Memory enhancement: consolidation, reconsolidation and insulin-like growth factor 2

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Life and societies would change significantly if memory capacity or persistence in health and disease could be enhanced. It has been known for many years that memory can be improved and strengthened. Substances known to enhance memory include hormones, neurotransmitters, neuropeptides and metabolic substrates. Recently, attention has been given to identifying the molecular mechanisms and targets whereby memory enhancement can be achieved. One approach would be to target the physiological changes that are induced by learning and naturally required for memory strengthening via consolidation and reconsolidation. Here, we review approaches that boost memories by targeting the cAMP response element binding protein-CCAAT enhancer binding protein (CREB-C/EBP) pathway and/or its recently identified target gene insulin-like growth factor 2 (IGF2).

Introduction
The continuing increase in life span is unfortunately associated with an increased prevalence of cognitive dysfunctions, including memory loss and diminished ability to form new long-term memories. Aging-related memory impairment is a common condition characterized by mild symptoms of cognitive decline. The aged population becomes slower in processing, storing and recalling new information, and shows impairments in cognitive functioning, including memory, concentration and organization. ‘I forget names so easily now’ or ‘I forget where I put things all the time’ are common complaints of the aged. More dramatic and devastating are the cognitive and memory losses in neurodegenerative diseases, such as Alzheimer’s disease (AD), in which the impairments interfere with normal life functioning. The economic impact of AD in the USA alone is estimated to be more than US$100 billion annually [1]. Thus, it is imperative to identify or develop approaches that can restore or prevent memory impairments. Furthermore, ethical debates aside, the possibility of speeding normal learning and boosting memory retention is appealing to the healthy population. Hence, identifying memory enhancers is of great importance and interest.

Numerous studies have indicated that memory can be enhanced with many types of strategy or drug that can either boost the baseline of the biological systems or modulate memory strengthening or retrieval. These drugs modulate neurotransmission, neurotransmitter receptors, neuropeptide [2–4], stress and related hormones [5,6] and metabolism [7,8]. The literature on this topic and the list of effective drugs is vast. Here, we focus our discussions on an ensemble of mechanisms that can be targeted to boost memories because they underlie the memory processes known as consolidation and reconsolidation. Indeed, one approach that can be employed to search for mechanisms and targets of memory enhancement is to identify and exploit the biological mechanisms by which learned information transforms into long-lasting memory. Newly learned information becomes a long-term memory through a process known as consolidation. This process converts the new labile memory trace into a stronger one that is resilient to disruption [9]. Consolidation requires an initial phase of de novo gene expression that in animal models of temporal lobe-dependent memories takes place over the first 24 h after training; its interruption results in memory loss [10,11]. Moreover, once stable and resilient to disruption, memories can again become labile and sensitive to interference if they are reactivated by, for example, recalling the memory. Memories then regain their resilience to interference by undergoing a process known as reconsolidation [12–15]. In several types of memory, reconsolidation has been found to be temporally limited. In fact, in rodents, only young memories (weeks-old), not old (months-old) ones are lost after amnesic agents are given together with memory reactivation [16–20]. It has been proposed that reconsolidation is a phase of lingering consolidation that in rodents can last for weeks [15,21]. If memory is retrieved during this sensitive phase, it undergoes reconsolidation to strengthen the memory itself, indicating that an important function of the post-retrieval reconsolidation process is to promote memory strengthening and persistence [15,22]. Thus, in principle, a strategy that potentiates either the initial consolidation or the reconsolidation phases may be successfully exploited as an effective cognitive enhancer. In agreement with this hypothesis, various gene mutations targeting the pathways known to be involved in long-term plasticity or memory formation result in memory
enhancement. Excellent reviews have summarized and discussed these mutants [23,24].

In this review, we discuss the hypothesis that memory can be enhanced by targeting a variety of steps of the cAMP-CREB-C/EBP-dependent gene cascade, which has been shown to be required for memory consolidation in numerous species [25]. After a brief summary of current knowledge, we expand on the effect of a target gene, IGF2, (also known as IGFl), which is regulated by the CREB-C/EBP pathway [26]. We also discuss memory enhancement in reconsolidation and relative temporal constraints and conclude with some unresolved questions about memory enhancement and its potential clinical applications.

Promoting memory enhancement by targeting the cAMP-CREB-C/EBP pathway

Studies of the molecular bases of long-term memory formation started with the findings in simple invertebrates that cAMP and the induction of CREB- and C/EBP-dependent gene expression have a key function in synaptic plasticity and memory formation [27–29], particularly during the temporal window of consolidation, when memory is still labile [9,30,31]. Specifically, in invertebrates, including Aplysia californica and Drosophila melanogaster, the molecular disruption of CREB or C/EBP isoforms leads to memory impairment [25]. Similarly, molecular and genetic disruptions of either CREB or C/EBP isoforms in rodents impair different types of long-term memory, including spatial, contextual, appetitive and aversive, leading to the conclusion that the cAMP-CREB-C/EBP pathway has an evolutionarily conserved, fundamental role in long-term memory formation [25,32–34]. Some authors have found that the essential function of CREB in memory formation may be overcome by genetic backgrounds and that CREB-independent forms of long-term plasticity and memory exist [35]. Despite the fact that some form of memory and/or long-term potentiation (LTP) may indeed be CREB independent, these contrasting effects may be due to the result of protein compensation of the transgene that can overcome the requirement for CREB. Hence, in summary, numerous studies across species, brain regions and types of learning paradigm indicate that CREB is critical for memory formation. Despite the possibility that memory consolidation may also be enhanced independently of the CREB pathway, in this review, we focus on discussing nodes of the CREB-C/EBP pathway that represent important targets for promoting memory enhancement and persistence.

Several intracellular signaling pathways have been shown to activate the CREB-C/EBP cascade [25] (Figure 1), which include important targets for both memory disruption and strengthening. Selective modulation of specific components of this pathway may lead to the enhancement of memories and, furthermore, make them more persistent. Indeed, studies have demonstrated that this is the case.

The expression of an activated form of CREB in both Drosophila melanogaster and neuronal cultures of Aplysia californica was found to lead to long-term memory and/or plasticity from learning or stimulation paradigms that evoke only short-term memory or plasticity [28,36] (but see [37]). Similarly, overexpression of C/EBP in Aplysia neurons converted short-term plasticity into long-term plasticity [38]. These findings were the first evidence that, if the molecular cascade involved in long-term plasticity and memory formation is overstimulated, memory retention can be enhanced or made more persistent.

Similar results were found in mammalian systems. Overexpression of a neuronal-specific adenyl cyclase 1 (AC1), an enzyme that catalyzes the synthesis of cAMP, in the forebrain of mice, resulted in enhanced object recognition and contextual fear-conditioning memories [39]. Injections of a protein kinase A (PKA) activator, Sp-cAMPs, into the amygdala enhanced reward-related learning in a dose-dependent manner [40]. Furthermore, rolipram, an inhibitor of the phosphodiesterase 4 (PDE4) family of enzymes, which catalyze cAMP hydrolysis and thereby increase cAMP concentrations and CREB phosphorylation, significantly enhanced memory retention in rodent contextual fear conditioning [41], object recognition [42] and several spatial tasks [43]. In agreement with these results, recent studies have shown that both PDE4 subtype D (PDE4D)−/− deficient mice and mice in which PDE4D in the dorsal hippocampus was knocked down by lentiviral vectors, have enhanced spatial and object recognition memories [44]. Inhibitors of other PDEs, for example, the selective PDE5 inhibitors sildenafil, zaprinast and vardenafil, which can lead to an increase in CREB phosphorylation, have also shown enhancing effects in spatial object recognition and active avoidance memories in rodents [45,46], although others have reported contrasting findings [47].

Memory-enhancing effects have also been found by directly increasing the expression of CREB. Viral vector-mediated overexpression of CREB in the rat amygdala or mouse hippocampus enhanced cued and contextual fear conditioning, respectively [48,49]. Transgenic overexpression of dominant active forms of CREB in the forebrain resulted in enhanced memory retention in a variety of tasks, including fear conditioning, social recognition, the Morris water maze and passive avoidance [50].

Boosting CREB-dependent transcription activity is likely to promote memory enhancement by targeting many mechanistic levels. For example, CREB regulates the transcription of proteins that are necessary for the stabilization of memory after learning. Thus, enhancing CREB activity may augment the levels of these critical proteins, resulting in stronger memories. A series of elegant studies has increased the understanding of how CREB regulates memory formation, thereby mediating memory retention. In the lateral amygdala, neurons with viral-mediated overexpression of CREB are more likely to be active after memory retrieval than are their neighboring neurons, and selective ablation of these CREB-overexpressing neurons leads to memory impairment [51,52], suggesting that auditory fear conditioning can recruit CREB-expressing neurons in the lateral amygdala to establish fear memory formation. Furthermore, viral-mediated overexpression of CREB in the amygdala enhances synaptic transmission and increases neuronal excitability [53]. Similar increases in neuronal excitability are seen in the dorsal hippocampus of transgenic mice expressing a constitutively active form of CREB [54], leading to the conclusion that memory enhancement correlates
with increased neuronal excitability that is mediated, at least in part, by increased CREB expression and/or its functions.

Furthermore, activation of the CREB-C/EBP cascade is regulated by inhibitory constraints [55–58], the removal of which results in stronger or more persistent memories. For example, mutant mice with reduced expression of the general control non-repressed 2 (GCN2) kinase, an eukaryotic initiation factor 2 α (eIF2α) kinase, have suppressed expression of activating transcription factor 4 (ATF4) mRNA, a repressor of CREB-mediated gene expression, and display enhanced spatial memory, as assessed in the Morris water maze [59]. Likewise, mutant mice with reduced phosphorylation of eIF2α exhibit enhanced memory in a variety of tasks [60]. Similarly, transgenic mice with forebrain expression of a general dominant negative inhibitor of the C/EBP/ATF family (EGFP-AZIP) have enhanced spatial memory retention. EGFP-AZIP expression presumably relieves the inhibitory regulators and results in a lower threshold for LTP induction as well as increased spatial memory retention [57]. In summary, any molecular step that regulates the induction of the CREB- and/or C/EBP-dependent gene expression may, in principle, be a good target for promoting memory enhancement.

As with all transcription factors, CREB and C/EBP functions on gene expression are controlled by the state and regulation of chromatin. Chromatin modification can modulate the transcription necessary for memory formation by regulating access of transcriptional regulatory proteins to DNA. For example, histone acetylation is a chromatin modification that regulates DNA–histone interactions via two opposing classes of enzyme: histone acetyltransferases (HATs), which acetylate histone tails and promote transcription, and histone deacetylases (HDACs), which deacetylate histones and promote genes silencing [61].

One important function of CREB is to recruit CREB-binding protein (CBP), a cofactor with intrinsic histone acetyl transferase activity, which results in increased gene transcription [62]. Genetic mutations in CBP are associated with Rubinstein-Taybi syndrome, which is characterized by facial
abnormalities, broad thumbs and mental retardation [63]. Mutant mice lacking one functional copy of CBP exhibit impaired long-term inhibitory avoidance and contextual fear-conditioning memories [64]. More specifically, transgenic mice with a functional loss of the HAT activity of CBP have impaired long-term memory formation [65], whereas injections of HDAC inhibitors, which would compensate for deficits in CBP activity in CBP-mutant mice, rescue the memory impairment [65,66]. Similarly, PDE4 inhibitors, which enhance CREB signaling, also dose dependently rescue the long-term memory defects in CBP mutants [67]. These studies suggest that modulating CREB-dependent signaling or the state of chromatin via HDAC inhibitors are potential therapeutic options for cognitive disorders associated with CBP dysfunction. In agreement with this hypothesis, in wild-type mice, HDAC inhibitors have been found to enhance long-term memory in a variety of tasks, including contextual fear conditioning [68], water maze [69], fear-potentiated startle [70], extinction of fear memory [71] and novel object recognition [72]. Several extensive reviews have been published on HDAC inhibitors and their potential applications to treat cognitive disorders [73–75].

One step downstream of the activation of CREBs and C/EBPs is regulation of the expression of their target genes. Some of these target genes may include secreted factors or membrane receptors that can be more easily targeted by pharmacological approaches. One of these genes, IGFl2, was recently identified [26].

**IGF2**, a C/EBP target gene required for memory consolidation, promotes memory enhancement

IGF2 has been recently identified as a target gene of C/EBPB during memory consolidation in rats [26]. IGF-2 is a mitogenic polypeptide that is structurally similar to insulin and IGF-1. Insulin, IGFs and their respective receptors, together with IGF-binding proteins (IGFBP), constitute the IGF-related system (Figure 2). This protein system is important for normal somatic growth and development, tissue repair and regeneration [76]. Compared with the other family members, IGF-2 is more abundantly expressed in the adult brain and is found in regions that are critically involved in memory consolidation, such as the hippocampus and cortex [77]. The mechanism of action of IGF-2 is not clear and may be multifaceted [78]. The highest affinity of IGF-2 is for IGF-2 receptor (IGF-2R). Beside IGF-2, IGF-2Rs binds a large number of ligands, including lysosomal enzymes, transforming growth factor-β (TGF-β), granzyme B, plasminogen, glycosylated leukemia inhibitory factor and retinoids [79]. Most IGF-2Rs, also known as cation-independent mannose 6 phosphate receptors, are located intracellularly where they facilitate the trafficking of lysosomal enzymes between the trans-Golgi network, endosomes and lysosomes [79]. Cell-surface IGF-2Rs sequester circulating and local levels of IGF-2, thereby leading to IGF-2 degradation. IGF-2Rs can also mediate the endocytosis of extracellular lysosomal enzymes [80]. IGF-2 can also bind with lower affinity to IGF-1 receptors

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**Figure 2.** The insulin-like growth factor (IGF)-related system components. The IGF-related system comprises three ligands: insulin, IGF-1 and IGF-2. IGF-1 and IGF-2 levels in circulation are regulated by IGF-binding proteins (BPs). There are multiple receptor conformations. Insulin and IGF-1 receptor (IR and IGF-1R) are tyrosine kinase receptors. Two isoforms of IR, IR-A and IR-B, are found in the brain [109]. For simplicity, only signaling initiated by the activated IGF-1R is shown. Activation of IR or IGF-1R leads to phosphorylation of adaptor proteins belonging to the IR substrate (IRS) family or Src homology 2 domain-containing transforming protein (SHC) [110]. Activation of IRS and SHC leads to activation of Raf/Ras/mitogen-activated protein kinase (MAPK)/MAP kinase kinase (MEK) and phosphatidylinositol 3-kinase (PI3K)/Akt pathways. Phosphorylation of Akt leads to subsequent activation of mammalian target of rapamycin (mTOR), eukaryotic translation initiation factor 4E (eIF4E) and p70S6 kinase (S6K). Activation of these pathways leads to enhanced proliferation, survival and metastasis in cancer cells [110]. IGF-2 binds IR-A [111], IGF-1R and IGF-2R [110], and with the highest affinity to IGF-2R. IGF-2R is structurally distinct from IR and IGF-1R, and is not a receptor tyrosine kinase. Once IGF-2 binds, IGF-2R targets IGF-2 to endocytosis-mediated lysosomal degradation as well as effecting signal transduction [85] (Figure 4). Abbreviations: Grb2, growth factor receptor-bound protein 2; PDKI, phosphoinositide-dependent kinase-1; PIP3, phosphatidylinositol (3,4,5)-trisphosphate.
Avoidance terals is required when receiving injections and thereafter when the induction of C/EBPα to IGF-2 promoters [26], in agreement with previous findings indicating that the IGF-2 promoters bear C/EBP consensus sequences [81]. Furthermore, either antisense-mediated knockdown of IGF-2 in the dorsal hippocampus or injections of a function-neutralizing anti-IGF-2R antibody significantly impaired long-term inhibitory avoidance memory, suggesting that the learning-dependent increase in IGF-2 (via IGF-2R) is required for inhibitory avoidance memory consolidation [26]. Conversely, injections of recombinant IGF-2 into the dorsal hippocampus immediately after training, but not 24 h later, significantly and dose-dependently enhanced long-term inhibitory avoidance and contextual fear memory retention (Figure 3a,b), as well as preventing memory loss (Figure 3a) [26]. In fact, rats given a single hippocampal injection of IGF-2 immediately after training still had significant memory retention 3 weeks after training, whereas vehicle-injected control rats had largely forgotten (Figure 3a) [26]. Injections of IGF-2 into the amygdala had no effect on memory retention, suggesting that the mechanisms regulated by IGF-2 in memory enhancement target selective regions, such as the hippocampus, but not the amygdala [26]. Furthermore, hippocampal injections of IGF-2 have also been found to facilitate fear extinction in mice (Figure 3d) [82], confirming and extending the conclusion that IGF-2 promotes memory enhancement. It will be important to investigate other brain regions as targets

![Figure 3](image-url)

**Figure 3.** Insulin-like growth factor 2 (IGF-2) enhances fear as well as fear extinction memories. (a) Rats underwent inhibitory avoidance training, immediately after which they received bilateral intrahippocampal injections of IGF-1 (grey bars), IGF-2 (black bars), or vehicle solution (white bars; indicated by ↓). Memory retention was tested at 24 h (Test 1) and 7 days (Test 2) after training. Rats that received IGF-2, compared with IGF-1 and vehicle solution, had significantly higher memory retention at both tests [26]. Similar intrahippocampal injections of IGF-2 immediately after inhibitory avoidance training, compared with vehicle solution, significantly prevented memory forgetting. Rats injected with IGF-2 immediately after inhibitory avoidance training showed significantly higher memory retention at 3 weeks after training (Test), whereas the rats that received vehicle solution showed significant memory retention decay [26]. (b) Rats underwent contextual/auditory fear conditioning, in which they associate a context and a tone to a footshock and freeze in subsequent exposure to the training context or to the tone in a different context. Immediately after conditioning, the rats received bilateral intrahippocampal injections of IGF-2 (black bars) or vehicle (white bars; indicated by ↓). Rats that were injected with IGF-2, compared with vehicle solution, had a significantly higher freezing score when they were tested in the training context. By contrast, no effect was seen on the auditory fear-conditioning test, as rats injected with IGF-2 had freezing scores similar to rats injected with vehicle [26]. (c) Rats underwent contextual fear conditioning and were divided into two groups. Twenty-four hours later, one group received a bilateral intrahippocampal injection of either IGF-2 (black bars) or IGF-1 (grey bars), whereas the other group underwent inhibitory avoidance memory retrieval (Test 1) and immediately thereafter received similar bilateral intrahippocampal injections (indicated by ↓). All groups were tested for memory retention 48 h after training (Final test). IGF-2 had no effect when given 24 h after training (NoR final test), but, if given in concert with memory retrieval, compared with IGF-1, significantly enhanced memory retention [26]. (d) Mice underwent contextual fear conditioning and context-dependent freezing was assessed 24 h later. Extinction of contextual fear was performed on consecutive days at 24 h intervals consisting of re-exposure to the training context without footshock. Fear extinction was significantly enhanced in mice that received injections of IGF-2 (red circles) into the dorsal hippocampus immediately after each extinction trial (indicated by ↓) compared with the vehicle-injected group (grey squares) [82]. Data are shown as mean latency or % freezing ± s.e.m.; *P < 0.05, **P < 0.01, ***P < 0.001. Adapted, with permission, from [26] (A–C) and [82] (D).
of IGF-2-mediated memory enhancement, such as the cortex. Such future studies will help to address questions of region-specific mechanisms and/or selective effects on specific memory systems in memory enhancement.

IGF-2 is an interesting candidate for potential clinical applications because it is a naturally produced growth factor that readily crosses the blood–brain barrier. In the adult rat, IGF-2 mRNA can be found in the brain, heart, kidney, uterus and liver [83], and peripheral IGF-2 can be transported into the brain via IGF-2Rs localized in brain capillaries [84]. The temporally limited effect of hippocampally injected IGF-2 that occurs from the time of training (i.e. <24 h) as well as that of the post-retrieval effect in young but not old memories (discussed below) indicates that both consolidation and reconsolidation mechanisms can be targeted to produce memory enhancement. Mechanistically, IGF-2-dependent memory enhancement requires the function of IGF-2R, but not IGF-1R [26]. IGF-2R, unlike insulin- and IGF-1R, is not a tyrosine kinase receptor [85]. Whereas insulin and IGF-1R mediate growth and activate the ras/raf/mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K)/Akt pathways (Figure 2), IGF-2R/mannose-6-phosphate receptor binds IGF-2 extracellularly and/or transports lysosomal acid hydrolase precursors from the Golgi apparatus to the lysosome [79] (Figure 4). IGF-2Rs from both the cell surface and Golgi traffic to the early endosome, where the relatively low pH environment causes the release of the IGF-2R cargo. The IGF-2Rs are recycled back to the Golgi by the retromer complex. The cargo proteins are then trafficked to the lysosome via the late endosome independently of the IGF-2Rs [79] (Figure 4). How these and potentially other functions of IGF-2Rs lead to memory enhancement is currently unknown.

IGF-2-mediated memory enhancement also requires de novo protein synthesis. Although IGF2 is a target gene of CREB3, IGF-2-mediated memory enhancement does not depend on de novo C/EBPβ synthesis and does not correlate with increased levels of pCREB and C/EBPβ [26]. This suggests that IGF-2 does not exert its effects by amplifying these transcriptional mechanisms, but rather by acting on translational and synaptic mechanisms. Interestingly, one of the newly synthesized proteins required for IGF-2-mediated memory enhancement is activity-regulated cytoskeletal-associated protein (Arc; also known as Arc 3.1) [26]; its functions are needed for long-term plasticity and memory formation. For example, Arc is required in the hippocampus and the amygdala for the consolidation of spatial memory and cue–fear conditioning, respectively [86,87]. Studies have suggested that Arc is critical for long-term plasticity and memory because, by interacting with the endocytic machinery proteins endophilin and dynamin, it regulates membrane trafficking of AMPA receptors [88,89]. AMPA receptor trafficking is critically involved in LTP, long-term depression (LTD) and memory. It is thought that AMPA receptor subunits are rapidly transported in and out of synapses to strengthen or weaken synaptic activity during plasticity and learning [90,91]. In agreement with this hypothesis of a positive correlation between plasticity and memory formation and increased trafficking of synaptic AMPA receptors, IGF-2-mediated memory enhancement was found to correlate with increased levels of synaptic GluA1 AMPA receptor subunits [26].

Endocytosis is also regulated by glycogen synthase kinase 3β (GSK3β), a serine/threonine kinase that was originally identified as a regulator of cell metabolism but has since been implicated in various functions, including proliferation, cell survival, neural development, and shaping nerve terminal development and function [92]. In fact, endocytosis was found to be dependent on the phosphorylation of dynamin I (at the Ser 774 residue) by GSK3β [93]. IGF-2-mediated memory enhancement correlates with the activation of GSK3β and requires the activity of GSK3 [26], supporting the hypothesis that the memory-enhancing effect critically targets functions of the endocytic pathway. Previous findings suggested a correlation between increased endocytosis and increased GSK3 activation and Arc expression [88,93]. Such findings appear to contrast with the finding that memory enhancement is accompanied by increased synaptic expression of certain receptor subunits (e.g. GluA1) [26]. However, it is possible that endocytosis of other receptors as well as restructuring of synapses are also critical for enhancing synaptic strength. Moreover, increased endocytosis may play an important role in homeostatic synaptic scaling, which may enhance the system capacity. Future studies will hopefully extend current knowledge regarding the role of endocytosis in memory enhancement.

An intriguing property of IGF2 and IGF2R is that they are both imprinted genes. Imprinted genes are expressed only by one allele, either maternal or paternal, and this
pattern of expression is maintained epigenetically in almost all tissues. How imprinted genes participate in behavior is a topic of great interest and recent investigations [94–96].

Interestingly, insulin and other insulin regulatory proteins have been shown to promote memory enhancement in rodents as well as in humans [97–99]. However the mechanisms by which this effect occurs seem to be different from that of IGF-2. Further studies are needed to better dissect the mechanisms of these effects.

**Targeting reconsolidation to enhance memory**

Why does memory become labile after retrieval and undergo reconsolidation? The biological purpose of reconsolidation has been the topic of debates and investigations since its rediscovery more than 10 years ago [13]. One debated issue in the reconsolidation field is its relationship with the role of the passage of time [100]. In inhibitory avoidance conditioning, retrieval leads to protein synthesis-dependent reconsolidation only if the memory is less than 2 weeks old. By contrast, 2- or 4-week old memories do not become fragile after retrieval and, in fact, are resilient to protein synthesis inhibitor treatment administered either systemically or via intra-amygdala injections [18,101]. Similarly, contextual fear conditioning becomes increasingly resistant to post-retrieval administration of protein synthesis inhibitors with time [19]. By contrast, 45 or 60 day old auditory fear conditioning memories are still susceptible to disruption by protein synthesis inhibition in the amygdala after retrieval, and no temporal gradient of resilience has been described [102,103]. In inhibitory avoidance conditioning, whereas hippocampal protein synthesis is required for the initial consolidation, it is dispensable during reconsolidation, indicating that mechanisms in the amygdala, but not the hippocampus, are critical for memory reconsolidation [104]. Hence, in inhibitory avoidance and, perhaps, other temporal-lobe-dependent memories, hippocampal resistance to post-retrieval amnesic treatments may reflect the more cortically distributed representation of the memory trace. By contrast, in pavlovian fear conditioning, where the amygdala is necessarily involved in the acquisition, storage and expression of conditioned fear memory, reactivation of the very localized trace renders the memory labile. Whether this reflects differences in molecular and/or circuit-based mechanisms remains to be understood.

One of the hypotheses proposed to explain the function of reconsolidation is that memory reconsolidates to become stronger and longer lasting [14]. Supporting this hypothesis, studies have demonstrated that a second training trial strengthens a contextual fear memory [105]. This strengthening requires the function of transcription factor early growth response protein 1 (EGR-1; also known as Zif268) in the hippocampus, which is also essential for the reconsolidation, but not consolidation, of contextual fear conditioning [105]. Using inhibitory avoidance conditioning in rats, a recent study reported that unreinforced contextual reminders given when the memory is young, but not when it is older, enhance memory retention and prevent forgetting [22]. Such findings indicate that reconsolidation does indeed promote memory enhancement. Targeting reconsolidation mechanisms should therefore also provide opportunities to enhance memories.

Several studies have explored the possibility of enhancing memory by targeting mechanisms activated during reconsolidation. For example, activating PKA in the basolateral amygdala (BLA) with Sp-cAMP during reconsolidation leads to enhancement of rat auditory fear memory retention; conversely, inhibiting PKA by administering 6-BNZ-cAMP to the same brain region immediately after retrieval impairs reconsolidation and leads to amnesia [106]. Similarly, in the crab *Chasmagnathus*, blocking angiotensin II impairs reconsolidation, whereas exogenous administration of recombinant angiotensin II enhances it [107].

IGF-2, which enhances inhibitory avoidance memory when administered to the hippocampus following training (Figure 3a), also significantly strengthens inhibitory avoidance memory when administered in the same brain region after memory retrieval (Figure 3c) [26]. The effect is retrieval dependent and limited to the same exact temporal window during which inhibitory avoidance memory undergoes reconsolidation; that is, less than 2 weeks after training. Hence, although the hippocampus is not a substrate for post-retrieval memory disruption after inhibitory avoidance training, it is a substrate for post-retrieval memory enhancement that occurs during the reconsolidation-sensitive temporal window. We conclude that, in medial temporal lobe-dependent fear memories, retrieval activates the amygdala to control consolidation that is mediated by hippocampal mechanisms, leading to subsequent strengthening and cortical redistribution of the trace. These mechanisms can be targeted to promote memory enhancement. Thus, to effectively target mechanisms of consolidation or reconsolidation for memory enhancement it is important to keep in mind that there are temporal constraints. Hence, an enhancer that targets the hippocampal CREB-C/EBP-dependent cascade will probably be most effective either within the first day after training or after reactivation when reactivation leads to reconsolidation. However, as the effect of IGF-2 on cortical regions still needs to be determined, it is possible that memory-enhancing effects in the cortex may have different temporal evolutions.

**Concluding remarks**

Important questions arise concerning memory enhancement that targets consolidation and reconsolidation mechanisms (Box 1). First, are the enhancing effects selective for the active trace and, therefore, only affect an active memory? This would restrict their effect, eliminating them as treatments that might globally enhance all stored memories. However, these types of pharmacological treatment could perhaps be paired with brain stimulation, which would activate the memory traces, allowing them to respond to the treatment. Second, from the results obtained, one can infer that if consolidation or reconsolidation is impaired by, for example, neurodegenerative disease, memory enhancers may be ineffective. Thus, it is important that memory enhancers are tested and investigated in disease models at multiple stages of disease progression. Memory enhancers targeting consolidation...
and reconsolidation may hopefully be able to compensate for, or rescue, defects that may well be the cause of the disease, thus significantly slowing the progression of the disorder. In other words, prevention would be the most, or perhaps the only, successful strategy. Third, as memory-enhancing treatments would be needed for non-aversive memories, the consolidation and reconsolidation mechanisms of appetitive or problem-solving memories will have to be better investigated and understood. Finally, a safe and clinically applicable memory enhancer would also need to be highly specific and have minimal adverse effects. Importantly, studies on memory enhancement should examine more carefully the effect of treatment on multiple tasks and brain functions to assess whether memory enhancement compromises the flexibility of memory or brain plasticity. Given that memory formation is such a dynamic process, gaining a more complete understanding of the anatomical and temporal dynamics of the molecular and systems changes required after learning and retrieval to consolidate memories will enable researchers to develop the most specific and efficacious memory enhancers.

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