Molecular and Cellular Mechanisms of Cognitive Function: Implications for Psychiatric Disorders

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Recent studies on the molecular and cellular basis of learning and memory have brought us closer than ever to understanding the mechanisms of synaptic plasticity and their relevance to memory formation. Genetic approaches have played a central role in these new findings because the same mutant mice can be studied with molecular, cellular, circuit, and behavioral tools. Therefore, the results can be used to construct models that cut across levels of analytical complexity, forging connections from the biochemistry of the modified protein to the behavior of the mutant mice. These findings are not only improving our understanding of learning and memory, they are also enriching our understanding of cognitive disorders, such as neurofibromatosis type I. Mechanisms underlying long-term changes in synaptic function are likely to be at the heart of many cognitive and emotional processes in humans. Therefore, molecular and cellular insights into learning and memory undoubtedly will have a profound impact on the understanding and treatment of psychiatric disorders. Biol Psychiatry 2000;47:200–209 © *2000 Society of Biological Psychiatry*

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Introduction

To understand learning and memory, investigators must develop explanations that are not restricted to one level of analysis (e.g., glutamate receptor function) but that can be connected to behavioral plasticity. For example, hypothesis about the role of *N*-methyl-D-aspartate-receptor (NMDAR) function in learning and memory, should include, at minimum, information about the cellular processes that it mediates and a possible connection with the behaviors modulated by these receptors. This multilayer approach is powerful because hypotheses can be constrained easily. Until recently, however, it has been difficult to test the impact of most molecular processes in learning and memory because of the lack of specific agents capable of disrupting candidate molecular and cellular processes. The introduction of gene targeting has circumvented this limitation, and it is now possible to disrupt any molecular process of interest. In addition, powerful new strategies (e.g., microarrays) enable us to identify genes involved in specific cellular processes (i.e., genes required for long-term changes in synaptic function), and once cloned, these genes can be manipulated in different ways in mice. Here, we will review recent studies investigating mechanisms of synaptic plasticity and their relation to learning and memory. We will also review how these insights are used in the study of cognitive disorders, such as the learning disabilities associated with neurofibromatosis type I.

Connecting Molecular and Cellular Mechanisms with Learning and Memory

Traditionally, studies of learning and memory (L&M) have attempted to make causal links between the animals behavior, the brain regions recruited, the circuits involved, the physiological mechanisms activated, and the molecular processes that support these mechanisms. This is a complex and lengthy process, but the history of neuroscience has demonstrated that it is possible to make significant progress even before all of the desirable links between behavior, neuroanatomy, circuitry, and neuronal and molecular processes are established.

What are the criteria to determine whether any one mechanism has been linked to L&M? We propose that it is essential to obtain three different types of experimental evidence and that it is critical to develop reasonable, realistic, and testable models that account for the proposed connection. For illustrative purposes, we will consider the type of evidence that would demonstrate a causal link between synaptic plasticity and learning. First, lesions that disrupt mechanisms of plasticity should impair L&M. Second, changes in synaptic function should be documented in appropriate regions of the brain during L&M. Third, triggering plasticity mechanisms should, under certain circumstances, enhance or cause learning. This last type of experiment is perhaps the most difficult. In addition to these three different types of experiments, there

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should be a reasonable explanation of how synaptic plasticity could be used for the processing and storage of information. It is important to note that these explanations must be rigorously testable, but they do not need to account for every finding on the role of plasticity in memory. The studies reviewed below are focused on recent developments that have brought us closer than ever to affirming with certainty that synaptic plasticity is critical for L&M. None of these studies provides definitive evidence that plasticity is central to learning. Taken together, however, they make an impressive case for the role of plasticity in the processing and storage of information in neuronal systems.

Lesions Disrupting Plasticity Affect Learning

Of the experimental categories mentioned above, lesion approaches have been used most often to test the connection between synaptic plasticity and L&M. The only two methods available for making molecular lesions are pharmacology and genetics. The work summarized below elucidates further our understanding of the mechanisms of long-term potentiation (LTP), and it strengthens their connection to L&M.

The Connection between LTP, the Stability of Place Fields, and Learning

LTP, as commonly studied in hippocampal slices, is only a model system for the far more complex and highly regulated long-term synaptic changes that may accompany learning in vivo in structures such as the hippocampus. LTP measured in vitro does not always reflect faithfully its in vivo counterpart, nor does it capture fully its properties. For example, inhibitory circuits, so critical to synaptic function, are disrupted in hippocampal slice preparations. Even LTP measured in vivo is only an experimental approximation of synaptic changes that may accompany learning because the induction conditions used almost certainly do not simulate those present during learning. Nevertheless, measurements of LTP in hippocampal slices are a highly useful model.

One of the key properties of L&M is associativity. During learning, previously unrelated information becomes bound by a series of associative links that reflect an individual's experience. Remarkably, the induction of LTP depends on the activation of a glutamate-gated receptor with associative properties. The *N*-methyl-D-aspartate receptor (NMDAR) requires two distinct events for activation: postsynaptic depolarization that removes a magnesium block, and presynaptically released glutamate. These tantalizing properties suggest that the NMDAR may be a coincidence detector for associative learning (Bliss and Collingridge 1993). A critical role of the NMDAR in L&M has been suggested by pharmacologic blockade (i.e., Gewirtz and Davis 1997; Maren et al 1996; Miserendino et al 1990; Morris et al 1986; but see Bannerman et al 1995; Saucier and Cain 1995), and more recently by genetic lesions. For example, mice with a null mutation of the NMDA receptor subunit NR2A require more synaptic stimulation than do control mice for induction of similar levels of hippocampal LTP. These mutants also need more training than do control mice for induction of similar levels of contextual conditioning, a form of learning sensitive to hippocampal lesions (Kiyama et al 1998). This result is consistent with the idea that (within certain ranges) the facility with which synaptic potentiation is induced parallels the facility with which learning is triggered.

Models of hippocampal function suggested that associative synaptic changes underlie the ability of hippocampal neurons to fire in a place-specific manner (O'Keefe and Dostrovsky 1971). Remarkably, recent pharmacologic studies with CPP, an NMDAR antagonist, have suggested that the function of this receptor is required for the stability (but not the induction) of place-specific neuronal firing in the hippocampus (Kentros et al 1998). Additionally, a CA1-specific deletion of the NMDAR1, which results in deficits in both CA1 LTP and in spatial learning (Tsien et al 1996), was shown to alter the properties of place cells in this region. The place fields of these mutants are enlarged, have decreased spatial selectivity, and show uncoordinated firing (McHugh et al 1996). These results indicate that the loss of NMDAR function in different hippocampal synapses affects place fields in different ways, attesting to the importance of these receptors in the processing of spatial information in the hippocampus. Thus, NMDAR function is required for LTP, place fields, and place learning.

The influx of Ca^{2+} through the NMDAR channel activates the calcium/calmodulin (Ca^{2+}/CaM) dependent kinase II (αCaMKII), a kinase enriched in postsynaptic densities. A number of pharmacologic and genetic studies have demonstrated that this kinase modulates synaptic plasticity in a variety of different organisms. Ca^{2+}/CaM is required for the activation of αCaMKII and for its translocation to the membrane (Shen and Meyer 1999). Following activation, this oligomeric kinase can become autophosphorylated at threonine (T) 286, which allows it to continue to be active even at basal levels of calcium (De Koninck and Schulman 1998; Meyer et al 1992). Therefore, the autophosphorylation of this kinase has been proposed to serve as a molecular memory for recent synaptic activity (Lisman and Goldring 1988). To test this hypothesis, a mutant mouse was derived in which T286 of

αCaMKII was replaced with alanine (A), an amino acid that cannot be phosphorylated (Giese et al 1998). Interestingly, the T286A mutant mice showed no NMDAR-dependent LTP in the CA1 region of hippocampal slices and likely lacks this form of plasticity in other hippocampal and neocortical regions (Giese et al 1998; Glazewski, Fox, and Silva, unpublished results). Recent biochemical studies have suggested that αCaMKII modulates synaptic function by phosphorylating and increasing the ionic conductance of AMPA receptors (Barria et al 1997). This kinase can also modulate the slow afterhyperpolarization, a major determinant of neuronal excitability, and therefore another mechanism by which this kinase could affect synaptic function (Muller et al 1992).

Consistent with the NMDAR-blocking studies mentioned above, the αCaMKII T286A mutation also impaired the stability, but not the generation, of place fields in the hippocampal pyramidal region (Cho et al 1998; Giese et al 1998). Place fields were observed in the mutant mice, but they were more unstable than in control mice (Cho et al 1998). Together, the studies reviewed above suggest that both NMDAR and αCaMKII function are required for long-term potentiation, and these synaptic changes are essential for the stability of spatial representations in the hippocampus and, consequently, for spatial learning. These results are exciting because they provide for the first time an experimental link between molecular events at the synapse (calcium entrance through NMDARs, autophosphorylation of αCaMKII, phosphorylation of AMPA receptors, and enhancement of synaptic currents), synaptic plasticity (LTP), circuit events (stabilization of place fields), and hippocampal-dependent learning (spatial learning).

cAMP, Plasticity, and Memory

Memories and synaptic changes can last for weeks or months, or they can decay and dissipate within minutes or hours. There is considerable evidence that cAMP signaling activates transcription factors (of the CREB family) required for the stability of synaptic changes and memory (Bailey et al 1996; Matthies 1989; Silva et al 1998). Recent findings have shown that cAMP signaling also has a key role in determining which synaptic changes become stable and long lasting. The evidence suggests that a balance between the activities of cAMP-dependent protein kinase A (PKA) and phosphatases PP1 and PP2A "gate" the long-term stability of synaptic changes. For example, inhibitors of PKA impair early stages of LTP, and this inhibition can be overcome by inhibitors of phosphatases PP1 and PP2A (calcineurin; Blitzer et al 1995). The data suggest that at low levels of cAMP, the Ca^{2+}/CaM dependent phosphatase calcineurin dephosphorylates target proteins, including PP1 phosphatase inhibitor 1 (I-1). Dephosphorylation by calcineurin inactivates I-1 and allows PP1 in turn to dephosphorylate a wide range of target proteins, such as αCaMKII, that are required for LTP induction. Therefore, phosphatase activity seems to block long-term synaptic changes. In contrast, when the concentration of cAMP (as produced by Ca^{2+}-stimulated adenylyl cyclase) is high, PKA promotes long-term synaptic changes by phosphorylating and therefore activating I-1, which then blocks PP1 (Blitzer et al 1998).

The role of PKA and calcineurin in gating LTP and memory has also been studied in mutant mice (Abel et al 1997; Mansuy et al 1998a, 1998b; Winder et al 1998). Transgenic mice expressing an inhibitory form of the regulatory subunit of PKA show unstable LTP and memory (Abel et al 1997; Bourtchouladze et al 1998). In agreement with the model described above, overexpression of this inhibitory PKA seems to impair long-term synaptic changes required for long-term memory. Similarly, increasing phosphatase activity also blocks later phases of LTP. Transgenic expression of an activated form of calcineurin increased phosphatase activity, resulting in unstable LTP and memory (Winder et al 1998). Importantly, the LTP deficits could be ameliorated by either inhibitors of PP1 or by pharmacologic activation of the PKA pathway. Thus, these pharmacological and genetic studies suggest that the balance between calcineurin and PKA activity gate the long-term stability of synaptic changes required for long-term memory.

Direct Observation of Synaptic Changes During Learning

To demonstrate that long-term changes in plasticity have a role in learning, it is crucial to document that these synaptic changes take place during learning in relevant circuits. This is difficult to accomplish, perhaps because most memories may involve only a small number of synaptic sites (but see below); in most cases, it is unclear where exactly these synaptic changes could be recorded. Direct observations of synaptic changes during learning have been made in simpler invertebrate systems where the key sensory and motor pathways are known. In systems such as Aplysia, it is possible to isolate the central nervous system, mimic the conditioned and unconditioned stimulus by direct neuronal stimulation, record the conditioned response, and monitor the strength of key synapses. Thus, these reduced preparations have identified a number of presynaptic mechanisms that support short- and long-term changes in synaptic function underlying associative and nonassociative learning in Aplysia (Byrne and Kandel 1996). Some of these synaptic changes even resemble LTP (Murphy and Glanzman 1997). Not surprisingly, similar

studies are far more difficult in mammalian systems; however, recent results have brought us a step closer to direct observations of synaptic plasticity during mammalian learning.

Synaptic Changes in the Amygdala during Fear Conditioning

The amygdala has a critical role in emotional memory, but until recently, there was no direct evidence of synaptic changes in the amygdala during the formation of emotional memories (Davis et al 1993; LeDoux 1995; Rogan and LeDoux 1995). Fear conditioning is a powerful model of emotional memory. In Pavlovian fear conditioning, an animal learns to fear a conditioned stimulus (i.e., a tone) after its association with an unconditioned stimulus, such as a foot shock. Two groups have shown that fear conditioning leads to increases in the strength of synapses between neurons of the auditory thalamus and the lateral amygdala (McKernan and Shinnick-Gallagher 1997; Rogan et al 1997). The lateral amygdala is though to be one of the sites where information about the conditioned and unconditioned stimuli converge (Romanski et al 1993). Recent electrophysiologic studies in vivo showed that pairing the tone with shock increases the field potentials triggered by the tone in the lateral amygdala. This increase is proportional to the magnitude of the conditioned response (freezing), is not present when the shock and the tone are unpaired, and is stable (Rogan et al 1997). Experiments with brain slices also uncovered evidence for training-dependent increases in the strength of auditory thalamus-lateral amygdala synapses in conditioned rats (McKernan and Shinnick-Gallagher 1997).

Synaptic Changes Associated with Contextual Learning in the Hippocampus

There have been few hippocampal studies similar to the ones described above for the amygdala (but see Doyere et al 1993). Recent studies, however, found indirect evidence for hippocampal changes in synaptic strength during learning (Xu et al 1998). After induction of hippocampal LTP in vivo, animals were exposed to a novel environment. The exposure to this novel environment reduced or even erased the LTP of CA3-CA1 synapses (Xu et al 1998). Control experiments showed that this decrease in synaptic strength is not caused by handling stress. Twenty-four hours after induction of LTP, exposure to a novel environment did not affect the potentiated synapses, demonstrating that with time (at least 24 hours) changes in synaptic strength become protected from the erasure process. Thus, these results suggest that hippocampal learning involves extensive decreases in synaptic strength that may act in tandem with the more restricted (and therefore more difficult to find) long-lasting increases in synaptic function.

A recent study uncovered indirect evidence that these long-lasting increases may indeed take place during hippocampal-dependent learning. If information is encoded by increases in synaptic strength, then an artificially induced increase in synaptic strength before training should prevent further synaptic strength increases brought about by training and thus block learning. Consistent with this hypothesis, previous studies showed that saturation of hippocampal synapses with tetanic stimuli prevents hippocampal-dependent learning (McNaughton 1982). These studies were not easily reproducible, however, perhaps because complete saturation of hippocampal synapses is difficult, and remaining unsaturated synapses could have sufficed to support learning in some of the tasks used in these experiments (Barnes et al 1994). A recent study circumvented this problem by lesioning one hippocampus and using a more comprehensive protocol for saturating the synapses of the spared hippocampus (Moser et al 1998). Thus, these results show that saturating the strength of hippocampal synapses prevents hippocampal-dependent learning, suggesting that changes in synaptic strength underlie learning. Stable decreases (i.e., long-term depression or LTD) in synaptic strength could also be involved in learning, and much of the arguments supporting a role for LTP in learning could also be made for LTD.

Enhancing Synaptic Strength and Learning

The results reviewed above showed that the disruption of mechanisms underlying synaptic plasticity with either pharmacology or genetics impairs learning and that synaptic changes occur during learning in different organisms and in different brain structures. To strengthen the connection between synaptic plasticity and learning, however, it is critical to show that induction or enhancement of mechanisms of plasticity should, under certain circumstances, either promote or facilitate L&M.

Deletion of the nociceptin receptor results in enhanced LTP measured in hippocampal slices (Manabe et al 1998). This mutation seemed to improve learning and memory in the water maze and in a passive avoidance task, suggesting that facilitation of LTP improves learning. By contrast, a recent study has shown that the disruption of the ionotropic glutamate receptor type 2 (GluR2) gene resulted in a large enhancement in hippocampal synaptic potentiation, but also in learning deficits, demonstrating that the facilitation of synaptic transmission does not always lead to better learning (Gerlai et al 1998). The large enhancements in synaptic transmission in the GluR2 mutants appear to result in increases in circuit excitability (perhaps even seizures) that could disrupt learning.

The example above illustrates an important quagmire in making connections between phenomena as complex as synaptic plasticity and learning. Because the modulation of synaptic function likely affects a number of functions in the brain other than L&M, it is not surprising that the relation between synaptic plasticity and learning is complex. Therefore, it is impossible to assess the merit of the connection between plasticity and learning with single experiments. Instead, various experiments of different kinds (see above) are needed. The studies presented above showed that 1) disruption of the mechanisms of LTP affect L&M, 2) changes in synaptic strength accompany learning, and 3) facilitation of synaptic potentiation can result in the facilitation of L&M.

Molecular Mechanisms of Learning: Possible Involvement in Depression and Schizoprenia

Learning and its many transfigurations are at the heart of cognitive function in humans, and therefore, it is not surprising that insights into mechanisms of L&M will have a profound impact in the understanding and treatment of many psychiatric disorders. The mechanisms responsible for the physiological stability of memory may also support long-term neuronal adaptations triggered by the genetic functional loss or gain of function associated with diseases such as schizophrenia and depression. Below, we will give two examples of how disruptions in the molecular mechanisms of LTP and learning also could affect depression and schizophrenia.

CREB, α7-Nicotinic Receptor and Schizophrenia

Schizophrenia is a mental illness that affects 1% of the population. Dopamine agonists produce symptoms that are similar to the positive symptoms of schizophrenia, and most antipsychotic drugs have dopamine-antagonist activity. The dopaminergic system is known to modulate adenylate cyclase, and the activity of this enzyme is one of the key determinants of CREB activation. Therefore, it is possible that dopaminergic dysfunction in schizophrenia leads to changes in CREB activity and to deregulation of CREB-dependent genes.

Human and animal studies have suggested that a decrease in the expression of the α7-nicotinic receptors could account for sensory-gating deficits observed in schizophrenic patients (Adler et al 1982). There is evidence for familial transmission of this sensory-gating deficit, and it is possible that the α gene is involved (Adler et al 1998). The promoter of the human α7-nicotinic receptor gene has a cAMP-responsive-element (CRE) site that seems to be conserved across species (Gault et al 1998). Accordingly, mice lacking the α and the Δ CREB isoforms have a decreased level of the α7-nicotinic receptor (Stitzel et al 1998). These mice also show deficits in LTP and in L&M (Bourtchuladze et al 1994). Because pharmacologic experiments showed that a specific α7-nicotinic receptor agonist enhances LTP (Hunter et al 1994), it possible that the LTP and learning deficits in CREB mutants are partially lower α7-nicotinic receptor expression. Therefore, the decreased α7-nicotinic receptor expression also may underlie the L&M deficits observed in schizophrenic patients. Nonetheless, mice deficient in the α7-nicotinic receptor did not show abnormal hippocampal-dependent learning or abnormal sensorymotor gating (Paylor et al 1998). Powerful new approaches, such as gene-expression analysis using microarrays ("DNA-chip"), could be used to detect additional CREB-dependent gene-expression changes in schizophrenic patients.

cAMP Signaling and Depression

Recent studies demonstrated that long-term antidepressant treatments result in sustained activation of the cAMP pathway in specific brain regions, including upregulation of CREB (Dowlatshahi et al 1998; Duman et al 1997; Nibuya et al 1996). It is possible that the molecular alterations produced by chronic antidepressant treatment result in long-lived alterations in the strength of specific synaptic connections within neural circuits (Hyman and Nestler 1996). Long-term antidepressant treatment also results in increased expression of neurotrophins. For example, expression of brain-derived neurotropic factor (BDNF), a target gene of CREB, is increased in certain populations of neurons in the hippocampus and cortex (Duman et al 1997; Nibuya et al 1996). These findings are interesting because stress usually decreases the expression of BDNF and leads to atrophy of the same populations of neurons (Mamounas et al 1995).

Expression of the neurotrophin receptor TrkB, is increased in the hippocampus in response to electroshock seizures and long-term antidepressant treatments (Nibuya et al 1995; Nibuya et al 1996). TrkB activation results in the activation of the Ras MAPK pathway (Qian et al 1998) which results in the activation of several target proteins, including CREB (Impey et al 1999). The involvement of the Ras signaling pathway in neuropsychological disorders is also suggested by studies of neurofibromatosis type I, as discussed below.

Involvement of the Neurofibromin-Ras Pathway in Neuropsychologic and Psychiatric Deficits

Neurofibromatosis type I (NF1) is one of the most commonly inherited neurologic disorders in humans, affecting

approximately one in 4000 individuals (Gutmann and Collins 1994; Huson and Hughes 1994). The gene encodes a 250 KDa protein (neurofibromin), which includes a GTPase-activating (GAP) domain that can stimulate the GTPase activity of $p21^{ras}$ (Ballester et al 1990; Martin et al 1990; Xu et al 1990). Neurofibromin can also regulate $p21^{ras}$ mutant proteins resistant to GTPase stimulation, indicating that it may also regulate Ras signaling by mechanisms independent of its GAP function (Johnson et al 1994). In addition, studies in the larval neuromuscular junction of *Drosophila melanogaster* suggested that neurofibromin modulates the rutabaga-encoded adenylyl cyclase (Guo et al 1997; The et al 1997), indicating that neurofibromin may be involved in the regulation of multiple signaling pathways. In contrast, a mutation in the Nf1 gene that specifically abolishes the Ras-GTPase-activating function of neurofibromin without affecting the ability to bind Ras causes the multisymptomatic NF1 phenotype (Klose et al 1998), suggesting that the loss of GAP function is crucial for the development of the disease.

Although the NF1 gene is expressed in a variety of tissue and cell types during development, the expression of this gene in adults is largely restricted to neuronal tissues (Datson et al 1992; Nordlund et al 1993, 1995). Several isoforms of NF1 are expressed in the adult brain, including an isoform (exon 9a), which is exclusively expressed in neurons and an isoform that has less GAP activity (NF1 type II, containing exon 23a; Danglot et al 1995; Geist and Gutmann 1996; Gutmann et al 1995).

Neurons devoid of neurofibromin develop and survive in the absence of neurotrophins because of upregulation of phosphatidylinositol-3 (PI-3) kinase downstream of Ras (Klesse and Parada 1998; Vogel et al 1995). Furthermore, NF1 associates with microtubules, and this association is disrupted by specific mutations in the GAP domain (Xu and Gutmann 1997). Neurofibromin also seems to mediate the modulation of potassium currents by the neuropeptide PACAP38 (pituitary adenylyl cyclase-activating polypeptide) at the Drosophila neuromuscular junction (Guo et al 1997), and it is possible that it may have a related function in the nervous system. Finally, NF1 can result in learning abnormalities, such as impairments in spatial cognitive function in mice and humans (see below).

Neurological and Psychiatric Effects of Mutations of the NF1 Gene

Neurofibromatosis results in a variety of symptoms that typically include benign neurofibromas, hyperpigmentation of melanocytes, and hamartomas of the iris. Some patients with NF1 may also show neural pathology, such as optic pathway gliomas (Gutmann and Collins 1994; Huson and Hughes 1994) and astrogliosis (Nordlund et al 1995). Learning disabilities occur in 30 to 45% of patients with NF1, even in the absence of any apparent neural pathology. These learning disabilities may include visuoperceptual problems, executive dysfunction and impairments in spatial cognitive abilities, expressive and receptive language and motor coordination, often resulting in a depression in mean IQ scores (Chapman et al 1996; Eldridge et al 1989; Eliason 1986, 1988; North 1993; North et al 1995, 1994; Varnhagen et al 1988; Zoller et al 1997).

Brain magnetic resonance imaging (MRI) studies of NF1 patients revealed areas of increased signal intensity in T2 weighted images. These unidentified bright objects (UBOs) occur throughout the brain, and some studies have reported a correlation between the presence of UBOs and learning impairments in NF1 patients (Denckla et al 1996; Hofman et al 1994; North et al 1994; but see Duffner et al 1989; Ferner et al 1993; Legius et al 1995). These UBOs are present in children but tend to disappear in the adulthood. Unfortunately, the neuroanatomic basis for UBOs is still unclear. They may reflect areas of abnormal brain parenchyma, either hamartomas, heterotopias, or local areas of brain dysplasia.

Recent studies revealed a higher incidence of psychiatric disorders in NF1 patients than in the general population (Zoller and Rembeck 1999). One third of the NF1 patients studied were affected by at least one of the psychiatric disorders tested. In 21% of the cases, the Nf1 mutation was accompanied by dysthymia. Other psychiatric disorders affecting NF1 patients at higher rates included organic personality disorder and dementia (associated with alcoholism). Curiously, the NF1 patients that escaped psychiatric deficits seemed to outperform healthy control subjects in positive self-evaluation tests. They also showed significantly more socialization and less aggressive feelings and irritability than control subjects. Previous studies showed that although basic motor speed was unaffected in psychiatrically healthy adult NF1 patients, it was impaired in NF1 patients with dysthymia (Zoller et al 1997), which is consistent with the idea that depressive symptoms may affect the speed with which motor behaviors are executed (Williams 1988).

Parallels between the Behavioral Effects of the NF1 Mutation in Mice and in Humans

The mouse and human neurofibromin are highly homologous (98% sequence similarity; Bernards et al 1993). Additionally, DNA sequences in the 5′ and 3′ end of the NF1 gene are also conserved between mice and humans, suggesting that the transcriptional regulation of this gene is also conserved across species (Bernards et al 1993;

Hajra et al 1994). In mice (and perhaps in humans), the complete loss of neurofibromin is lethal (Brannan et al 1994; Jacks et al 1994). With age, mice heterozygous for a targeted disruption of the Nf1 gene ($Nf1^{\pm}$) have an increased incidence of phaeochromocytomas and myeloid leukemias, both of which are known to occur frequently in NF1 patients. Nevertheless, these mice do not show all of the tumor types that are characteristic of neurofibromatosis type I (Brannan et al 1994; Jacks et al 1994).

Consistent with the observation in human NF1 brains, $Nf1^{\pm}$ mice develop region-specific astrogliosis (Rizvi et al 1999). Our behavioral analysis shows that the $Nf1^{\pm}$ mutation also affects learning in mice (Silva et al 1997). There are a number of parallels between the behavioral effects of the NF1 mutation in mice and in humans. First, the NF1 mutation seems to affect some brain functions more than others. For instance, it does not seem to disrupt simple associative learning in mice but does impair specifically some more complex forms of learning, such as spatial learning. Second, in both humans and mice, the $Nf1^{\pm}$ mutation does not affect cognition in all individuals (incomplete penetrance), and remedial training can compensate for it (Eldridge et al 1989; Eliason 1986, 1988; North 1993; North et al 1995; Silva et al 1997; Varnhagen et al 1988). Third, the severity of the NF1 phenotype is thought to be affected by genetic variation, which exacerbates the condition of NF1 patients without having a noticeable impact on normal siblings (Easton et al 1993). Consistent with this, we showed that a heterozygous mutation of the *N*-methyl-D-aspartate receptor ($NMDAR1^{\pm}$) increases the severity of the learning deficits of $Nf1^{\pm}$ mice without affecting learning in litter-mate control subjects (Silva et al 1997). Finally, in agreement with the observation of motor coordination problems in a significant percentage of NF1 patients, mice lacking the 23a Nf1 isoform also show impaired motor skills (RMC and AJS, unpublished data). It would be interesting to determine whether Nf1 mutant mice show evidence of psychiatric symptoms analogous to those shown by NF1 patients (i.e., depression).

Because the analysis of Nf1 mutant mice shows that they model important features of the learning disabilities caused by the mutation of the NF1 gene in humans, we set out to find clues about the molecular mechanisms underlying the NF1 learning deficits. Patients with a mutation that specifically disrupts the Ras-GAP activity of NF1 showed learning impairments (Klose et al 1998). This suggests that the up-regulation of Ras activity could underlie the learning impairments. We tested this hypothesis by decreasing Ras activity in NF1 mutant mice. Our results showed that decreasing Ras activity with either a drug or with null mutations in Ras genes expressed in the brain ameliorated the learning impairments of NF1 mutant mice (Kogan et al 1998). These experiments show that the Nf1 mutants are powerful tools for understanding the molecular basis of the NF1 learning impairments, and they suggest that similar studies could be conducted for inherited psychiatric disorders, such as schizophrenia and depression, once the culprit genes are identified.

Conclusions

We have discussed a number of findings that strengthen considerably the connection between synaptic plasticity and L&M. These reports have shown that disruption of mechanisms underlying long-term changes in synaptic strength affect L&M, that changes in synaptic strength accompany learning, and that the facilitation of synaptic potentiation can result in the facilitation of L&M. It is possible that deregulation of these L&M mechanisms may also contribute to psychiatric disorders such as schizophrenia and depression. The deregulation of the cAMP/CREB signaling pathway, for example, disrupts L&M and may likewise impact schizophrenia and depression. Unfortunately, insights into the mechanisms underlying these psychiatric diseases are still limited. The exciting genetic investigations underway promise to find the genes causing these disorders. Once identified, these genes could be used in a multitude of studies, including the generation of animal models. Our findings with the $Nf1^{\pm}$ mutant mice demonstrate that animal models of cognitive disorders can be useful in unraveling the molecular and cellular mechanisms underlying these disorders. In the near future, we may be able to use these and other powerful molecular approaches to develop and fine tune treatments for these tragic disorders.

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