. Frankland, and Jeffrey H. Kogan
Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724

Learning and remembering the location of food resources, predators, escape routes, and immediate kin is perhaps the most essential form of higher cognitive processing in mammals. Two of the most frequently studied forms of place learning are spatial learning and contextual conditioning. Spatial learning refers to an animal’s capacity to learn the location of a reward, such as the escape platform in a water maze, while contextual conditioning taps into an animal’s ability to associate specific places with aversive stimuli, such as an electric shock. Recently, transgenic and gene targeting techniques have been introduced to the study of place learning. In contrast with the abundant literature on the neuroanatomical substrates of place learning in rats, very little has been done in mice. Thus, in the first part of this article, we will review our studies on the involvement of the hippocampus in both spatial learning and contextual conditioning. Having demonstrated the importance of the hippocampus to place learning, we will then focus attention on the molecular and cellular substrates of place learning. We will show that just as in rats, mouse hippocampal pyramidal cells can show place specific firing. Then, we will review our evidence that hippocampal-dependent place learning involves a number of interacting physiological mechanisms with distinct functions. We will show that in addition to long-term potentiation, the hippocampus uses a number of other mechanisms, such as short-term-plasticity and changes in spiking, to process, store, and recall information. Much of the focus of this article is on genetic studies of learning and memory (L&M). However, there is no single experiment that can unambiguously connect any cellular or molecular mechanism with L&M. Instead, several different types of studies are required to determine whether any one mechanism is involved in L&M, including (i) the development of biologically based learning models that explain the involvement of a given mechanism in L&M, (ii) lesion experiments (genetics and pharmacology), (iii) direct observations during learning, and (iv) experiments where learning is triggered by turning on the candidate mechanism. We will show how genetic techniques will be key to unraveling the molecular and cellular basis of place learning.

INTRODUCTION

Learning and remembering the location of food resources, predators, escape routes, and immediate kin is perhaps one of the most essential forms of higher cognitive processing in mammals (O’Keefe and Nadel, 1978). Understandably, there is an extensive behavioral, neuroanatomical, and electrophysiological literature on the neural systems and mechanisms supporting place learning. Two of the most often studied forms of place learning are spatial learning and contextual conditioning. Spatial learning refers to an animal’s capacity to learn the location of a reward, such as the escape platform in a water maze (Morris,
SUBSTRATES OF PLACE LEARNING

1981), and contextual conditioning taps into an animal's ability to associate specific places with aversive stimuli, such as an electric shock (Fanselow, 1989). Both spatial learning and contextual conditioning depend on the animal's ability to learn and remember details about the attributes and features of specific places (e.g., maze room, conditioning chamber), and therefore it is not surprising that they share much in common. However, there are also many differences between these two types of associative learning that make appropriate comparisons between the two both interesting and revealing.

Recently, transgenic and gene targeting techniques have been introduced to the study of place learning (Silva, Paylor, Wehner, & Tonegawa, 1992a; Silva, Stevens, Tonegawa, & Wang, 1992b). It is now feasible to add, delete, or modify any gene, and techniques currently under development will allow complete control over when and where these genetic lesions take place. The studies carried out so far demonstrate that mutations of neuronal genes can yield very specific and informative phenotypes. This specificity is crucial for forging connections between the molecular, cellular, neuroanatomical, and behavioral phenotypes of the mutant mice. Such integrative analyses of mutant mice have a crucial role in uncovering mechanisms of learning and memory (L&M), such as those supporting place learning.

In contrast with the abundant literature on the neuroanatomical substrates of place learning in rats, very little has been done in mice (Chen, Kim, Thompson, & Tonegawa, 1996; Logue, Paylor, & Wehner, 1997). This lack of information is problematic for the interpretation of transgenic and gene targeting studies of place learning in mice. Additionally, there are several issues that need to be resolved concerning the involvement of structures such as the hippocampus in place learning. For example, despite a well-established role of the hippocampus in spatial learning, its involvement in contextual conditioning is still controversial. This is puzzling since both spatial learning and contextual conditioning seem to require rapid processing of polymodal information (the multiple cues in the room or in the conditioning chamber), a function that has traditionally been assigned to the hippocampus. Thus, in the first part of this article, we review our studies on the involvement of the hippocampus in both spatial learning and contextual conditioning (Cho, Friedman, & Silva, 1998a; Frankland, Cestari, Filipkowski, McDonald, & Silva, 1998). We show that although the hippocampus is normally crucial for processing information about place, mice with hippocampal lesions can use alternative strategies/systems to recognize specific places.

Having demonstrated the importance of the hippocampus to place learning, we then focus our attention on the molecular and cellular substrates of place learning. We show that just as in rats, mouse hippocampal pyramidal cells can show place specific firing (Cho, Giese, Silva, & Eichenbaum, 1998b). Then, we review our evidence that hippocampal-dependent place learning involves a number of interacting physiological mechanisms with distinct functions. We show that in addition to long-term potentiation (LTP), the hippocampus uses a number of other mechanisms, such as short-term plasticity (Silva, Rosahl, Chapman, Marowitz, Ciocchi, Sudhof, & Bourchuladze, 1996) and changes in spiking (Giese, Storm, Reuter, Fedorov, Shao, Leicher, Pongs, & Silva, 1998b), to process, store, and recall information. Much of the focus of this article is on genetic studies of L&M, but we also show that there is no single experiment that can unambiguously connect any cellular or molecular mechanism with
L&M. Instead, several different types of studies are required to determine whether any one mechanism is involved in L&M, including (i) the development of biologically based learning models that explain the involvement of a given mechanism in L&M, (ii) lesion experiments (genetics and pharmacology), (iii) direct observations during learning, and (iv) experiments where learning is triggered by turning on the candidate mechanism. We show how genetic techniques will be a key to unraveling the molecular and cellular basis of place learning.

NEUROANATOMICAL SUBSTRATES OF PLACE LEARNING

The Hippocampus Is Required for Spatial Learning in Mice

Rats with damage to the hippocampus and related structures are unable to show spatial learning in the Morris water maze (Morris, Garrud, Rawlins, & O’Keefe, 1982; Sutherland, Kolb, & Whishaw, 1982). In this maze animals search for a submerged platform in a round pool filled with murky water (Morris, 1981). We have found the same spatial learning impairment in hippocampus-lesioned mice (Cho et al., 1998a). The lesioned animals are, however, fully capable of swimming to a platform marked with a visible cue, indicating that their vision, motor coordination, and motivation to escape the water are unaffected by the lesions.

Interestingly, studies with ibotenic acid in rats showed that only lesions that deleted more than 80% of the dorsal hippocampus resulted in significant water maze deficits (Moser, Moser, Forrest, Andersen, & Morris, 1995). In contrast, our studies with ibotenic-lesioned mice show that even small lesions (<50% of dorsal hippocampus) result in profound spatial learning deficits (Cho et al., 1998a). However, our studies also show that prior training in the visible-platform task (nonspatial) improved the performance of the hippocampus-lesioned mice during spatial training (Cho et al., 1998a), suggesting that damage to the hippocampus may not entirely prevent all spatial learning. Indeed there is evidence that the cingulate cortex (Sutherland, Whishaw, & Kolb, 1988), the caudate nucleus (Packard, Hirsh, & White, 1989), and the parietal cortex (DiMattia and Kesner, 1988) are involved in spatial learning. Nevertheless, studies in rats, mice, and even humans (e.g., Aguirre, Detre, Alsop, & Esposito, 1996; Cammalleri, Gangitano, D’Amelio, Raieli, Raimondo, & Camarda, 1996; Maguire, Burke, Phillips, & Staunton, 1996a; Maguire, Frackowiak, & Frith, 1996b) have demonstrated the central involvement of the hippocampus in spatial learning.

The Hippocampus Is Not Essential for Recognition of an Aversive Context

Contextual fear conditioning is a form of associative learning, where animals learn to recognize the place [conditioned stimulus (CS)] where they were previously shocked [unconditioned stimulus (US)]. Contextual conditioning is identified as an increase in a range of conditioned responses, including autonomic (e.g., increased heart rate) and behavioral (e.g., freezing) changes, that are associated with fear when an animal is in the training environment. Freezing, a cessation of all bodily movement aside from respiration, has been the most widely measured conditioned response in this task. Studies in our laboratory showed that the very same hippocampal ibotenate lesions that abolished
spatial learning did not block contextual conditioning in the lesioned mice (Cho et al., 1998a). We also obtained similar results with electrolytic lesions of the dorsal hippocampus (Frankland et al., 1998). Our results are consistent with recent reports in rats and mice (Logue et al., 1997). This indicates that pretraining damage to hippocampal neurons does not abolish contextual conditioning. However, it is important to note that our results show evidence for attenuated conditioning freezing in hippocampus-lesioned mice. Therefore, lesioning the hippocampus attenuates, but does not block, both spatial learning and contextual conditioning, indicating that nonhippocampal systems are less efficient at processing place information.

The Hippocampus Normally Suppresses Other Systems That Can Mediate Recognition of an Aversive Context

In contrast with pretraining lesions, results in our laboratory showed that recognition of an aversive place (contextual conditioning) is completely abolished by posttraining lesions of the hippocampus (Frankland et al., 1998). This demonstrates that while the hippocampus is not essential for recognition of the place/context in which the animals were shocked, it is normally involved in processing contextual information. In addition, the results demonstrate that strategies mediated by nonhippocampal systems are normally suppressed when the hippocampus is functional. If there was no suppression of processing by nonhippocampal systems, then pre- and posttraining lesions would have both failed to block place recognition. Our conclusion is in agreement with an earlier study suggesting that spatial strategies mediated by hippocampal systems suppress the learning of a conditioned cue preference in a radial arm maze (McDonald and White, 1995). But, why would the brain have a system capable of processing place information that is only functional in animals with hippocampal lesions? The answer is that nonhippocampal systems are normally used for processing very salient cues that are tightly associated with the US (see below). Other less salient cues are normally overshadowed by hippocampal suppression.

Single-Cue Strategies versus Polymodal-Cue Strategies in Place Recognition

Previous studies proposed that the hippocampus is essential for integrating different modalities of information (tactile, olfactory, auditory, etc.) into a single polymodal CS (Nadel, Willner, & Kurz, 1985; Sutherland and Rudy, 1989), which could then be associated with the US. In contrast, associations between single cues within the context and the US may also be sufficient for an animal to recognize an aversive context. Therefore, the results in our laboratory suggest that in animals with hippocampal lesions, context conditioning may be mediated by associations between single cues in the context and the US. Nevertheless, our results also show that nonhippocampal strategies (e.g., cue-based) result in weaker conditioning (Frankland et al., 1997). This could be because individual cues from a conditioned context may occur in other neutral contexts, thus decreasing their predictive value. In contrast, a context includes a unique combination of cues, and the exact same combination is less likely to occur elsewhere by chance. Therefore, in general, contextual CSs should have greater predictive value than single cues from within that context.
Pretraining Lesions of the Hippocampus Do Not Block Context Conditioning, but They Do Block Discrimination between Similar Contexts

Other recent findings in our laboratory provide evidence that animals with hippocampal lesions are using strategies based on single cue/US associations (Frankland et al., 1997). This was done by testing mice with pretraining lesions of the hippocampus on a task that should be optimally solved by context-based strategies: contextual discrimination (McDonald, Koerner, & Sutherland, 1995). In this task, mice were trained to discriminate between two similar (but not identical) chambers: one in which they were shocked, the other in which they were not. Because a large number of potentially salient cues (e.g., shock grid floor) were shared, reliance on single associations between these and the presence or absence of shock should result in equal levels of conditioned freezing in the two chambers. In contrast, context-based strategies should lead to higher levels of conditioning in the chamber where the animal was shocked. Consistent with this hypothesis, pretraining lesions of the hippocampus disrupted context discrimination. While control mice exhibited greater levels of freezing in the chamber associated with shock, hippocampus-lesioned mice showed robust, but equal, levels of freezing in both chambers (shock and non-shock chambers). Therefore, our results demonstrate that while nonhippocampal systems can support recognition of an aversive context, they are inefficient at discriminating between two similar contexts. Interestingly, consistent with the lesion results, mice with heterozygous mutations for the neurofibromatosis type I and the N-methyl-D-aspartate receptor (NMDAR) genes (Nf1/Nmdar1) are impaired in context discrimination but not in context recognition (Frankland et al., 1998). These mutant mice were previously shown to have deficits in the hippocampal-dependent version of the water maze (Silva, Frankland, Marowitz, Friedman, Lazlo, Cioffi, Jacks, & Bourtchuladze, 1997). The neuroanatomical and genetic lesion studies reviewed highlight the critical role that the hippocampus has in integrating polymodal contextual information. Even though context recognition can be processed elsewhere, perhaps supported by single cue associations, the hippocampus seems to be essential for polymodal representations of context.

Hippocampal Place Cells in Mice

The most convincing evidence that the hippocampus processes spatial information is the existence of place cells there (O'Keefe, 1976; O'Keefe and Dostrovsky, 1971). These pyramidal cells fire more actively when an animal is within a specific area referred to as a place field. Place cells were first described in rats, but recent studies have also demonstrated them in mice (Cho et al., 1998b; McHugh, Blum, Tsien, Tonegawa, & Wilson, 1996; Rotenberg, Mayford, Hawkins, Kandel, & Muller, 1996). Importantly, in mice and rats place cells appear to encode a polymodal representation of place and not simply salient landmarks in the animal's surroundings (Cho et al., 1998b).

MOLECULAR AND CELLULAR SUBSTRATES OF PLACE LEARNING

Most studies investigating the cellular mechanisms underlying place learning have focused on the possible role of hippocampal LTP. There is an extensive literature on the possible role of LTP in L&M, and therefore further experi-
ments, including genetic studies, are easy to frame within that rich experimental tradition. In contrast, studies examining the possible role of other cellular mechanisms in L&M face greater problems. Although there are numerous experimental results demonstrating that LTP could not possibly be the sole cellular mechanism used by the brain to process, store, and recall information (see below), comparatively little has been done to explore the potential roles of other mechanisms. In this section, we review studies illustrating the utility of genetics in unraveling the molecular and cellular basis of place learning. Altogether our studies reviewed here implicate LTP, short-term plasticity, and changes in spike properties in hippocampal processing of place information.

LTP Is Necessary but Not Sufficient for Place Learning

The interest in LTP as a mechanism of L&M is based on the Hebbian hypothesis that memories can be stored in neural circuits by adjusting synaptic strengths (Hebb, 1949). Thus, LTP is comfortably entrenched within the rich neuroconnectionist modeling tradition (Churchland and Sejnowski, 1992). This is an eloquent demonstration of the essential role of biological modeling in studies of L&M. Biologically based models can strengthen the connection between a given mechanism and L&M, since they provide an explanation of how the two are connected. Without a clear hypothesis it is difficult both to interpret the results of L&M experiments and to plan future studies.

The hippocampus expresses various forms of LTP and long-term depression (LTD). These electrophysiological phenomena have the very properties thought to be crucial for memory formation (associativity, specificity, reversibility and stability) (Bliss and Collingridge, 1993; Malenka, 1994). Additionally, electrophysiological observations during learning revealed that hippocampal neurons fire in patterns that are ideal for the induction of LTP (Larson, Wong, & Lynch, 1986; Otto, Eichenbaum, Wiener, & Wible, 1991). Although these findings indicate that LTP-like synaptic phenomena may occur during place learning, they do not show that LTP is required for learning.

The induction of some forms of LTP involves an NMDAR-dependent, postsynaptic increase in \([\text{Ca}^{2+}]\) (Bliss and Collingridge, 1993; Huang, Nguyen, Abel, & Kandel, 1996). This \([\text{Ca}^{2+}]\) increase activates protein kinases and other enzymes that trigger a stable increase in synaptic transmission (Bliss and Collingridge, 1993; Huang et al., 1996). Pharmacological blockers of LTP in rats (e.g., APV, an NMDAR blocker) were shown also to impair place learning (e.g., Davis, Butcher, & Morris, 1992; Kim, Fanselow, DeCola, & Fernandez, 1992), suggesting that a block of NMDAR-dependent LTP affects learning. Results from genetic lesion studies are consistent with a possible role for LTP in place learning. For example, mutations of either \(\alpha\)-Ca\(^{2+}\)-calmodulin kinase II (\(\alpha\text{CaMKII}\)) (Silva et al., 1992a), Fyn tyrosine kinase (Grant, O’Dell, Karl, Stein, Soriano, & Kandel, 1992), type I adenylate cyclase (Wu, Thomas, Villarces, Xia, Simmons, Chavkin, Palmiter, & Storm, 1995), NMDAR \(\epsilon_1\) subunit (Sakimura, Kutsuwada, Ito, Manabe, Takayama, Kushiya, Yagi, Aizawa, Inoue, Sugiyama, et al., 1995), or metabotropic glutamate receptor 5 (mGluR5) (Lu, Jia, Janus, Hendersen, Gerlai, Wojtowicz, & Roder, 1997) impair both LTP in the hippocampal CA1 region and place learning.

Our studies of mice lacking the \(\alpha\) and \(\Delta\) isoforms of the cAMP-responsive element binding protein (CREB\(^{\alpha,\Delta}\)) also revealed strong parallels between
LTP and place learning. These mutants have normal short-term memory (up to 1 h), but long-term memory is severely impaired. Similarly, LTP induced with a 100-Hz, 1-s tetanus (at 2 × pulselength of baseline) is present initially but disappears within 60 to 90 min (Bourtchuladze, Frenguelli, Blendy, Cioffi, Schutz, & Silva, 1994). Further studies showed that extended spaced training can rescue the memory deficits of these mutants (Kogan, Frankland, Blendy, Coblenz, Marowitz, Schutz, & Silva, 1996). In striking parallel, the deficits in LTP can also be rescued with additional spaced tetani (10-min intervals) (unpublished results). Massed tetani (1-min intervals) do not rescue LTP in the CREBΔ− mutants, just as massed training does not rescue their memory deficits (Kogan et al., 1996).

Thus, the evidence described above shows that (i) modeling studies, (ii) observations during learning, and (iii) two types of lesion experiments (pharmacological and genetic) in two different species (mice and rats) indicate that LTP is necessary for place learning. But is LTP alone sufficient for L&M? The studies reviewed below demonstrate that it is not.

If LTP were the only cellular mechanism involved in place learning, then all manipulations that affect LTP would also affect place learning. However, there is extensive evidence that this is not the case. For example, mutants lacking either the β1 isoform of the protein kinase A catalytic subunit (Cβ1−) (Qi, Zhuo, Skalhegg, Brandon, Kandel, McKnight, & Idzerda, 1996) or the β isoform of the inhibitory subunit (RIβ−) of this kinase (Brandon, Zhuo, Huang, Qi, Gerhold, Burton, Kandel, McKnight, & Idzerda, 1995) have deficient CA3 LTP, CA1 LTD, and CA1 LTP depotentiation (Brandon et al., 1995; Huang, Kandel, Varshavsky, Brandon, Qi, Idzerda, McKnight, & Bourtchuladze, 1995; Qi et al., 1996). However, tests with the water maze, Barnes maze, and contextual conditioning showed that place learning is normal in these two mutants (Huang et al., 1995). Additionally, recent pharmacological experiments suggest that the effects of NMDAR blockers on place learning tests, such as the water maze (Davis et al., 1992; Kim et al., 1992), may have been confounded by performance impairments caused by side effects of the drugs used (Cain, Saucier, Hall, Hargreaves, & Boon, 1996; Saucier and Cain, 1995). In addition, APV-treated rats pretrained on the behavioral demands of the water maze appeared to perform normally, indicating that NMDAR function (and, by consequence, NMDAR-dependent LTP) is not essential for spatial learning (Bannerman, Good, Butcher, Ramsay, & Morris, 1995; Cain et al., 1996; Saucier and Cain, 1995). Nevertheless, there are various forms of LTP in the hippocampus, and only some are dependent on the function of NMDARs (Huang et al., 1996). There are many other potential explanations for the failure to see learning deficits in animals treated with blockers of LTP (e.g., the tasks used may not be sensitive enough, the animals may compensate with some other mechanisms, the LTP block was not complete enough). The danger is that the very complexity that makes unraveling mechanisms of place learning extremely difficult also hinders the falsification of established models.

To Understand the Role of LTP in Place Learning, It Is Essential to Develop Insights into the Roles of Other Cellular Mechanisms

Paradoxically, one of the problems with determining the role of LTP in L&M is that most of the effort has been focused on LTP itself, and comparatively
very little attention has been given to other concurrent processes that may interact, complement, compensate for, and modify the role of LTP in L&M. It is impossible to determine where and how a single piece fits in a large puzzle if all the other pieces are missing.

The critical consequence of LTP between two neurons is that activation of the presynaptic neuron is more likely to induce an action potential in the other. Consequently, LTP can have an immediate effect on the dynamics of spiking in neural circuits. Since information in the brain is encoded by the number and/or pattern of action potentials, LTP should have a pronounced and lasting impact on neuronal networks. In addition to LTP, neurons have a myriad of other mechanisms capable of modulating spiking. For example, changes in the number or activity of most ion channels can also affect the dynamics of spike trains. Consequently, modulation of these channels could also have a role in learning. Just like LTP, short-term plasticity is also known to have a pronounced (although briefer) effect on spike dynamics. The studies that follow provide evidence that in mammals, mechanisms other than LTP have a role on place learning. Understanding how these mechanisms affect place learning will be crucial for better defining the role of LTP.

Short-Term Plasticity Is Involved in Place Learning

Short-term plasticity (STP) (Fisher, Fisher, & Carew, 1997; Zucker, 1989) could be responsible for short-term memory (Little & Shaw, 1975). Just as LTP is thought to be required for long-term memory, short-term changes in synaptic strength may store information for brief periods. For example, in any trial in the water maze, the animal has to continuously remember the places it just visited to organize its searches for the platform. This form of working memory may require synaptic changes (short-term plasticity) that can be induced and erased quickly. Long-term storage of this type of information could overwhelm the capacity of hippocampal networks with superfluous information. STP may also be critical for encoding the timing or sequence of events in neuronal networks (Buonomano & Merzenick, 1995). For example, the relative sequence of spatial information that the animals are exposed to, as they search for the hidden platform, may be critical for learning the spatial relationships among landmarks in the room. Since the animals cannot be exposed simultaneously to all the landmarks that surround the pool, the specific sequence of visual information acquired during their searches for the platform could be critical for learning the positions of these guiding landmarks.

Studies in our laboratory implicated STP in place learning. We studied four different lines of mutant mice lacking key presynaptic proteins that are known to affect the regulation of neurotransmitter release (Silva et al., 1996). Mice heterozygous for the α-calcium±calmodulin kinase II (αCaMKII+/−) have lower paired-pulse facilitation (PPF) and increased augmentation (Aug) (Chapman, Frenguelli, Smith, & Chen, 1995). Mice mutant for synapsin II (SyII−/−), as well as synapsin I and II mutants (SyI/II−/−), reveal normal PPF, but lower Aug. In contrast, mice lacking synapsin I (SyI−/−) show enhanced PPF, but normal Aug (Rosahl, Geppert, Spillane, Herz, Hammer, Malenka, & Sudhof, 1993; Rosahl, Spillane, Missler, Herz, Selig, Wolff, Hammer, Malenka, & Sudhof, 1995). Importantly, hippocampal CA1 LTP seemed unaltered in all of these mutant mice (Rosahl et al., 1993, 1995; Silva et al., 1996). The deletion of
synapsin II also does not disrupt LTP in the hippocampal mossy fiber pathway (Spillane, Rosahl, Sudhof, & Malenka, 1995). Consistent with a role for STP in learning, Sy II−/−, SyII−/−, and αCaMKII+/− mutants with a decrease in either PPF or Aug reveal place learning deficits, whereas the PPF enhancement does not disrupt place learning in Sy I−/− mice (Silva et al., 1996).

There is also evidence from other organisms suggesting that STP has a role in learning (Fisher et al., 1997; Zucker, 1989). Short-term changes in synaptic efficacy seem to underlie habituation of withdrawal responses in Aplysia (Castellucci, Pinsker, Kupfermann, & Kandel, 1970; Fisher et al., 1997) and the habituation of escape responses in vertebrate and invertebrate species (Auerbach and Bennett, 1969; Zilber-Gachelin and Chartier, 1973; Zucker, 1972). In addition, electrophysiological studies in the neuromuscular junction of Drosophila learning mutants revealed impairments in STP (Broadie, Rutherford, Skoulakis, & Davis, 1997; Zhong and Wu, 1991). It is conceivable that the central nervous systems (CNS) of these mutants also have similar impairments in STP and that these deficits are responsible for their L&M abnormalities (Tully, 1991).

The work summarized above supports the idea that STP is critical for learning. First, there are biologically based models that propose a role for STP in learning. Second, electrophysiological studies in invertebrate and vertebrate circuits showed that STP has a role in simpler forms of learning (Fisher et al., 1997). Third, our genetic lesion studies of STP also uncovered evidence (different mutants and different tasks) indicating that a decrease in either Aug or PPF results in learning impairments. Altogether these findings indicate that STP plays a role in learning.

A-Type K+ Currents Modulate Spiking and Learning

A-type K+ channels were proposed to have a role in invertebrate learning, such as in the conditioning of the phototactic behavior of Hermissenda. This type of conditioning reduces A-type K+ channel currents and increases excitability in neurons involved in the conditioned response (Alkon, 1984). Remarkably, single-electrode voltage-clamp studies in the motor cortex (layers III and V) of cats revealed a decrease in A-type K+ current and an increase in spike discharges in cells that developed a “conditioning response,” but not in cells that did not (Woody, Gruen, & Birt, 1991). Thus, just as in Hermissenda, Pavlovian conditioning in the motor cortex of cats results in a decrease of A-type K+ currents and in an increase in spike discharges (Woody et al., 1991). These results demonstrate changes in A-type K+ currents during learning, but they do not demonstrate that these changes are critical for learning.

Consistent with the hypothesis that A-type K+ channel function is required for learning, studies in Drosophila showed that the mutation of a Shaker A-type K+ channel impairs olfactory conditioning (Cowan and Siegel, 1986). To determine whether disruptions of A-type K+ channel function affect place learning, our laboratory derived a mouse mutant for an auxiliary β subunit (Kvβ1.1) (Giese et al., 1998b) that confers A-type inactivation on otherwise noninactivating Shaker-related K+ channels (Rettig, Heinemann, Wunder, Lorra, Parcej, Dolly, & Pongs, 1994). Analyses of the mutant mice suggest that the loss of Kvβ1.1 transformed some A-type K+ channels into noninactivating, delayed rectifier-type K+ channels. The Kvβ1.1 mutation, however, did not
eliminate all A-type K⁺ currents in the hippocampus, since there are α subunits capable of A-type fast inactivation (Sheng, Tsau, Jan, & Jan, 1992). Importantly, the deletion of Kv1.1 in the mutants resulted in a decrease both in spike broadening during repetitive firing and in the slow afterhyperpolarization (sAHP) (Giese et al., 1998b). Since the cumulative inactivation of A-type K⁺ channels during a spike train decreases the repolarization of later spikes, thereby increasing their duration (Ma and Koester, 1996), the decrease in the inactivation of A-type K⁺ channels in the mutant mice could be directly responsible for their reduction in spike broadening. Spike broadening can control Ca²⁺ influx during a spike train (Jackson, Konnerth, & Augustine, 1991). Therefore, the decrease in sAHP observed in the mutants may result from reduced spike broadening.

Consistent with the hypothesis that A-type K⁺ channel function is required for place learning, the Kv1.1 mutant mice showed impairments in the Morris water maze (Giese et al., 1998b). Remarkably, the Kv1.1 mutant mice learn the Morris water maze normally, but once trained could not relearn a new location for the hidden platform as well as controls. The mutation did not affect other behaviors tested, such as fear conditioning and exploratory behavior in an open field.

The analyses of the Kv1.1 mice demonstrate that A-type K⁺ channels affect the sAHP, and it suggests that the decrease in the sAHP is responsible for their impaired ability to reverse recently learned information. Consistent with the idea that the sAHP is involved in learning, previous studies in rabbits demonstrated that trace eye-blink conditioning, a hippocampus-dependent form of learning, results in a reduction of the sAHP lasting several days in the CA1 region of the hippocampus (Disterhoft, Thompson, Moyer, & Mogul, 1996). Perhaps this reduction of the sAHP is found in other systems after learning. For example, the increases in spike discharges observed in conditioned cells of the motor cortex of cats could be due to a reduction in the sAHP (Woody et al., 1991).

Based on the findings summarized above, we propose that during learning the transient A-type K⁺ currents are modulated so that they lose their fast inactivation and behave more like delayed rectifiers. This modulation results in a decrease in the width of spikes in a spike train (Ma and Koester, 1996), which then results in decreased calcium influx during the spike train, thus reducing the calcium-dependent sAHP (Storm, 1990). The sAHP is a key determinant of spike train characteristics. Large sAHPs suppress neuronal firing, while neurons spike more readily under conditions that reduce the sAHP. Importantly, a variety of biologically based models have proposed a key role for the sAHP in L&M (Hasselmo and Bower, 1993; Lisman and Idiart, 1995). For example, acetylcholine is known to suppress synaptic transmission and decrease the sAHP. This dual function has been proposed to serve as a filtering device that prevents the contamination of new memories with old memories inadvertently reactivated during learning (Hasselmo and Bower, 1993). The reduction of the sAHP may also serve to stabilize memory (Berner, 1991) by increasing the excitability in neurons encoding the learned information and ensuring their future coactivation.

How could the lower sAHP of the mutants result in their inability to modify learned information? The key behavioral finding is that while “naïve” mutants learned the location of a hidden platform in the water maze test, once trained
the mutants were impaired in their ability to learn a new platform location. Since the mutants already have a low sAHP, further reductions during learning (Disterhoft et al., 1996) may prevent any additional learning. Perhaps, in the mutants the increase in excitability is such that the networks coding for the old information are inescapably activated by any related new information. This reactivation leads to reinforcement of the old information, thus compromising any possible changes during relearning.

The results presented above support the hypothesis that the modulation of A-type K⁺ channels is critical for learning. First, a large number of modeling and electrophysiological studies demonstrate that information in the brain is encoded by the number and/or pattern of spike trains. A-type K⁺ channels have a key role in the modulation of spike trains since they affect both spike broadening during a spike train and the sAHP (hyperpolarization inhibits neuronal firing). Therefore, it is reasonable to propose that the modulation of A-type K⁺ channels may be critical for learning. Second, direct electrophysiological observations in vertebrate and invertebrate preparations documented decreases in A-type K⁺ currents after learning. Third, genetic lesions of A-type K⁺ channels in Drosophila and mice impaired learning.

The Important Role of Place Cell Studies

The studies described above involved mostly correlations between in vitro electrophysiology and behavioral analyses of lesioned animals. Typically, a molecular component is disrupted by either a pharmacological or genetic lesion; the effects are characterized in electrophysiological experiments with brain slices and then correlated with behavioral changes observed in the animals studied. However, there is a very large gulf between processes in brain slices and working brains, and it is often difficult to establish close connections between the results of these two types of analyses. To narrow this gap, recently, a number of laboratories have used single-unit recordings of place cells in genetically modified mice (Cho, Giese, Silva, & Eichenbaum, 1998b; McHugh et al., 1996; Rotenberg et al., 1996). The principal goal of these studies has been to test the connection between LTP, place cells, and spatial memory. Studies involving our laboratory examined the spatial firing patterns of hippocampal cells in αCaMKIIΔ⁴⁸⁶A (Giese, Fedorov, Filipkowski, & Silva, 1998a) and CREBΔ⁴ mouse mutants (Bourtchuladze et al., 1994; Kogan et al., 1996) because these mutants differ substantially in the severity of their defects in synaptic plasticity and learning. αCaMKIIΔ⁴⁸⁶A mice are severely impaired in spatial learning as well as in NMDAR-dependent LTP in the CA1 region of the hippocampus (Giese et al., 1998a). In contrast, CREBΔ⁴ mice have reduced LTP and milder spatial learning deficits (Bourtchuladze et al., 1994; Kogan et al., 1996). Consequently, we expected to find more profound abnormalities in the spatial representations of αCaMKIIΔ⁴⁸⁶A mice than in CREBΔ⁴ mutants. In striking parallel with the LTP and spatial learning deficits, these studies showed that place cell stability is more severely affected in the αCaMKIIΔ⁴⁸⁶A mice than in the CREBΔ⁴ mutants. While place fields in CREBΔ⁴ mutants were capable of preserving their spatial selectivity across trials, those in αCaMKIIΔ⁴⁸⁶A mutants could not. Additionally, our analysis showed that in both mutants their place fields were more easily disrupted by changes in environmental landmarks than in their wild-type littermates (Cho
Interestingly, two independent mutations affecting the autophosphorylation of αCaMKII at threonine 286 (Mayford, Wang, Kandel, & O'Dell, 1995; Rotenberg et al., 1996; Giese et al., 1998a) disrupted LTP, place cell fine tuning and stability, and place learning. Therefore, these results demonstrate the importance of the autophosphorylation of this kinase for LTP, and they suggest that LTP is crucial for the stability of place fields and that hippocampal place field stability is required for spatial learning. These findings also demonstrate that place cell studies will be a key component of the analyses of place learning in genetically modified mice.

THE WIZARDRY OF GENETIC TECHNIQUES: WHERE TO GO FROM HERE?

Most genetic studies of L&M have used techniques that do not allow control over the time and the place of the mutations. It is often difficult to determine whether the effects of mutations are due to alterations in development or to changes in the adult function of the altered/deleted proteins. Similarly, since mutations tend to affect several brain regions, traditional gene targeting techniques cannot pinpoint the exact brain structures responsible for the behavioral deficits of the mutants. Methods currently under development promise to overcome these limitations.

Region-Specific Mutations

A newly developed technique allows the restriction of a mutation to specific regions of the brain (Tsien, Chen, Mercer, Anderson, Mayford, Kandel, & Tonegawa, 1996). This technique takes advantage of promoters with a restricted expression in the brain (Tsien et al., 1996). For example, the αCaMKII promoter is mostly active in specific regions of the postnatal forebrain (Mayford et al., 1995). In transgenic mice, this promoter can be used to control the expression of the Cre recombinase (Sauer, 1993), an enzyme that deletes genes flanked by loxP sites (small DNA sequences recognized by Cre) (Tsien et al., 1996). Thus, deletion of the gene of interest (flanked by loxP sites) takes place only in tissues of the transgenic mouse where the Cre recombinase is expressed (Tsien et al., 1996). This technique was used to derive a deletion of the NMDAR restricted to the CA1 region of the hippocampus. Mutation of this glutamate gated ion channel led ion channel to LTP deficits in the CA3–CA1 synapse, to abnormal CA1 place cell properties, and to impaired spatial learning. This study is an elegant demonstration of the importance of NMDAR-dependent LTP in CA3–CA1 synapses (Tsien et al., 1996).

Inducible Expression of Transgenic Constructs

Another new genetic strategy combines a tissue-specific promoter (e.g., αCaMKII) with the tetracycline transactivator system (Furth, St. Onge, Boger, Gruss, Gossen, Kistner, Bujard, & Hennighausen, 1994). With this system it is possible to have some control over the time (and place) when a given mutant gene is expressed in mice (Mayford, Bach, Huang, Wang, Hawkins, & Kandel, 1996). This technique allows direct comparison of the same transgenic mice with or without the mutant protein. Therefore, with this technique it may be possible to separate the developmental effects of a genetic lesion from its effects
on function in adult animals. So far this approach was used to inducibly suppress the expression of a transgene encoding a constitutively active form of \( \alpha \text{CaMKII} \) (Mayford et al., 1996). Before suppression of the transgene, the mutants were impaired in CA1 LTP, in spatial learning, and in cued and contextual conditioning (Mayford et al., 1996). Remarkably, the inducible suppression of the transgene reversed these phenotypes (Mayford et al., 1996). This surprising result showed that the expression of the mutant \( \alpha \text{CaMKII} \) affected LTP and L&M, but it did not cause irreversible developmental damage (Mayford et al., 1996), as it may have been expected. In the future, the tet system may also be used for controlling the expression of Cre recombinase. Thus, soon it may be possible to have both region restricted and inducible deletions of genes of interest.

The Study of Mice with Point Mutations Will Be Key to Determining Which Functional Domains of a Given Protein Are Critical for L&M

The ability to target specific domains within a protein will be also crucial for genetic studies of L&M. The studies published to date used mutants in which entire proteins were deleted. Therefore, with this strategy it was impossible to determine which aspects of protein function were critical for L&M. For example, the complete deletion of \( \alpha \text{CaMKII} \) in mice (\( \alpha \text{CaMKII}^{\text{null}} \)) disrupts hippocampal LTP and place learning (Silva et al., 1992a, 1992b). Besides its \( \text{Ca}^{2+}/\text{calmodulin (CaM)} \)-dependent activity, \( \alpha \text{CaMKII} \) can undergo autophosphorylation, resulting in CaM-independent activity. The complete deletion of the \( \alpha \text{CaMKII} \) protein in \( \alpha \text{CaMKII}^{\text{null}} \) mutants disrupts both the CaM-dependent and -independent activity of this kinase. To determine whether the CaM-independent activity of the \( \alpha \text{CaMKII} \) was critical for learning, we used a novel gene targeting procedure (Pointlox Procedure) to introduce a point mutation into the \( \alpha \text{CaMKII} \) gene (Giese et al., 1998a) This mutation (a substitution of threonine 286 for alanine or T286A) blocked the autophosphorylation of this kinase and its CaM-independent activity, without affecting its CaM-dependent activity. Despite normal CaM-dependent activity, the \( \alpha \text{CaMKII}^{T286A} \) mutants showed no NMDAR-dependent LTP and no spatial learning in the Morris water maze, demonstrating that the autophosphorylation of \( \alpha \text{CaMKII} \) is required for LTP and learning (Giese et al., 1998a).

Mice with specific amino acid substitutions in genes of interest may also be critical to test hypotheses of L&M. For example, in addition to their deficit in LTP, the \( \alpha \text{CaMKII}^{\text{null}} \) mutants revealed a dramatic increase in augmentation. Thus, without further experiments it would be impossible to determine whether the deficit in LTP or the increase in augmentation was responsible for the learning impairments. This problem, however, was addressed by analysis of the \( \alpha \text{CaMKII}^{T286A} \) mutants: These mutants are deficient in LTP and learning, but have normal augmentation (unpublished observations), demonstrating that the deficit in LTP alone could account for the learning impairments observed in the \( \alpha \text{CaMKII}^{\text{null}} \) mutants. Thus, just as inducible mutants will provide a better indication for when a protein is critical for L&M, the study of mice with point mutations will help to determine which biochemical properties of a given protein are critical for L&M.

CONCLUSIONS

The studies summarized above indicate that LTP may be a key contributor to the synaptic changes that encode place memories in hippocampal circuits.
However, these studies also indicated that other cellular mechanisms complement and interact with LTP. For example, CA1 neurons show place-specific firing even in mutants lacking LTP, demonstrating that LTP is not essential for the establishment of place cells. However, the spatial representations of these mutants are noisier and unstable, indicating that LTP is required for the fine tuning and stability of place cells. We have also reviewed evidence demonstrating that LTP is necessary but not sufficient for processing and encoding information in the hippocampus. The studies with the Kv1.1 mice suggested that the modulation of the sAHP is also crucial for place learning. Perhaps, decreases in the sAHP after learning stabilize memory formation by increasing the excitability (and therefore the coactivation) of neurons encoding the memory. Before memories can be encoded, however, the required information has to be processed. STP may not only be essential for working memory, but it could also be crucial for processing temporal information in hippocampal circuits. Undoubtedly, other hippocampal mechanisms (e.g., inhibition) will also have a role. The challenge that lays ahead is not simply to increase the list of involved mechanisms, but to understand how they interact.

The studies reviewed above also showed that even though genetics will have a key role in unraveling the molecular and cellular substrates of place learning, a multitude of other approaches will also be needed. For example, the studies reviewed here implicating LTP, STP, and changes in sAHP in place learning combined evidence from a variety of different approaches. Pharmacological and genetic lesions studies, direct observations during learning in several species, and modeling experiments support the hypothesis that learning (including place learning) involves changes in LTP, STP, and A-type K$^+$ currents. Individually, each of the studies reviewed is difficult to interpret because there are many possible explanations for the results. For example, it is difficult to rule out explanations based on artifacts specific to the techniques used (e.g., developmental effects in genetic lesions). The interpretation of these experiments, however, is far more constrained when findings from different approaches are considered together. The convergence of data from experiments with multiple species and techniques strengthens arguments for the involvement of a given mechanism in L&M. The studies presented here demonstrate that genetics will be key to unraveling the molecular and cellular basis of place learning.

REFERENCES


Frankland, P. W., Cestari, V., Filipkowski, R., McDonald, R., & Silva, A. J. (1998). The hippocam-
pus is essential for contextual discrimination but not for context recognition in mice. Behavioral Neuroscience (in press).


Mayford, M., Wang, J., Kandel, E. R., & O'Dell, T. J. (1995). CaMkII regulates the frequency-
response function of hippocampal synapses for the production of both LTD and LTP. Cell, 81, 891–904.


Silva, A. J., Frankland, P. W., Marowitz, Z., Friedman, E., Lazlo, G., Cioffi, D., Jacks, T., &


