Memory enhancement: the search for mechanism-based drugs

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Rapid progress has been made in understanding the synaptic changes required for memory encoding. Several companies are now attempting to use information about the induction and consolidation phases of this process to build memory-enhancing drugs. These efforts have produced novel compounds that improve retention scores across a broad range of tests and species. Initial clinical results are encouraging. Issues now arise about appropriate applications of candidate drugs and optimal cellular targets for future development.

The idea that memory is encoded by activity-driven changes in synapses dates almost from the beginning of modern neuroscience. A central tenet of this argument was belatedly satisfied by the 1973 discovery[1] that brief periods of intense activity cause an extremely persistent (weeks at least) increase in the strength of excitatory synapses in cortex, hippocampus, and other forebrain structures. Subsequent work established numerous correspondences between memory and the long-term potentiation (LTP) effect and showed that manipulations of the latter have predictable effects on the former[2]. The emergence of LTP as a widely accepted substrate of many (though not all) commonplace forms of memory, coupled with rapidly expanding information on the molecular biology of synaptic plasticity in general, has resulted in a new effort to invent mechanism-based memory drugs. The present review summarizes the status of these efforts, emphasizing projects that have progressed as far as clinical testing. This focus necessarily precludes discussion of the full range of potential targets for drugs directed at memory deficits.

Stages in production of memory-related synaptic changes

Production of stable synaptic changes is typically divided into induction, expression and consolidation phases. These relate to steps in memory formation introduced into the psychological literature with the discovery that electroconvulsive shocks applied shortly after learning erase memory[3]. Subsequent decades of work confirmed that memory encoding involves two distinct steps: an acquisition process requiring a few seconds, followed by a series of changes that consolidate the new information against disruption and decay, which requires hours or even days. The manifestation of memory in behavior (such as recognition) constitutes a third component emerging immediately after acquisition and in advance of consolidation. LTP, as summarized below, seems to pass through a similar sequence of stages, two of which are logical targets for mechanism-based memory drugs.

The consensus induction model involves the two classes of transmitter receptors co-localized at excitatory (glutamatergic) synapses. AMPA-type glutamate receptors generate depolarization needed to unblock voltage sensitive NMDA-type glutamate receptors, which then admit calcium into the dendritic side of the synapse (**Fig. 1**).

Today most researchers hold that the enhanced postsynaptic currents that define LTP expression are caused by changes in AMPA receptors, but there is disagreement about how they are changed. Simply adding new receptors to the synapse[4] would increase the response to a given amount of transmitter; one variant of this idea posits that recycling is co-opted so as to alter receptor number[5]. The alternative is that extant receptors are modified so as to enhance their operation. The leading candidate for this process is serine phosphorylation of two sites on the cytoplasmic domain of the receptor[6].

An important advance in consolidation came with the discovery[7] that LTP is easily erased immediately after its induction but not after delays of 30-60 minutes. As with memory, the progressive resistance of LTP to disruption (consolidation) seems to have multiple phases. Adhesion proteins are implicated in steps beginning within minutes of induction[8,9], whereas protein synthesis is needed for phases occurring an hour or more later[10,11].

The current search for memory drugs begins with the assumption that steps in memory formation correspond to steps in synaptic modification. This is not to argue that the behavioral phenomena can be reduced to plasticity: for example, the critical questions of memory organization and retrieval are barely addressed by current biological models. However, to the degree that initial encoding and later consolidation of memory depend upon the induction and stabilization of LTP, then drugs that enhance these cellular effects are expected to promote their behavioral reflections.

Drugs that facilitate induction

Repetitive release of transmitter over a period of 30–50 milliseconds allows AMPA receptors to generate sufficient depolarization to unblock NMDA receptors and thereby induce LTP (**Fig. 1**). Increasing the amount of glutamate released during stimulation or enhancing the effects of the transmitter on AMPA receptors can reduce this requirement. Work on the latter approach—positive modulation of AMPA receptors—is now well advanced and has resulted in competing families of drugs.

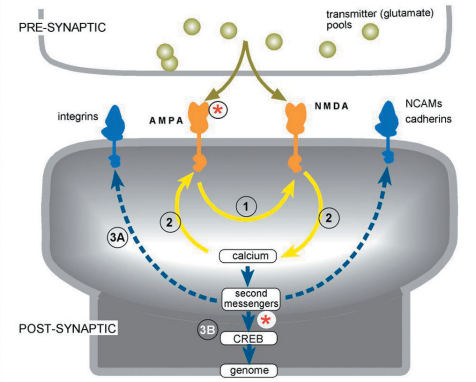

Fig. 1. Targets for the development of memory-enhancing drugs. The production of memory-related synaptic changes occurs in three stages. Step 1: induction. Released transmitter binds to AMPA-type glutamate receptors, which then depolarize the postsynaptic region and unblock NMDA-type receptors. Step 2: expression. NMDA receptors admit calcium and thereby modify AMPA receptors so as to increase the size of subsequent excitatory currents. Step 3: consolidation. NMDA receptors also trigger changes that stabilize the modifications to the AMPA receptors. A rapidly developing aspect of this (3A) involves adhesion receptors, whereas a more delayed component requires genomic events (3B). Current strategies for drug development (red asterisks) target the AMPA receptor component of induction or the gene-signaling component of consolidation.

Ampakines™ were the first allosteric modulators of AMPA receptors to augment excitatory transmission in brain[12]. They have no detectable agonist or antagonist actions but instead modify two aspects of receptor biophysics—desensitization and deactivation—that terminate the synaptic current[13]. By slowing these two processes, ampakines enhance and prolong the synaptic currents generated by release of glutamate from axon terminals (**Fig. 2**).

The drugs freely enter the brain, where they increase both glutamatergic transmission and the aggregate activity of cortical neurons controlling complex behaviors[14]. As described in an extensive literature, these effects are accompanied by substantial improvements in retention scores on diverse tests that sample memories lasting for minutes, hours or weeks. Positive results are reported for tasks dependent upon different brain structures, types of rewards and training regimens. They also hold across species (rats, rabbits, monkeys). In all, positive modulation of AMPA receptors reduces the requirements for the encoding of memory, whether the memory is of a type that normally persists for only a few minutes or instead normally lasts for an indefinite period.

Being modulators, ampakines affect only those AMPA receptors activated by endogenously released transmitter and thus only those networks engaged by the brain's present activity. This feature, coupled with the absence of targets outside the central nervous system, presumably accounts for their positive effects on memory at dosages well below those that produce notable side-effects. (Seizures are the most severe risk factor.)

Additional modulators with chemical structures distinct from those of the ampakines have been discovered, beginning with the benzothiadiazides. Similar to the ampakines, one set of derivatives is reported to enhance excitatory transmission, promote the formation of LTP, and improve object recognition memory[15]. Another series of biarylpropylsulfonamide variants[16,17] is notable for its potency, with some members of the group being effective in the low nanomolar range. The diversity of these chemical structures points to the conclusion, now supported with experimental evidence[18], that AMPA receptors have multiple modulatory sites. Linking chemical structures and modulatory sites with particular physiological[19], and thus presumably behavioral, effects constitutes one of the more exciting prospects for drug development.

In all, the search for positive modulators has led to a rich pharmacology and a number of promising candidates for memory therapeutics. Several companies (Cortex, Lilly, Organon, Servier) have aligned themselves with particular modulator strategies, giving the impression that something of a race is on to produce an AMPA receptor–based memory drug.

Drugs that improve consolidation

This represents a logical alternative (or complementary) approach to improved encoding as a route for developing memory-enhancing drugs. Most efforts at promoting consolidation deal with the late, protein synthesis–dependent phase rather than the adhesion phase that develops too quickly to be a consequence of gene activation. The CREB family of transcription factors has received particular attention in this regard. CREB is phosphorylated and activated by the cyclic AMP-dependent protein kinase and then binds to the cyclic AMP response element of target genes. It is essential for long-term facilitation in *Aplysia*[20] and contributes to the maintenance of the later stages of LTP[21]. As expected from these physiological results, several studies have documented a role for CREB in long-term memory in *Drosophila*[22] and mice[23]. Other behavioral work implicates CREB in late-stage consolidation[24] (not early stage), and shows that enhancing CREB functioning does indeed enhance the stable encoding of long-term memory[25,26], which is of crucial importance for drug development.

Multiple efforts are now underway to develop drugs that facilitate CREB's contributions to long-term memory. Under some conditions, inhibitors of the phosphodiesterase isozyme PDE-IV, which is responsible for hydrolysis of cAMP, increase CREB phosphorylation, CREB binding to DNA, and memory scores in rats[27]. Although the clinical use of current PDE IV inhibitors is restricted by unacceptable side effects, more potent, and perhaps selective, compounds are under development[28]. Given the ubiquitous distribution of CREB, target selectivity (brain/forebrain) will be a critical requirement for drugs acting upon it to produce cognitive effects. At least two biotechnology companies (Memory Pharmaceuticals, Helicon) are pursuing CREB-based strategies.

Applications

An FDA advisory panel recently decided that Mild Cognitive Impairment (MCI) is an acceptable category for clinical treatment. Persons with MCI have memory impairments relative to their age and education, but have significant preservation of everyday activities. Several studies have shown that subjects diagnosed as having MCI progress to Alzheimer's disease at a much higher rate (~10–15% per year) than age-matched controls. MCI is targeted along with Alzheimer's by most memory drug programs. These are enormous markets, involving perhaps as many as 10 million people in the US alone.

Application beyond these well-characterized disorders depends on unresolved public policy issues. A substantial literature shows that deterioration in memory functioning is part of brain aging in mammals, including humans. Assuming the new mechanism-based memory drugs have minimal side effects, then

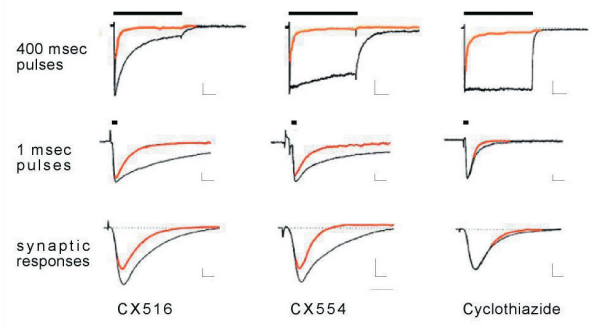

Fig. 2. Positive modulators of AMPA receptors. Experiments using membrane patches excised from mature hippocampal neurons are shown in the top two rows; synaptic responses from the same structure are described in the bottom row. Long pulses of glutamate (black bar) were applied to the patches to test desensitization, brief pulses (1 ms; middle row) to sample deactivation rates. Responses in the presence of drug are indicated with the darker traces. The ampakine CX516 had relatively weak effects on desensitization but strong effects on deactivation; the benzothiadiazide cyclothiazide produced the opposite pattern. The second ampakine (CX554, middle column) had good effects on both phenomena. Cyclothiazide did not markedly affect single synaptic responses but does enhance high-frequency responses (not shown; adapted from ref. 13).

the question could arise as to whether they are appropriate for treating conditions that, although disturbing, are part of the normal course of life. The answer to this question will determine the size of the market for memory drugs.

Finally, the new pharmacology could be used to produce chronic improvements in MCI and early-stage Alzheimer's by inducing increased neurotrophic support. Regional changes in neuronal activity and trophic support are widely cited as potential causes for the neuronal atrophy found in the aged brain. Glutamatergic transmission regulates neurotrophin expression, raising the possibility that positive modulators of glutamate receptors will increase trophic factor levels and thereby offset age-related declines. These positive modulators increase transcription of brain-derived neurotrophic factor (BDNF) in cortex and hippocampus of adult rodents[29,30]. Whether comparable effects are obtained in aged animals, and whether the increases are associated with reduced cortical atrophy, remains to be seen.

Clinical results

Clinical results for newer generation, mechanism-based memory drugs have been reported thus far only for the AMPA receptor compounds. For reasons of safety surrounding an entirely new class of drugs, initial multiple-dose ampakine experiments were conducted with young adults as subjects and dosages at the lower end of the anticipated effective range. Despite the latter point, small-to-moderate improvements were obtained in tests involving visual associations, recognition of odors, acquisition of a visuo-spatial maze, and location and identity of playing cards[31]. There were no changes in motor performance, and none of the subjects reported any subjective feelings after ingesting the drug. Subjects were not able to correctly guess whether they had received drug or placebo over successive days of testing. These findings accord with animal studies showing that ampakines selectively enhance the encoding of both short- and long-term memory.

Positive results have also been reported in a simple test of nonsense syllable recall using 65–75 year-old subjects who were not symptomatic for any psychiatric disturbances (including MCI)[32]. Average scores for the highest dose group were more than twofold greater than for the placebo group (**Fig. 3**). Importantly, blood levels of ampakine for this group fell close to the range predicted from animal studies to increase transmission through complex networks. There were no evident changes in self-assessment in any of 15 psychological variables sampled by a standard test instrument. Although the findings from these two human studies have to be viewed as preliminary, they do encourage the general idea that gains in understanding synaptic plasticity can lead to novel memory enhancing drugs. Large-scale clinical trials for MCI are in progress at Servier Pharmaceuticals and Cortex Pharmaceuticals. These efforts will undoubtedly provide the strongest test yet of the hypothesis that compounds that promote LTP will enhance the encoding of memory.

Not unexpectedly, the introduction of a new pharmacology for memory impairments was followed shortly by tests of its potential utility in treating other cognitive disorders. AMPA receptor modulators have positive effects in certain preclinical models of schizophrenia and of attention deficit/hyperactivity disorder[33]. Following from the former results, an ampakine used in human memory studies was tested in a 30-day trial with hospitalized schizophrenic patients maintained under an optimized dosage of the antipsychotic drug clozapine. The ampakine produced moderate to large improvements relative to placebo-treated patients in measures of attention and memory[34]. Subsequent to this study, Phase II (clinical efficacy) schizophrenia trials with an AMPA receptor modulator were announced by Organon. Clinical tests of ampakine effects on attention deficit/hyperactivity disorder were recently carried out by Shire Pharmaceuticals, but the results have not yet been described.

Beyond their potential significance to major disorders, the ADHD and schizophrenia trials establish the critical point that it will be possible to treat subjects with novel memory-enhancing drugs on a regular basis for extended periods[34]. This is a critical issue not only for the repeated dosing needed for acute, daily improvements in memory but also for the potential use of the drugs to obtain chronic changes via increased neurotrophism.

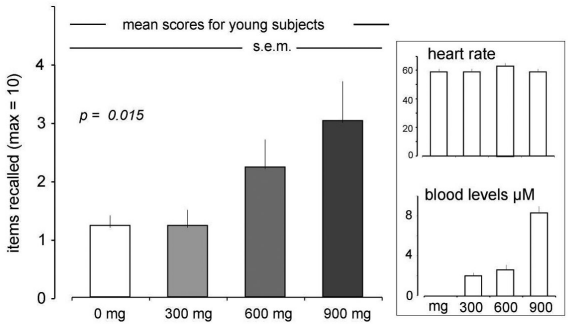

Fig. 3. Effects of a positive modulator on the recall of nonsense syllables by aged subjects. Four groups of healthy 65–75 year old subjects were given a list of 10 nonsense syllables and then asked to recall them without prompting 5 minutes later. Three of the groups were given the ampakine CX516 at the indicated dosages. Retention scores (mean ± s.e.m.) are shown; the differences were significant (ANOVA). Right, the drugs did not detectably affect heart rate; blood values in the low micromolar range were associated with improved memory scores (adapted from ref. 31).

Conclusions

Drugs based on known mechanisms of memory-related plasticity have been developed. In preclinical studies, some of the compounds have strong positive effects that hold across tasks and species. Interest is high, and a number of companies are pursuing work in this area. Initial clinical results can be described as encouraging and provide proof of principle that basic research on the substrates on memory encoding has reached the point at which it can yield interesting drug leads. Whether the current candidates are addressing the most appropriate phases of memory formation (encoding, expression, consolidation), or the optimal entry points within them, now become questions of great interest.

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1. Bliss, T. V. P. & Lomo, T. Long-lasting potentiation of synaptic transmission in the dentate area of the anesthetized rabbit following stimulation of the perforant path. *J. Physiol. (Lond.)* **232**, 334–356 (1973).
2. Martin, S. J., Grimwood, P. D. & Morris, R. G. Synaptic plasticity and memory: an evaluation of the hypothesis. *Annu. Rev. Neurosci.* **23**, 649–711 (2000).
3. Duncan, C.P. The retroactive effect of electroshock on learning. *J. Comp. Physiol. Psychol.* **42**, 32–44 (1949).
4. Lynch, G. & Baudry, M. The biochemistry of memory: a new and specific hypothesis. *Science* **224**, 1057–1063 (1984).
5. Malinow, R. & Malenka, R. C. AMPA receptor trafficking and synaptic plasticity. *Annu. Rev. Neurosci.* **25**, 103–126 (2002).
6. Lee, H. K., Barbarosie, M., Kameyama, K., Bear, M. F. & Huganir, R. L. Regulation of distinct AMPA receptor phosphorylation sites during bidirectional synaptic plasticity. *Nature* **405**, 955–959 (2000).
7. Barrionuevo, G., Schottler, F. & Lynch, G. The effects of repetitive low frequency stimulation on control and "potentiated" synaptic responses in the hippocampus. *Life Sci.* **27**, 2385–2391 (1980).
8. Dityatev, A., Dityateva, G. & Schachner, M. Synaptic strength as a function of post- versus presynaptic expression of the neural cell adhesion molecule NCAM. *Neuron* **26**, 207–217 (2000).
9. Kramar, E. A., Bernard, J. A., Gall, C. M. & Lynch, G. Alpha3 integrin receptors contribute to the consolidation of long-term potentiation. *Neuroscience* **110**, 29–39 (2002).
10. Nguyen, P. V. & Kandel, E. R. A macromolecular synthesis-dependent late phase of long-term potentiation requiring cAMP in the medial perforant pathway of rat hippocampal slices. *J. Neurosci.* **16**, 3189–3198 (1996).
11. Frey, U. & Morris, R. G. Synaptic tagging and long-term potentiation. *Nature* **385**, 533–536 (1997).
12. Staubli, U., Rogers, G. & Lynch, G. Facilitation of glutamate receptors enhances memory. *Proc. Natl. Acad. Sci. USA* **91**, 777–781 (1994).
13. Arai, A., Kessler, M., Rogers, G. & Lynch, G. Effects of a memory-enhancing drug on DL-alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor currents and synaptic transmission in hippocampus. *J. Pharmacol. Exp. Ther.* **278**, 627–638 (1996).
14. Hampson, R., Rogers, G., Lynch, G. & Deadwyler, S. Facilitative effects of the ampakine CX516 on short term memory in rats: correlations with hippocampal unit activity. *J. Neurosci.* **18**, 2748–2763 (1998).
15. Pirotte, B. *et al.* 4H-1,2,4-Pyridothiadiazine 1,1-dioxides and 2,3-dihydro-4H-1,2, 4-pyridothiadiazine 1,1-dioxides chemically related to diazoxide and cyclothiazide as powerful positive allosteric modulators of (R/S)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl) propionic acid receptors: design, synthesis, pharmacology, and structure-activity relationships. *J. Med. Chem.* **41**, 2946–2959 (1998).
16. Baumbarger, P. J., Muhlhauser, M., Zhai, J., Yang, C. R. & Nisenbaum, E. S. Positive modulation of alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors in prefrontal cortical pyramidal neurons by a novel allosteric potentiator. *J. Pharmacol. Exp. Ther.* **298**, 86–102 (2001).
17. Gates, M., Ogden, A. & Bleakman, D. Pharmacological effects of AMPA receptor potentiators LY392098 and LY404187 on rat neuronal AMPA receptors *in vitro*. *Neuropharmacology* **40**, 984–991 (2001).
18. Arai, A. C., Kessler, M., Rogers, G. & Lynch, G. Effects of the potent ampakine CX614 on hippocampal and recombinant AMPA receptors: interactions with cyclothiazide and GYKI 52466. *Mol. Pharmacol.* **58**, 802–813 (2000).
19. Arai, A. & Lynch, G. AMPA receptor desensitization modulates synaptic responses induced by repetitive afferent stimulation in hippocampal slices. *Brain Res.* **799**, 235–242 (1998).
20. Dash, P. K., Hochner, B. & Kandel, E. R. Injection of the cAMP-responsive element into the nucleus of *Aplysia* sensory neurons blocks long-term facilitation. *Nature* **345**, 718–721 (1990).
21. Barco, A., Alarcon, J. M. & Kandel, E. R. Expression of constitutively active CREB protein facilitates the late phase of long-term potentiation by enhancing synaptic capture. *Cell* **108**, 689–703 (2002).
22. Yin, J. C. *et al.* Induction of a dominant negative CREB transgene specifically blocks long-term memory in *Drosophila*. *Cell* **79**, 49–58 (1994).
23. Bourtchuladze, R. *et al.* Deficient long-term memory in mice with a targeted mutation of the cAMP-responsive element-binding protein. *Cell* **79**, 59–68 (1994).
24. Kida, S. *et al.* CREB required for the stability of new and reactivated fear memories. *Nat. Neurosci.* **5**, 348–355 (2002).
25. Yin, J. C., Del Vecchio, M., Zhou, H. & Tully, T. CREB as a memory modulator: induced expression of a dCREB2 activator isoform enhances long-term memory in *Drosophila*. *Cell* **81**, 107–115 (1995).
26. Josselyn, S. A. *et al.* Long-term memory is facilitated by cAMP response element-binding protein overexpression in the amygdala. *J. Neurosci.* **21**, 2404–2412 (2001).
27. Nagakura, A., Niimura, M. & Takeo, S. Effects of a phosphodiesterase IV inhibitor rolipram on microsphere embolism-induced defects in memory function and cerebral cyclic AMP signal transduction system in rats. *Br. J. Pharmacol.* **135**, 1783–1793 (2002).
28. Zhu, J., Mix, E. & Winblad, B. The antidepressant and antiinflammatory effects of rolipram in the central nervous system. *CNS Drug Rev.* **7**, 387–398 (2001).
29. Lauterborn, J. C., Lynch, G., Vanderklish, P., Arai, A. & Gall, C. M. Positive modulation of AMPA receptors increases neurotrophin expression by hippocampal and cortical neurons. *J. Neurosci.* **20**, 8–21 (2000).
30. Mackowiak, M., O'Neill, M. J., Hicks, C. A., Bleakman, D. & Skolnick, P. An AMPA receptor potentiator modulates hippocampal expression of BDNF: an *in vivo* study. *Neuropharmacology* (in press).
31. Ingvar, M. *et al.* Enhancement by an ampakine of memory encoding in humans. *Exp. Neurol.* **146**, 553–559 (1997).
32. Lynch, G. *et al.* Evidence that a positive modulator of AMPA-type glutamate receptors affects delayed recall in aged humans. *Exp. Neurol.* **145**, 89–92 (1997).
33. Gainetdinov, R. R., Mohn, A. R., Bohn, L. M. & Caron, M. G. Glutamatergic modulation of hyperactivity in mice lacking the dopamine transporter. *Proc. Natl. Acad. Sci. USA* **98**,11047–11054 (2001).
34. Goff, D. C. *et al.* A placebo-controlled pilot study of the ampakine CX516 added to clozapine in schizophrenia. *J. Clin. Psychopharmacol.* **21**, 484–487 (2001).