Genetic and Genomic Strategies in Learning and Memory

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Abstract: Learning and memory is a property of central importance in the nervous system, yet many of the molecular mechanisms for this behavior remain enshrouded in mystery. Despite the daunting nature of the problem, a number of complementary strategies have been employed to unravel the complexities of learning and memory, ranging from genetics to biochemistry. One of the most recent tools brought to bear in this area is genomics. Here, we review some of the most significant insights that have been so far obtained in learning and memory, and we suggest possible areas of future progress.

Key Words: Complex traits, Down syndrome, Engineered mice, Expression profiling, Functional genomics, Genetics, Learning and Memory, Microarrays.

INTRODUCTION

While the memory of our evolutionary past is stored in our genome, memory on the scale of a lifetime or less is a property of the brain. Learning and memory is important not only for successful interaction with the environment for most animals, but also helps define our unique individuality as humans. The search for a scientific explanation for this complex process has been a long-standing objective [1], but despite many noteworthy advances much remains to be accomplished. The complexity of learning and memory can be appreciated by consideration of some of the multiple mechanisms involved: there must be pathways for storage and recall, for which memories should be kept and which ones should be erased, for assigning temporal order to memories, and so on.

Many different disciplines have been employed to unravel the mechanisms of learning and memory, including biochemistry, genetics, behavior, anatomy and physiology. The most recent addition to this armamentarium is genomics. In this review, we will discuss the insights obtained into learning and memory from this diverse group of technologies and give a brief look to the future.

BIOCHEMISTRY AND PHARMACOLOGY

Pioneering work using *Aplysia*, a marine snail with a simple nervous system, showed that learning and memory could be productively investigated in a less complex setting than the vertebrates [2]. Biochemical and pharmacological studies using this organism have given very important insights into the molecular aspects of learning and memory, including the identification of functional changes in the strength of pre-existing synaptic connections in short-term memory storage, and recognition of proteins essential for the long-term memory formation [1, 3].

Most work has taken advantage of the gill withdrawal reflex which made it possible to identify and characterize the role of individual neurons in memory acquisition, as well as allowing biochemical and molecular investigations at the single cell level [4]. Sensitization of the gill withdrawal reflex, a simple form of learning and memory, was found to depend upon serotonin (5-hydroxytryptamine, 5-HT) mediated activation of post-synaptic G-protein coupled receptors. Once activated, the 5-HT receptors stimulated adenylate cyclase, leading to increased levels of cAMP and activation of cAMP-dependent protein kinase (PKA). Electrophysiologically, the resulting long-term facilitation can persist for days and is accompanied by the growth of new synaptic connections [4-6]. Accompanying these events, mitogen-activated protein kinase (MAPK) is recruited by PKA dependent phosphorylation, and PKA and MAPK in turn phosphorylate and activate transcription factors in the nucleus belonging to the cAMP response element-binding (CREB) protein family. The long-term transcriptional program induced by the CREB family is implicated as one of the most conserved mechanisms in long-term synaptic plasticity. At the synaptic level, growth of new connections associated with the learning and memory process is mediated by rearrangement of structural proteins. One important example is the cell adhesion molecule ApCAM, for which internalization is a necessary step in the growth of new synapses [5].

GENETICS

Genetics can provide especially powerful evidence for cause and effect, in contrast to biochemical and pharmacological studies, which on their own are usually correlative. From classical screens to conditional knockouts, a variety of genetic approaches have been successfully applied to the identification and characterization of the molecular components of learning and memory. Because of its facile

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1389-2029/03 $41.00+.00

genetics, the fruit fly *Drosophila melanogaster* is the most studied invertebrate model of learning and memory. However, interesting insights have also been obtained from study of the nematode worm, *Caernorhabditis elegans*. Among the vertebrate models, the mouse stands preeminent because of the possibility of genetic engineering and the high degree of homology of its genome to that of the human [7-9].

Forward genetic screens in *Drosophila* have successfully identified genes that play important roles in learning and memory. Such screens have been more slowly adopted in the mouse. However, in 1994 a breakthrough study used random mutagenesis in the mouse to identify the *Clock* gene as playing an important role in circadian rhythms [10-12]. This investigation has inspired further large-scale efforts to use random mutagenesis to identify learning and memory genes in this model organism [13-18].

Despite the power of the genetic screens to look for behavioral genes, there are some drawbacks. One major disadvantage is the life cycle of the organism, which places an inherent limitation on the throughput of the screens. In addition, it can be impossible to identify recessive mutations in genes affecting behavior that have redundant functions or are homozygous lethal. These difficulties of genetic screens extend to gene knockout studies, although the advent of tissue specific knockouts now allows behavioral studies of knockouts that are otherwise homozygous lethal. Overall, gene knockout technologies have provided extraordinary insights into the relationship between individual genes in mammals and learning and memory. However, their greatest strengths lie in testing pre-existing hypotheses, rather than exploring unexpected regulatory and interdependent relationships between families of genes and their role in behavior.

The next few paragraphs are devoted to a discussion of some of the most salient findings from the various models and genetic approaches available.

Invertebrate Models

In *Drosophila*, genetic screens employed Pavlovian conditioning paradigms, assays where an odor is paired with an electric shock [19-23]. These screens led to the isolation of a number of mutants affecting learning and memory. Examples include *dunce* [24], *rutabaga* [25], *amnesiac* [26], and *radish* [27], all of which display impaired associative learning. The first three genes, *dunce*, *rutabaga* and *amnesiac*, participate in the same cAMP-PKA biochemical pathway implicated in learning and memory in *Aplysia*. Long-term memory in *Drosophila* was found to consist of two independent forms, anesthesia resistant memory, dependent on the *radish* gene, and protein synthesis dependent memory, dependent on CREB. The latter pathway was demonstrated through the use of both a dominant-negative CREB allele, which blocked the formation of long-term memory [28], and over-expression of the wild-type allele, which enhanced long-term memory [29]. It was also found that CREB was required for the induction of functional synaptic plasticity [19], while structural plasticity was regulated via *FasII*, a neural cell adhesion molecule [30].

In a recent study, genes involved in long-term memory formation were identified through two complementary strategies: behavioral screening of transposon insertion strains for memory phenotypes and the use of DNA microarrays to assess global transcriptional response differences during memory formation in groups of normal flies subject to massed or spaced training [31]. Relevant genes identified by the microarray study were confirmed through quantitative PCR. Interestingly, both the genetic and microarray approaches suggested the involvement of the *pumilio*/*staufen* pathway in long-term memory formation. These genes had been originally identified as maternal effect genes important in embryonic development. It was found that the expression of *pumilio*, a transcript-specific translational repressor, was regulated during long-term memory formation, and some learning mutants carried lesions in the same gene. Similar findings were obtained for *staufen*, a gene involved in mRNA translocation in both neurons and oocytes. Furthermore, long-term memory was specifically abolished in temperature-sensitive alleles of *staufen*.

An especially promising prospect in *Drosophila* is the development of new approaches for specific spatial and temporal genetic inactivation of neural communication in targeted pathways [32-34]. One technique uses the Gal4-UAS system for spatial targeting [34] of the *shibire*^{ts1} temperature sensitive allele [35]. The Gal4-UAS system provides spatial specificity, while shifts in temperature provide temporal specificity, through blockade of synaptic vesicle trafficking via the semi-dominant effects of the *shi*^{ts} allele.

A novel technique that also inactivates targeted neural pathways uses the "electrical knockout" channel (EKO channel). This method inhibits electrical activity in excitable cells using a genetically modified Shaker K^+ channel in combination with the spatial specificity of the Gal4-UAS system. It has been shown that this system can provided graded inhibition of cellular excitability *in vivo* in targeted cells (neurons, muscles, and photoreceptors) in *Drosophila*, resulting in consonant physiological and behavioral effects [36].

The small worm *C. elegans* has a nervous system consisting of 302 neurons, and its synaptic connections have been anatomically mapped. Despite its simple nervous system, it is able to display short and long-term habituation, which are nonassociative forms of learning involving a single stimulus [37]. Furthermore, the completion of its genome [38] facilitates the application of reverse and forward genetic techniques to illuminate the molecular mechanisms of habituation. Molecular genetic analysis of chemotaxis and thermotaxis (the ability to detect chemical compounds and temperature respectively), have revealed that the molecular elements involved in olfaction, taste, and thermosensation are very similar to the sensory signaling found in vertebrates [39].

C. elegans is capable of remembering its temperature of cultivation: in a thermal gradient, it will move towards and track isotherms near this temperature (isothermal tracking). In a recent investigation, Samuel *et al.* [40], studied the AFD neuron which has a role in thermotactic behavior. They monitored the fluorescence of pH-sensitive green fluorescent protein localized to synaptic vesicles in AFD and measured the rate of synaptic release in worms cultivated in a range of temperatures, and subjected to a fixed ambient temperature in the same range. They found that the rate of synaptic release is high if either the ambient temperature is higher or

lower than the temperature of cultivation, but the rate is low if these temperatures are similar, suggesting that AFD encodes a comparison of ambient and cultivation temperatures.

In another study investigating isothermal tracking, Gomez *et al.* [41] showed that the neuron-specific calcium sensor-1 (NCS-1) is essential for this behavior, as ncs-1 knockout worms exhibited major defects in isothermal tracking but preserved normal chemotactic, locomotor and thermal avoidance behaviors. They also showed that NCS-1 overexpression enhanced isothermal tracking performance as well as improving learning and memory, strongly suggesting that proper signaling via NCS-1 is a fundamental pathway for this behavior.

In an investigation of long-term habituation, Rose *et al.* [42] have shown that there is a contribution from presynaptic glutamate release. The authors tested habituation to mechanical tapping in wild-type worms and worms with a mutation in a vesicular glutamate transporter in the sensory neurons that respond to tapping (*eat-4*). They found that the mutant worms showed no evidence of long-term habituation.

Vertebrate Models

Since the work in *Drosophila* and *Aplysia* had emphasized the importance of conserved molecular mechanisms in learning and memory, it was natural to ask whether similar pathways existed in mammals. From a neuroanatomical viewpoint, the celebrated case of patient H.M. [43] identified the crucial role of the hippocampus in long-term memory function in humans, and influenced the later genetic studies carried out in mice. Also focusing attention on the hippocampus was the discovery of long-term potentiation (LTP) [44]. This activity-dependent form of synaptic plasticity [45, 46], paralleled long-term facilitation in *Aplysia* and was an attractive *in vitro* model of learning and memory. Early pharmacological and electrophysiological studies revealed different phases and molecular elements in LTP. An early and transient form of potentiation produced by a single high-frequency train of excitatory stimuli (E-LTP), produced modification of preexisting proteins and strengthening of synaptic connections. However, multiple tetanic applications of high-frequency trains of stimuli resulted in a more persistent long-term form of LTP (L-LTP). This phase required cAMP, PKA, MAPK and CREB, leading to the production of new proteins and later the growth of new synaptic connections. These molecular pathways are reminiscent of those described in *Aplysia* and *Drosophila*.

With this background, the advent of knockout and transgenic technologies in the mouse gave the opportunity to critically examine the relationship between the behavior of learning and memory and hippocampal electrophysiology, as well as the potential molecular pathways for these phenomena [47-49].

One investigation suggesting a link between LTP and learning and memory was of CREB knockouts in mice. As we have seen, CREB is one of the transcription factors activated during learning, is required for long-term memory and plays this role in diverse species [50]. A complete deletion of the CREB gene is lethal in mice [51]. Nevertheless, deletions of only the alpha and delta splicing isoforms reduce CREB levels by 75% and these mice survive to adulthood [52]. The α/δ CREB knockout mice showed intact short-term memory but impaired long-term memory. Consistent with these findings, hippocampal LTP showed an abnormally rapid decay to baseline after tetanic stimulation, but paired-pulse facilitation and post-tetanic potentiation were normal. A recent investigation has used a different strategy to confirm a role for CREB in hippocam-pus-dependent learning in genetically modified mice [53]. The CREB family of transcription factors (CREB, CREM, ATF1) were all inhibited in region CA1 of the dorsal hippocampus by creation of transgenic mice expressing KCREB, a mutant CREB that is a potent inhibitor of all three CREB family transcription factors. Spatial and temporal specificity was obtained by using the CaMKIIα promoter and the tetracycline-regulated tTA transactivator, respectively. Expression of the mutant CREB impaired learning in a task, the Morris water maze, that required the dorsal hippocampus but the transgene did not interfere with context-dependent conditioning, which is independent of the dorsal hippocampus. Several forms of late-phase LTP were normal in these animals, but forskolin-induced and dopamine-regulated potentiation were not, suggesting that some experimental forms of plasticity circumvent the requirement for CREB. Despite this observation, the uncovered abnormality in LTP together with the deficits in learning and memory is evidence linking these two dissimilar phenomena.

Another relevant study was of the NMDA receptor. Deletion of the NR1, NR2A, or NR2B subunits resulted in neonatal lethality. To avoid this problem, the NR1 subunit was deleted specifically in the CA1 region of the hippocampus [54, 55] using the Cre/LoxP system under the regulation of αCaMKII promoter [56, 57]. This tissue specific knockout produced spatial learning deficits when tested using the Morris water maze. Further strengthening the link between LTP and learning and memory there were also abnormalities in LTP in these mice, which specifically lacked LTP at CA1 synapses in the hippocampus [54, 55]. Conversely, mice genetically engineered to produce more of the NMDA receptor 2B (NR2B), led to facilitation of synaptic potentiation and these mice displayed better learning and memory than controls [58].

The neurotrophins play important roles in neuronal plasticity in the brain. They include nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), neurotrophin-3 (NT3) and neurotrophin-4 (NT4) [59]. The principal receptor for BDNF and NT4 is TrkB, a receptor tyrosine kinase and a member of the Trk family of receptors. Mice with a targeted homozygous deletion of TrkB exhibit increased apoptosis in hippocampus and cortex during development. This potential source of artifact was avoided by construction of a specific regional and postnatal temporal deletion in the hippocampus, using a floxed TrkB allele and the Cre recombinase expressed from the αCaMKII promoter. These mice showed impaired memory when tested with hippocampus-dependent tasks, and decreased LTP [60]. Consistent with these findings, mice lacking BDNF also had impaired hippocampal LTP [61].

The mechanisms involved in the BDNF/TrkB pathway for the regulation of the synaptic strength are still not very

well understood. There is evidence that BDNF and TrkB are important in the regulation of presynaptic release of neurotransmitters [62, 63], as well as postsynaptically [64], but the topic remains highly controversial [65]. One investigation favoring a presynaptic role showed increased numbers of docked vesicles in the presence of BDNF [66]. Docking and priming (preparation of vesicles for neurotransmitter release) are possible ways to modulate presynaptic plasticity [67]. Recent investigations had shown that the RIM protein is involved in vesicle-priming in mammals [68, 69]. Investigation of a knockout of the RM1α gene, one of the two most abundant isoforms of RIM, revealed deficits in upregulation of neurotransmitter release during short-term synaptic plasticity in hippocampal CA1 cells [68] and also lack LTP of hippocampal mossy fibers [69]. Furthermore, RIM has been shown to interact with the Rab3A synaptic-vesicle protein, which is involved in docking [70]. RIM1α is likely to be participating in both Rab3A-dependent and independent pathways in presynaptic plasticity [68, 69].

Homozygous NT4 knockout mice lack any overt phenotype, except for neuronal reductions in two peripheral ganglia. Interesting learning and memory deficits were found in these mice, which had normal short-term memory but striking deficits in long-term memory. Parallel with these findings, long-term, but not decremental, LTP was found to be diminished. These findings suggest the importance of NT4 in modulating the synaptic plasticity required for long-term learning and memory and is additional evidence for a link between LTP and this behavior [71].

Neurofibromatosis type I is an inherited autosomal dominant cancer disorder, for which the mechanism of dominance is haploinsufficiency. Neurofibromatosis type I is also one of the most frequent single-gene disorders resulting in learning deficits in humans [72]. The NF1 protein, neurofibromin, participates in Ras GTPase-activating (GAP) protein activity and adenylyl cyclase modulation. Mice carrying a heterozygous null mutation of the Nf1 gene ($Nf1^{+/-}$) model the disorder and show important characteristics of the associated learning deficits [73]. Recent work indicates that these abnormalities may be caused by excessive Ras activity, leading to impairments in long-term potentiation due to increased GABA-mediated inhibition [74, 75].

Erasure of memories may be an active process [76]. J.L. Borges in his short story "*Funes, el memorioso*" [77], described a man who, after falling from his horse, lost the ability to forget even the smallest details. Lacking the capacity for generalization and abstraction, he was almost unable to think. This characteristic of memory has been recently studied by Genoux *et al.* They investigated mice with a conditional knockout of protein phosphatase 1 (PP1), in which the expression of a natural inhibitor of PP1 was regulated using the reverse tetracycline transactivator system. These mice displayed better efficacy in learning and memory on hippocampus related tasks when the inhibitor was switched on and a faster memory decline when it was switched off. These results suggest that protein phosphatase 1 acts as a molecular antagonist of learning and memory [78]. Similarly, it has recently been shown that the cannabinoid receptor 1 plays an important role in erasing fear-related memories in mice [79].

The principal biochemical pathways and molecular elements of learning and memory discussed in this review are summarized in (Fig. **1**).

Complex Trait Mapping

The genetics of cognitive ability, including learning and memory, is complex; that is, many genes interact with environment to contribute to the ultimate phenotype [80]. A number of studies have successfully identified loci contributing to learning and memory abilities in mice [81-84]. However, as is common for such complex trait mapping experiments, the mapped regions were very large, and identifying the responsible genes will be a daunting proposition. Studies in humans suggest a relatively high degree of heritability for cognitive abilities [84]. However, in addition to the technical difficulties of gene identification, mapping of such traits in humans is mired in ethical concerns.

Smith *et al.* [85-87] have presented a novel technique that may allow the fine mapping and identification of candidate genes in complex traits. The method utilizes several overlapping large insert clones from a genomic region of interest, which are then employed to create a panel of transgenic mice. The collection of mice is called an *'in vivo'* library, since segments of the human genome are propagated in mice rather than the usual vehicles of *E. coli* or yeast. The *in vivo* library technique has been employed to identify genes from a 2 Mb region of chromosome 21q22.2 involved in learning disabilities of Down syndrome. Screening of the *'in vivo'* library singled out a particular YAC (yeast artificial chromosome) that caused learning and memory deficits as a result of extra gene dosage. Creation of mice with fragments of the YAC was then employed to identify the *minibrain* gene as being responsible for the learning and memory deficits of the mice and potentially also for the learning disabilities of Down syndrome. This method has promise for the dissection of other complex behavioral traits, in addition to learning and memory.

GENOMICS

The advent of DNA microarray technology has brought new possibilities to the design of experiments relevant to learning and memory, especially in mice [88]. This new technology permits high-throughput gene expression analysis in the brain and opens the door to genome-wide studies of behavior. Together with the recent completion of the mouse genome sequence [7], microarray technology should facilitate not only the identification of individual genes involved in learning and memory, but also a better understanding of more complex gene interactions and their role in behavior.

Mody *et al.* [89] used high-density oligonucleotide microarrays to analyze hippocampal gene expression in developing mice from embryonic day 16 to post-natal day 30 (time points: E16, P1, P7, P16 and P30). Regulated genes were identified that were involved in neuronal proliferation, differentiation and synapse formation. Sandberg *et al.* [90] used the same type of arrays to analyze gene expression in normal 129vEv and C57BL/6 brains. Genes were also

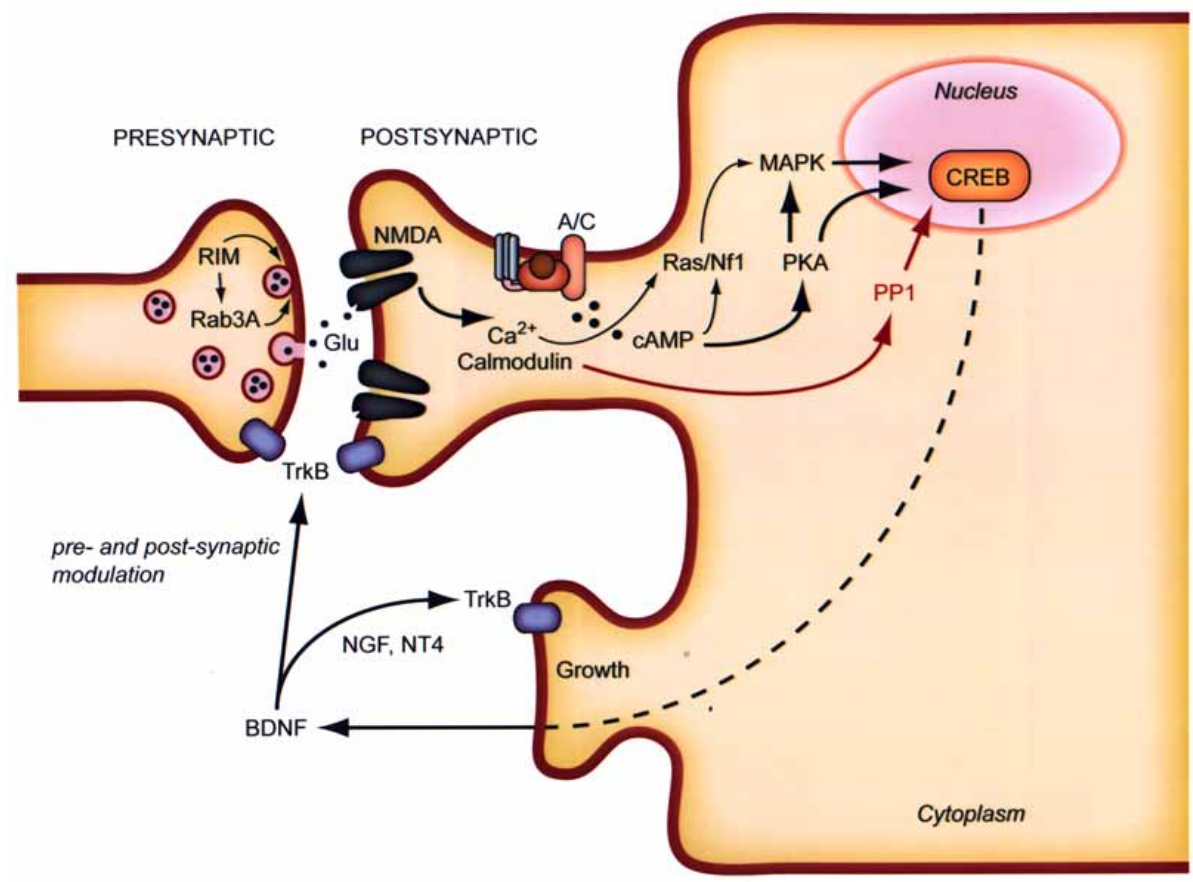

Fig. (1). Schematic representation of some of the principal molecular elements identified in learning and memory (see text for details).

identified that were differentially expressed in specific brain regions. These types of study, together with proper experimental validation, may help uncover new candidate genes for learning and memory.

One study using cDNA microarrays to specifically identify learning and memory genes investigated differences in hippocampal gene expression between two F1 hybrid mouse strains that performed well on the Morris water maze [47], a widely used test of learning and memory, and two inbred strains that performed poorly [91]. Singular value decomposition was used to identify groups of differentially expressed genes that separated the good and bad performing strains on this test, (see Fig. **2**). Most of the uncovered genes had unknown function. This strategy of using multiple strains together with microarray analysis may be a useful general approach in the future for narrowing down candidate genes in complex traits. Another promising approach is to combine the information from microarrays with knowledge of genetic loci identified by complex trait mapping [92, 93].

Microarrays have also been employed to identify genes in rodents induced in the hippocampus as a result of exercise. Rats exposed to an environment for three weeks with voluntary running opportunities were compared with sedentary animals. A large number of differentially expressed genes were found related to neuronal activity, synaptic structure and neuronal plasticity [94]. In rats exposed to a brief vigorous swimming task compared with passive controls, differences were found in hippocampal transcript levels related to cell cycle, development, differentiation and gene regulation [95]. Exposure to environmental novelty in mice resulted in expression changes for genes linked to neuronal structure, synaptic plasticity, and transmission [96].

In two independent studies specifically studying learning and memory, genes that were differentially expressed as a result of maze training in the rat hippocampus were identified using microarrays. Luo *et al.* [97], assessed gene-expression changes after training in a multiunit T-maze [98] using microarrays consisting of mouse cDNA clones. They found genes with increased expression related to Ca^{2+} signaling, Ras activation and kinase cascades, known pathways implicated in learning and memory. Cavallaro *et al.* [99], used the GeneChip Rat Neurology U34 arrays (Affymetrix, Santa Clara, CA) to identify genes differentially expressed after training in the Morris water maze. Their experimental design included a control group of rats subjected to a sham swimming task, in order to discriminate genes regulated by exercise and physical activity from memory-related genes. Gene expression profiles were com-

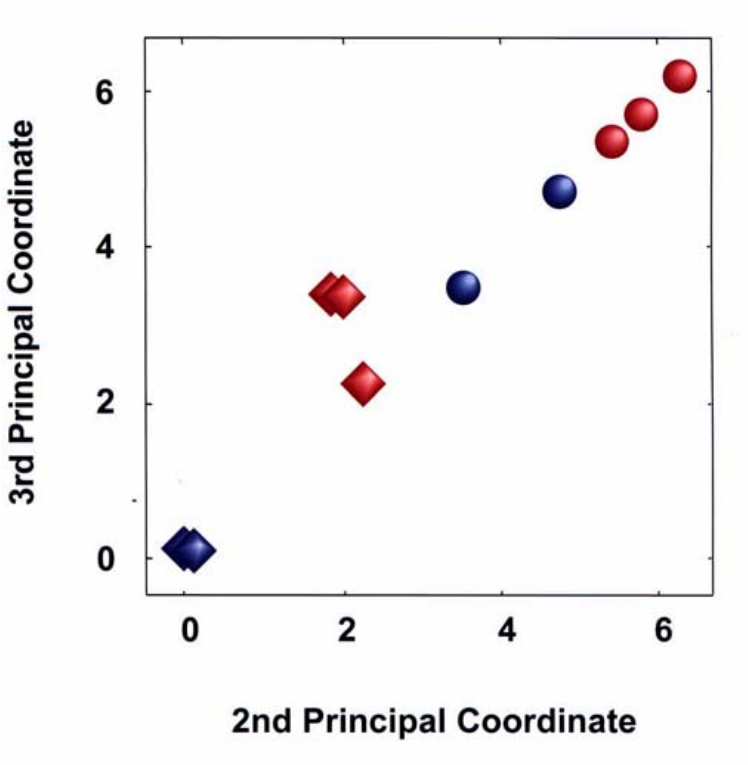

Fig. (2). Singular value decomposition (SVD) of differentially expressed genes in the hippocampus separates good from poor learning mouse strains [91]. The projection of gene expression levels in the 2nd and 3rd principal components of the SVD is shown. The two good learning strains are shown as red and blue circles, while the two poor learning strains are shown as red and blue diamonds.

pared between control and experimental groups at 1, 6 and 24 h after training. Many genes were found to be influenced by physical activity, but learning and memory specific genes showed distinct temporal patterns when clustered. All of the memory related genes had a known function and some of them were previously implicated in synaptic plasticity, memory, or cognitive disorders. The only gene uninfluenced by physical activity but increased at all time points in the active learning group was fibroblast growth factor 18 (FGF-18). The effect of this growth factor was further explored by giving an exogenous dose to a group of rats. Remarkably, this group showed significant improvements in learning when compared to non-treated animals.

In a conceptually similar study, microarrays were used to compare hippocampal gene expression profiles in mice undergoing active learning in the Morris water maze with mice undergoing a sham learning procedure [100]. Analysis of variance identified three genes significantly regulated as a result of the learning experience. One was the α subunit of the platelet derived growth factor (Pdgfra), another showed homology to DnaJ and CREB2, and the third was novel.

Changes in gene expression have also been investigated using the rabbit eye blink conditioning paradigm together with high-density cDNA microarrays [101]. Transcript levels from cerebellar lobule HVI and hippocampus of control and experimental groups were compared, and both novel genes and previously identified genes were found to be regulated as a result of the task.

Monogenic Traits in Humans

In contrast to the difficulties of complex trait analysis, study of single traits in humans leading to learning disabilities has allowed the identification of a number of genes that may be relevant to learning and memory [102]. One of the most commonly inherited forms of mental retardation is Fragile X syndrome [103]. This syndrome results from a triplet repeat expansion leading to silencing of the fragile X mental retardation 1 (FMR1) gene. A knockout mouse model of this disorder has been successfully created and replicated the deficits in learning and memory as well as others facets of the disorder [104]. A recent study has investigated brain gene expression profiles in the knockout model of this syndrome [105]. Many genes were identified that were differentially expressed between wild-type and FMR1 knockout mice, a number of which had been previously implicated in memory or learning. In another interesting study, mutations have been reported in the human angiotensin II receptor gene (AGTRII) in X-linked mental retardation [106], indicating an unexpected role for AGTRII in brain development and cognitive functions.

Recent studies of nonspecific X-linked mental retardation (MRX) have implicated the Rho- and Rab-GTPase pathways in learning and memory. MRX is a collection of genetically distinct retardation disorders grouped together by virtue of their common location in the X chromosome. MRX is non-syndromic, i.e. affected patients show cognitive impairment

but no other distinctive clinical o biochemical condition. The Rho- and Rab-GTPase proteins belong to the Ras superfamily of kinases and are implicated in regulation of the actin cytoskeleton and vesicle exocytosis respectively. Mutations in genes directly controlling the activation of the Rho cycle, oligophrenin-1 (OPHN1) [107] and ARHGEF6 [108], were found in patients affected by MRX. Another investigation showed that a point mutation in the tPAK3 (p21-activated kinase) gene, which encodes a serine-threonine kinase, was also found in MRX [109]. PAK proteins are essential effectors linking Rho-GTPases to cytoskeletal reorganization and to nuclear signaling. Related to Rab-GTPases, mutations were found in the GDI1 gene that encodes αGDI, a Rab-GDP dissociation inhibitor (GDI). This protein plays an essential role in the recycling of Rab GTPases required for vesicular transport through the secretory pathway [110]. All these results emphasize the association between cognitive impairment and defects in Ras-like GTPase signaling pathways important for vesicular release.

CONCLUSION

Learning and memory are complex and integrated processes involving many different levels of system organization. From the molecular components of a single synaptic connection between two neurons to the cellular architecture responsible for information storage, many problems await full understanding. Biochemistry, genetics and pharmacology have recently been joined by the newest genomic technologies in our attempt to unravel the mysteries of these processes. Perhaps the most difficult challenge in understanding learning and memory will be the transition from the molecular realm to the cellular and regional neuroanatomical domains. A number of related questions arise. Will it be possible to use the tools of genomics to map 3D patterns of gene expression to understand better learning and memory? Will this help us understand where memories are stored? Can we watch brain gene expression in the real time for the whole genome? Can we trace the path of every axon and dendrite responsible for memory storage and retrieval? Already the first halting steps are being taken to answer the first of these questions by the development of new approaches for high-throughput 3D mapping of gene expression in the brain [111-113]. However, development of the other technologies probably lies far in the future. Nevertheless, it is intriguing to contemplate that the recent successes of the genome project have allowed us to uncover the memory of evolutionary events encoded by the human genome, and that this may do much to illuminate how memories are stored during a lifetime.

ACKNOWLEDGEMENTS

Supported by the NIH (DA015802), Tobacco-Related Disease Research Program (11RT-0172), and Alzheimer's Association (IIRG-02-3609).

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