Neurobiology of Learning and Memory 105 (2013) 117–124

Contents lists available at SciVerse ScienceDirect

Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme

Pattern and predictability in memory formation: From molecular mechanisms to clinical relevance

Gary T. Philips *, Ashley M. Kopec, Thomas J. Carew

Center for Neural Science, New York University, 4 Washington Place, Room 809, New York, NY 10003, United States

ARTICLE INFO

Article history:
Available online 28 May 2013

Keywords:
Learning
Memory
Spacing effect
Training pattern

ABSTRACT

Most long-term memories are formed as a consequence of multiple experiences. The temporal spacing of these experiences is of considerable importance: experiences distributed over time (spaced training) are more easily encoded and remembered than either closely spaced experiences, or a single prolonged experience (massed training). In this article, we first review findings from studies in animal model systems that examine the cellular and molecular properties of the neurons and circuits in the brain that underlie training pattern sensitivity during long-term memory (LTM) formation. We next focus on recent findings which have begun to elucidate the mechanisms that support inter-trial interactions during the induction of LTM. Finally, we consider the implications of these findings for developing therapeutic strategies to address questions of direct clinical relevance.

1. Introduction

All animals must use their experience to create a statistical model of their world. This model is driven by both pattern and predictability. The regularity (or pattern) of an experience is predictive of the likelihood of an encounter with the same or related experiences in the future, and therefore facilitates the acquisition and maintenance of adaptive behavior. The maintenance of such a predictive model depends on the formation of long-term memory (LTM). Yet not all repeated experiences are retained in LTM. The timing of experiences is critical. In psychological terms, the benefit to LTM induction of temporally distributed experiences (trials), compared to more closely spaced trials, is often termed *the spacing effect* and can be traced to the earliest formal studies of human learning and memory by Hermann Ebbinghaus (1885/1913). Since these seminal observations more than a century ago, it has become increasingly evident that the spacing effect is a ubiquitous phenomenon that governs LTM formation in a wide range of species and across a wide variety of tasks. Yet even after decades of study, we still understand relatively little about the properties of neural circuits in the brain that determine the benefit of spaced training. In this review we will briefly discuss major findings that elucidate some of the cellular and molecular mechanisms that can, at least in principle, contribute to the spacing effect. We will then focus on recent studies that provide novel and fundamental insights into how effective spacing intervals are determined and may benefit LTM formation. Finally, we conclude with a discussion of the implications of experimental studies for the development of effective learning strategies in humans, as well as the potential for these studies to inform questions of direct clinical relevance.

2. General principles of the spacing effect

The benefit of spaced training to LTM formation is widely observed in both vertebrate and invertebrate model systems, and provides striking parallels to the general principles observed in humans. The spacing effect in LTM is observed across a variety of tasks, including spatial reference memory (Bolding & Rudy, 2006), working memory (Klapdor & Van Der Staay, 1998), appetitive associative conditioning (Colomb, Kaiser, Chabaud, & Preat, 2009), aversive associative conditioning (Amano & Maruyama, 2011; Williams, Frame, & LoLordo, 1991; Yin et al., 1994) and both sensitization and habituation (Carew, Pinsker, & Kandel, 1972; Pinsker, Carew, Hening, & Kandel, 1973; Sutton, Ide, Masters, & Carew, 2002). Effective training intervals appear to be task specific and are controlled by a number of factors, including the retention interval examined (e.g., Beck, Schroeder, & Davis, 2000; Gerber, Wustenberg, Schutz, & Menzel, 1998) and the relationship between trial duration and trial spacing (Gibbon, Baldock, Locurto, Gold, & Terrace, 1977). Finally, although a sufficient spacing of training trials is necessary to benefit LTM induction (with effective training intervals ranging from minutes to days; see Parsons & Davis, 2012), trials can of course also be spaced too far apart to support LTM acquisition (Bolding & Rudy, 2006; Gibbon et al., 1977; Parsons & Davis, 2012; Philips, Tzvetkova, & Carew, 2007). Thus, the benefit of spaced training is non-monotonic, in agreement with

* Corresponding author.
E-mail address: gp60@nyu.edu (G.T. Philips).

1074-7427/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.nlm.2013.05.003

studies in humans (Cepeda, Pashler, Vul, Wixted, & Rohrer, 2006; Ebbinghaus, 1885/1913).

Interestingly, although there is a general trend in both the human and animal literature describing a benefit from repeated spaced training trials, there is a large body of work studying LTM which forms following a single training session, so-called "flashbulb" memories (Diamond, Campbell, Park, Halonen, & Zoladz, 2007; van Giezen, Arensman, Spinhoven, & Wolters, 2005). Is this learning different from that which develops over repeated experiences? One-trial memories typically develop from emotionally salient events and may indeed rely on mechanisms that are different from those recruited during multi-trial learning (Irvine, von Hertzen, Plattner, & Giese, 2006; Radwanska et al., 2011). However, memory deficits on one-trial cued fear and passive avoidance tasks in mutant mice (that are alphaCAMKII autophosphorylation-deficient) can be rescued by providing additional spaced training trials (Irvine, Vernon, & Giese, 2005). Thus, the possibility exists that even one-trial learning tasks can benefit from mechanisms that subserve LTM formation across spaced training.

3. Cellular and molecular correlates of the spacing effect

Both vertebrates and invertebrates express memory across multiple temporal domains. Each domain has unique cellular and molecular mechanisms that support its induction. Short-term memory (STM) typically develops following a single experience (training trial), lasts on the order of minutes, and relies on the transient modification of pre-existing proteins to establish short-lasting plasticity within underlying neural circuits (Alkon & Naito, 1986; Barondes, 1970; Castellucci, Blumenfeld, Goelet, & Kandel, 1989; Scheibenstock, Krygier, Haque, Syed, & Lukowiak, 2002; Wittstock, Kaatz, & Menzel, 1993; Xia, Feng, & Guo, 1998). Following multiple training trials, both intermediate-term memory (ITM, lasting several hours) and LTM (lasting $\geqslant$24 h) are established. ITM induction requires ongoing protein synthesis, but does not require new gene transcription (Lukowiak, Adatia, Krygier, & Syed, 2000; Lyons, Green, & Eskin, 2008; Sangha, Scheibenstock, McComb, & Lukowiak, 2003; Stough, Shobe, & Carew, 2006; Sutton, Masters, Bagnall, & Carew, 2001). In contrast, LTM requires not only protein synthesis, but also gene expression to stabilize the new growth and enhanced cellular and synaptic plasticity required for LTM expression (Bailey, 1999; Bailey, Bartsch, & Kandel, 1996; Castellucci et al., 1989; Mozzachiodi, Lorenzetti, Baxter, & Byrne, 2008; Sangha et al., 2003; Sutton et al., 2001; Tully, Preat, Boynton, & Del Vecchio, 1994; Wustenberg, Gerber, & Menzel, 1998).

The spacing effect does not appear to regulate the acquisition or development of STM, but strongly regulates the induction of LTM in a variety of learning tasks in a wide range of species, including pigeon (Gibbon et al., 1977), rodent (Bolding & Rudy, 2006; Klapdor & Van Der Staay, 1998; Williams et al., 1991), honeybee (Gerber et al., 1998), *Drosophila* (Tully et al., 1994), *Hermissenda* (Rogers, Talk, & Matzel, 1994), *Lymnaea* (Lukowiak, Cotter, Westly, Ringseis, & Spencer, 1998), and *Aplysia* (Carew et al., 1972). The effect of training pattern on the formation of ITM is less well studied, but has shown to be of benefit in some cases (Sutton et al., 2002). Not surprisingly, spaced training is better than massed training at recruiting the cellular, molecular and structural signatures of LTM (for recent comprehensive reviews see Barco, Bailey, & Kandel, 2006; Lynch, Kramar, Babayan, Rumbaugh, & Gall, 2013; Naqib, Sossin, & Farah, 2012).

3.1. Cellular correlates

In vertebrate studies, the long-term potentiation (LTP) of synaptic signaling is the most often studied cellular correlate of LTM (although there are several instances described in which LTP induction and LTM induction are not correlated: Barnes, 1995; Pineda et al., 2004, and Shors & Matzel, 1997). LTP is observed at synapses in multiple brain regions, but LTP of the CA3 Schaffer collateral synapses onto area CA1 pyramidal neurons in the hippocampus has been most frequently studied (Bliss & Collingridge, 1993; Malenka & Bear, 2004). LTP induction at CA3/CA1 synapses and LTM share many mechanistic similarities, including the ability to be strengthened across spaced training and a sensitivity to the patterning of spaced training trials (Abraham, Logan, Greenwood, & Dragunow, 2002; Huang & Kandel, 1994; Kramar et al., 2012; Malenka, 1994; Winder, Mansuy, Osman, Moallem, & Kandel, 1998). At the molecular level, the requirements for a form of long-lasting LTP (L-LTP) are similar to those for LTM: both require cAMP, cAMP-dependent protein kinase A (PKA), the extracellular signal-regulated protein kinase (ERK) of the mitogen-activated protein kinase superfamily (hereafter referred to as MAPK) and CREB signaling (for review see Barco et al., 2006). Moreover, manipulations that remove inhibitory constraints on PKA, MAPK and CREB activation, support the induction of LTP and LTM with a reduced number of trials (Barad, Bourtchouladze, Winder, Golan, & Kandel, 1998; Genoux et al., 2002; Malleret et al., 2001). In studies in invertebrate model systems, where clear links between behavior and cellular signaling can be established, similar conclusions have been drawn between the training pattern sensitivity for the induction of structural plasticity (Wainwright, Zhang, Byrne, & Cleary, 2002), neuronal excitability (Mozzachiodi et al., 2008; Rogers et al., 1994) and synaptic plasticity (Mauelshagen, Sherff, & Carew, 1998) and LTM. There are several excellent recent reviews in this general area (Barco et al., 2006; Mayford, Siegelbaum, & Kandel, 2012; Mozzachiodi & Byrne, 2010; Naqib et al., 2012).

3.2. CREB

A conserved molecular target of the spacing effect appears to be the differential recruitment of the cAMP response element binding protein (CREB) signaling and CREB-mediated transcription by spaced, but not massed training patterns (reviewed in Naqib et al., 2012; Silva, Kogan, Frankland, & Kida, 1998; Yin & Tully, 1996). CREB-mediated transcription is a critical requirement for the development of long-lasting plasticity and LTM in many systems (Dash, Hochner, & Kandel, 1990; Pittenger et al., 2002; Taubenfeld, Milekic, Monti, & Alberini, 2001) and is upstream of the synthesis of cytoplasmic effectors such as synapsin I (Hart et al., 2011) as well as subsequent nuclear signaling mediated by the recruitment of genes which encode for additional transcription factors important for memory consolidation, including C/EBP (Alberini, Ghirardi, Metz, & Kandel, 1994; Taubenfeld et al., 2001) and CREB itself (Liu, Cleary, & Byrne, 2011). Removing the inhibitory constraints on CREB activation or overexpressing CREB during learning can support the formation of long-lasting forms of synaptic plasticity and LTM with reduced training trials (Bartsch, Casadio, Karl, Serodio, & Kandel, 1998; Bartsch et al., 1995; Genoux et al., 2002; Malleret et al., 2001; Yin, Del Vecchio, Zhou, & Tully, 1995). Thus, CREB recruitment is an important and highly conserved mechanism that contributes to establishing the training pattern requirements for memory formation.

Importantly, CREB phosphorylation on ser133 (Gonzalez & Montminy, 1989) is not always sufficient to induce its transcriptional activity. The recruitment of transcriptional coactivators such as the CREB-binding protein (CBP) and the CREB-regulated transcriptional coactivator 1 (CRTC1) help to regulate CREB-dependent LTP and LTM formation (Ch'ng et al., 2012; Hirano et al., 2013; Kovacs et al., 2007; Wood et al., 2005; Zhou et al., 2006). Evidence from LTP studies in rat has implicated CRTC1 in pattern detection (Zhou et al., 2006). Overexpression of CRCT1 is sufficient to lower the

threshold for late-LTP induction to a single high frequency train of stimulation. Additionally, the elevation of CRTC1 levels by fasting flies prior to exposing them to an aversive olfactory task is sufficient to lower the threshold for LTM induction from ten training trials to a single trial (Hirano et al., 2013). Since transcriptional coactivators are important signaling requirements for CREB-mediated transcription, the pattern-sensitive recruitment of these signaling partners during learning must also be considered as potential sites for pattern detection.

3.3. CaMKII, PKA and MAPK

Pattern sensitivity is also observed upstream of CREB in the recruitment of signaling cascades such as calcium/calmodulin-dependent protein kinase II (CaMKII), PKA and MAPK that can directly or indirectly phosphorylate CREB on ser133 and promote its transcriptional activity. All three kinase signaling pathways have been highlighted in LTP studies as candidate molecules involved in the integration of synaptic events over time. For example, both CaMKII (Coomber, 1998) and PKA (Roberson & Sweatt, 1996) are rapidly activated following single LTP-inducing stimuli and preferentially support LTP induction across either closely spaced or more distributed training stimuli, respectively (Kim, Huang, Abel, & Blackwell, 2010; Woo, Duffy, Abel, & Nguyen, 2003). MAPK is also activated transiently following a single LTP-inducing stimulus, reaching peak levels at approximately the same timing as the optimal inter-tetanus interval (5–10 min) between 3 tetani that result in maximal LTP induction (Ajay & Bhalla, 2004). Intriguingly, in *Aplysia*, the dynamics of inter-trial PKA and MAPK activation were recently used to design enhanced (and surprisingly non-intuitive) spaced training patterns based on predictions for the maximal accumulation and overlap of the two signals (Zhang et al., 2012). The recruitment and sustained signaling of CaMKII (Chen et al., 2012), PKA (Muller & Carew, 1998) and MAPK (Atkins, Selcher, Petraitis, Trzaskos, & Sweatt, 1998; Cammarota et al., 2000; Sharma, Sherff, Shobe, Bagnall, Sutton, & Carew, 2003b) are also observed following spaced, but not massed training and are known to be necessary for LTM formation.

3.4. G-proteins

The induction of small G proteins further upstream from the signaling cascades described above has also been shown to be sensitive to training pattern. For example, in *Aplysia*, MAPK can be activated by both spaced and massed exposures of the central nervous system to the neuromodulator serotonin (5HT; 5 pulses spaced by 15 min, or a single 25 min exposure). However, only the spaced training pattern is associated with the reliable induction of a MAPK-dependent long-term facilitation (LTF; Martin et al., 1997; Mauelshagen et al., 1998). Ye and colleagues (2008) showed that the selective recruitment of LTF-supporting mechanisms by spaced 5HT could be accomplished by a differential ratio of activity of the *Aplysia* homologues of the small G proteins Ras and Rap 1 (ApRas and ApRap), recruited by each training pattern. Increased ApRas (relative to ApRap) activity supported MAPK activation following spaced training. However, no such increase was recruited by massed training. Moreover, overexpressing ApRap during spaced training antagonized MAPK activation. A related finding is observed in a *Drosophila* homologue of the SHP2 phosphatase, *corkscrew*, which lies upstream of Ras/MAPK activation. Corkscrew phosphatase activity was recently shown to determine the pattern sensitive recruitment of MAPK and LTM induction in flies (Pagani, Oishi, Gelb, & Zhong, 2009). Collectively, these findings implicate the recruitment of the small G proteins, CaMKII, PKA and MAPK signaling cascades during learning as determinants of long-term synaptic strengthening and LTM over multiple spaced training experiences.

4. Why is massed training ineffective in recruiting the mechanisms of LTM consolidation?

Although spaced training clearly supports the recruitment of many signaling mechanisms that support LTM induction, an inverse question can be raised: Why is massed training ineffective? One answer is that it is simply unable to recruit the same signaling that supports LTM induction across spaced training. However, in many cases massed training actually recruits signaling which actively opposes LTM induction (Abel, Martin, Bartsch, & Kandel, 1998; Yin & Tully, 1996). In a recent study in *Drosophila*, the development of LTM during spaced training on an aversive olfactory conditioning task was shown to require the recruitment of slow synchronous calcium oscillations in two pairs of neuromodulatory dopaminergic neurons, specifically in the interval between spaced trials (Placais et al., 2012). Massed training, on the other hand, was shown to actively weaken these oscillations to support the induction of a second and opposing form of memory, anesthesia-resistant memory (ARM). Thus, in this case, massed training fails to allow the critical synchrony necessary to develop the neuromodulatory signaling required for LTM. In studies of LTP induced by theta burst stimulation (TBS) of Schaffer collateral CA3/CA1 synapses in acute hippocampal slices, Lynch and colleagues (Kramar et al., 2012; Lynch et al., 2013) recently identified a refractory period of 40–50 min that occurs following a single TBS stimulation that prevents a subsequent TBS (TBS2) from recruiting additional enhancements in synaptic strength. However, beyond the refractory period the spaced administration of additional rounds of TBS could recruit additional potentiation (up to 150% that typically observed following 3 TBS trains spaced 60 min apart). The increased potentiation was correlated with an increased number of spines on CA1 pyramidal neuron dendrites with evidence of synaptic remodeling. No such increase was observed if TBS2 was given during the refractory period. These authors further suggest that spines recruited for LTP by TBS1 may produce a diffusible factor (such as Ras-GTP or RhoA) that acts to "prime" nearby spines to express LTP in response to TBS2. Thus, sufficiently spaced training trials may benefit LTM by allowing the recruitment of additional synaptic plasticity and synapses not recruited during the initial experience, and massed training may fail to support LTM because subsequent training trials are administered too soon after earlier trials to support the recruitment of additional plasticity and synapses.

At the molecular level, the intracellular accumulation of calcium during massed training trials has been shown to recruit the phosphatases PP1 and PP2B (calcineurin), which can serve as "brakes" on LTM induction by antagonizing, for example, CREB phosphorylation at ser133 (Bito, Deisseroth, & Tsien, 1996). In rodent and invertebrate models, the removal of this phosphatase activity can serve to increase the recruitment of CREB during learning and can facilitate the induction of long-term plasticity and LTM from fewer training trials (Genoux et al., 2002; Koshibu et al., 2009; Malleret et al., 2001; Rosenegger, Parvez, & Lukowiak, 2008; Sharma, Bagnall, Sutton, and Carew, 2003a). Similar gain-of-function is observed in flies when CREB is overexpressed (Yin et al., 1995) and when the Ras/MAPK activation during learning is facilitated (Pagani et al., 2009). Finally, in *Aplysia*, an analog of massed training has been shown to preferentially support the sustained translocation of PKC to the plasma membrane which antagonizes the activation of PKA necessary to support LTF and LTM (Farah, Weatherill, Dunn, & Sossin, 2009). Intriguingly, Naqib et al. (2012) have proposed that the rates of rapid protein synthesis and degradation may determine both the failure of massed training and the success of spaced training to induce LTM in *Aplysia*.

5. Molecular windows in LTM formation

The studies reviewed thus far have principally focused on understanding how mechanisms known to be important for the induction of LTM are selectively recruited over repeated spaced experiences. However, an important question remains: How do temporally spaced experiences interact and build upon one another to support LTM formation? We will now focus our discussion on two recent studies [i.e., Philips, Ye, Kopec, and Carew (2013) and Parsons and Davis (2012)] that have begun to address this question.

The study of the integration of multiple repeated experiences in support of LTM induction has an inherent problem: It is difficult to determine the unique contribution of each individual experience to the final memory. For instance, in a spaced training pattern of ten trials, what is the unique contribution of trial 8 (compared e.g. to trial 7 or trial 9) to LTM induction? Philips et al. (2007) recently explored this general question in *Aplysia*. Specifically, they examined the individual contribution of distributed experiences to LTM induction through the development of a novel training paradigm which consisted of just two spaced training trials. Although four regularly spaced trials (ITI = 15 min) can support LTM induction (repeated-trial training; Sutton et al., 2002), Philips et al. reported that qualitatively similar LTM develops across just two trials spaced by 45 min, a temporal interval that brackets the total duration of the training episode that occurs across the repeated-trial training pattern (Philips, Sherff, Menges, & Carew, 2011; Philips et al., 2007). Two-trial training with intervals of 15 min or 1 h failed to induce LTM, providing a temporal boundary (with a clear onset and offset) for the successful LTM training "window". Thus, during two-trial training, the contribution of each trial to the final LTM can be easily identified: an initial experience (Trial 1) sets in motion a series of signaling events that must be inherited by a second experience (Trial 2) within a narrow temporal window. Using this paradigm, Philips et al. (2007) explored the molecular signaling established by the initial training trial that might be important for supporting the interaction with Trial 2 for LTM induction. They found that MAPK recruitment by Trial 1 was a molecular correlate occurring in the narrow temporal window within which Trial 2 could support the induction of LTM. MAPK was activated at 45 min following Trial 1, but was not observed at earlier (15 min) or later (1 h) time points. This finding is consistent with two previous observations. Ajay and Bhalla (2004) found a correlation of the peak activation of MAPK following individual training stimuli with effective spacing intervals for maximal LTP induction, and Pagani et al. (2009) observed a correlation between peak inter-trial activation of MAPK and effective LTM spaced training intervals in wild type flies. In *Aplysia*, since a single training trial was not, in itself, sufficient to support LTM induction, Philips et al. (2007) hypothesized that the MAPK signaling recruited by Trial 1 was necessary to establish the interactive window for trial 2 to support LTM induction. Indeed, this hypothesis was recently confirmed. Philips et al. (2013) demonstrated that the MAPK recruited by Trial 1 is required for LTM induction. The net effect of Trial 1 was to establish a "molecular context" at 45 min which included the nuclear translocation of MAPK and the recruitment of CREB signaling (activation of the CREB kinase p90rsk and the immediate early gene, *C/EBP*). Importantly, although in place at 45 min, this context was transient, terminating by 1 h. Thus, the opening and closing of the MAPK-dependent molecular context captures the time course for the opening and closing of the permissive interaction window for two-trial LTM induction.

The recruitment of CREB signaling and *C/EBP* induction following a single training trial (Philips et al., 2013) is unexpected, since these events are typically considered to be final stages involved in LTM formation. However, the recruitment of CREB-mediated transcription (Silva et al., 1998) and immediate early genes (Guzowski, 2002) have already been suggested as possible mediators of the successful integration of repeated experiences. For example, Silva et al. (1998) suggested that a sufficient inter-trial spacing might be determined by the timing for the recruitment of CREB and completion of an initial round of transcription, before subsequent training (and CREB activation) could recruit additional rounds of transcription to support LTM. Moreover, the ability of overexpressed CREB to modify the intrinsic excitability of neurons has prompted others to label it a "learning gene" (Benito & Barco, 2010). Intriguingly, CREB mutant mice lacking two of the three CREB activator isoforms (α and δ) demonstrate learning deficits on a spatial memory version of the Morris water maze task when trained across 5 spaced trials (ITI = 1 min), but normal memory when an alternate training strategy (only two training trials separated by 1 h) was employed (Kogan et al., 1997). Thus, the absence of two CREB isoforms, or possibly the elevated levels of the remaining CREB isoforms, appeared to alter the effective training pattern in these mice, but it did not eliminate the ability of these animals to acquire LTM. Collectively, these findings suggest that CREB/C/EBP signaling can be recruited much earlier during learning than is commonly appreciated and support the idea that they may function in the integration of inter-trial signaling during LTM formation.

Thus far we have discussed molecular windows in the range of minutes to perhaps hours. However, much longer permissive windows are possible. For example, in an intriguing recent study, Parsons and Davis (2012) reported that rats could be trained with two spaced training trials (each trial consisting of a single pairing of light and electrical shock that alone was not sufficient for the induction of STM or LTM) over a surprisingly broad permissive window, beginning at 1 h and extending for several days. They also observed that several targets of PKA were transiently activated in the amygdala after a single training trial, but were not persistently activated across the broad two-trial interaction window. However, when PKA activation in the amygdala was selectively disrupted during trial 1, this manipulation was sufficient to disrupt LTM induction following a second spaced trial at 24 h. Thus, Parsons and Davis (2012) proposed that PKA-dependent signaling following the initial training trial was necessary to "prime" the interaction with the second training trial to support LTM.

Collectively, the novel strategy of focusing on inter-trial interactions over just two training trials, Philips (2007, 2013) and Parsons and Davis (2012) has provided important new insights into how individual experiences can successfully interact over a broad range of temporal intervals to support the induction of LTM. These studies have also implicated a molecular framework of PKA/MAPK/CREB signaling as important determinants of the temporal windows that are permissive for LTM formation. As will be discussed below, these results may have direct translational implications that could enable the understanding and perhaps correction of learning deficits associated with aging and disease.

6. Effective versus optimal learning strategies

In reviewing the mechanistic insights gained from studies of the spacing effect in model systems, a final fascinating study by Zhang et al. (2012) warrants mention: These authors combined computer simulations with cellular and behavioral studies to ask whether a standard, regularly spaced training pattern of five sensitizing trials in *Aplysia* was the *optimal* training pattern for LTM induction. The investigators began with the assumption that the maximal recruitment of CREB during learning would support maximal

LTM induction, and that this would be downstream of coincident and maximal PKA and MAPK activity in single neurons. Using the kinetics for the recruitment of PKA and MAPK in neurons following a single training trial, they found that regularly spaced training intervals were not as effective as an expanded interval training pattern (i.e., the first few trials closely spaced, with final trials occurring at longer intervals, to maximize PKA/MAPK coincident signaling and CREB activation). Indeed, this new *optimal* training pattern produced better synaptic facilitation and LTM than the standard protocol using equally spaced trials. Collectively, this study and those that describe qualitatively similar LTP and LTM induction from training patterns that use as few as two training trials (Kramar et al., 2012; Parsons & Davis, 2012; Philips et al., 2007) suggest that although they are *effective*, many current training patterns employed in mechanistic studies of the spacing effect may not be *optimal*. Thus, an important question relevant to future progress is whether similar or unique mechanisms support LTM induction across *effective* versus *optimal* spaced training patterns.

7. Implications of spaced training in health and disease

In humans, the benefits of spaced training for memory formation in young healthy adults are well documented (for a recent meta-analysis see Cepeda et al., 2006), and also appear to benefit learning throughout the lifespan. Children, including infants as young as 3 months old, and both young and old cognitively intact adults, retain more information when it is presented in a spaced training pattern than when it is presented in a massed pattern (Galluccio & Rovee-Collier, 2006; Grassi, 1971; Jackson, Maruff, & Snyder, 2013; Karpicke & Bauernschmidt, 2011; Rovee-collier, Evancio, & Earley, 1995; Simone, Bell, & Cepeda, 2012). The spacing effect is observed across a wide variety of learning tasks including declarative (explicit) memory tasks (e.g., Simone et al., 2012), non-declarative (implicit) memory tasks (e.g., Greene, 1990; Shea, Lai, Black, & Park, 2000), and also the ability to generalize knowledge (Kornell & Bjork, 2008; Kornell, 2010; Vlach & Sandhofer, 2012). Spaced training is even a beneficial strategy in muscle strengthening (de Salles et al., 2009; Rahimi, Qaderi, Faraji, & Boroujerdi, 2010). Thus, the spacing effect transcends species, age and task.

Given the simplicity of implementing spaced training in patients' daily routines (e.g., Camp, Foss, O'Hanlon, & Stevens, 1996) and the widely accepted benefits it provides, it is not surprising that spaced training has become a therapeutic focus for a number of human disorders and disabilities. A variation of the spaced training procedures described thus far, spaced retrieval (Landauer & Bjork, 1978), is popular in treating persons with memory disorders (Balota, Duchek, Sergent-Marshall, & Roediger, 2006; Brush & Camp, 1998; Camp, Foss, O'Hanlon, & Stevens, 1996; Haslam, Hodder, & Yates, 2011; Small, 2012). Rather than exposing the subject to spaced presentations of a target stimulus during training, the subject studies the target stimulus, and then is asked to retrieve the target from memory soon after the stimulus is presented. If the subject correctly states the target response, the time to the next retrieval is increased and retrievals become increasingly spaced over time. If the subject has an incorrect retrieval, the time to the next retrieval is reduced to the most recent correct retrieval interval. While having some support in the literature, there is conflicting evidence as to whether expanding retrieval intervals results in superior memory compared to fixed retrieval intervals (Balota et al., 2006; Hopper et al., 2005).

Surprisingly, although spaced training is of clear benefit, a consistent operational definition of the boundary conditions that constitute effective spaced training is lacking in the literature. Experimental inter-trial intervals range from seconds to days, and even weeks (Cepeda et al., 2006; Raman et al., 2010). This discrepancy between studies may make it difficult to attain the most therapeutic benefit from spaced training, as different tasks and disorders could benefit from different inter-trial intervals. Thus, although spaced training has been shown to be beneficial using a range of training intervals, more systematic studies are necessary to determine whether more effective inter-trial intervals can be identified.

Despite vastly different inter-trial intervals, experimental designs, and spaced training paradigms (e.g., conventional spaced training versus spaced retrieval), one trend is completely clear: spacing is especially beneficial for young and old individuals with neurological disorders. Spaced training has been successfully implemented as a cognitive therapy in children with learning disabilities (Gettinger, Bryant, & Fayne, 1982; Riches, Tomasello, & Conti-Ramsden, 2005), cerebral palsy (Grassi, 1971), and autism (Dunlap & Koegel, 1980), and in adults with dementia (Brush & Camp, 1998; Camp, Foss, O'Hanlon, & Stevens, 1996; Haslam et al., 2011; Hopper, Drefs, Bayles, Tomoeda, & Dinu, 2010), Alzheimer's disease (Balota et al., 2006; Small, 2012), anomia (Abrahams & Camp, 1993; Morrow & Fridriksson, 2006), brain injury (Goverover, Arango-Lasprilla, Hillary, Chiaravalloti, & DeLuca, 2009a; Haslam et al., 2011), multiple sclerosis (Goverover, Hillary, Chiaravalloti, Arango-Lasprilla & Deluca, 2009b), amnesia (Cermak, Verfaellie, Lanzoni, Mather, & Chase, 1996), and public speech anxiety (Tsao & Craske, 2000).

Two important trends emerge from spaced training research in the context of therapy. First, spaced training can benefit poor-performing subjects more than healthy subjects. In children with Selective Language Impairment (SLI), spaced training benefited their verb production over massed training (Riches et al., 2005), while no significant benefit was observed in children without SLI. Additionally, Shoephoerster (1962) reported that children who were below average spellers, but not classified as learning disabled, benefited from spaced spelling instruction over massed spelling instruction. However, above average spellers did well no matter what instruction paradigm they experienced.

Second, effective spaced training patterns may differ between healthy subjects and age-matched individuals with neurological disorders. Jackson et al. (2013) administered 3 weeks of massed or spaced training on a visuo-spatial task to cognitively normal young and old adults, and a recall test was administered 24 h post-training. The cognitively normal (MMSE score of 28–30) older adult subjects were enrolled in a longitudinal study which included positron emission tomography brain imaging using Pittsburgh compound B (PiB), which binds to amyloid plaques in the brain (Klunk et al., 2004). Subjects were separated according to PiB status: PiB+ (those with amyloid build-up) and PiB− (those with no amyloid build-up). Unsurprisingly, young adults and older, PiB− adults showed better recall with spaced training than with massed training. Older, PiB+ adults, however, did not benefit from the spaced training even though they appeared cognitively similar to PiB− adults. The authors concluded that the spacing effect can be overshadowed by disease burden, in this case the accumulation of amyloid. Intriguingly, the spacing effect has been successfully utilized to treat patients with dementia and Alzheimer's (see above), who are likely to show similar amyloid pathology (Forsberg et al., 2008; Villemagne et al., 2008). Additionally, the younger adult subjects in this study exhibited a greater spacing effect than older, PiB− adults. Thus, although spaced training is beneficial for memory acquisition and retention in healthy individuals of all ages, and in persons with neurological disorders, several important questions remain unanswered: Do older individuals simply benefit less than their younger counterparts, or are learning strategies affected in normal aging such that the optimal intervals for young adults are not the same for old adults? Also, can disease alter effective learning strategies? These important questions remain to be answered to better understand and mitigate the learning dysfunction seen in aging and disease.

8. Could time be the best medicine?

As mentioned in the molecular component of this review, studies of animal models have begun to utilize knowledge of the molecular mechanisms of inter-trial interactions to predict the optimal training paradigms for LTM (Zhang et al., 2012). Given the newly emerging molecular framework of roles for the widely conserved PKA/MAPK/CREB signaling pathways in defining effective training windows over temporally distributed experiences, an important next step will be to incorporate these findings into our knowledge of the disrupted molecular signaling that occurs in aging and in neurological disorders, and to generate testable hypotheses (using the powerful animal model systems detailed in this review) focusing on effective learning strategies that may ultimately be employed in human disorders.

As a step in that direction, several animal studies have already provided evidence that disease states can change effective learning strategies, without impairing the ability to form LTM. For example, Deutsch and Rocklin (1972) hypothesized that anticholinesterase-induced amnesia measured on an active avoidance task could be rescued by increasing the intervals between training trials to allow acetylcholine concentrations to decrease. Indeed, increasing the inter-trial interval allowed the animals to exhibit normal memory. Additionally, Pagani et al. (2009) recently demonstrated that a fly model of the human genetic disorder, Noonan Syndrome (Tidyman & Rauen, 2009), which is associated with mutations in SHP2 that result in a dysregulation of Ras/MAPK signaling, exhibited profound learning deficits when trained on an aversive olfactory discrimination task at training intervals effective for wild type flies. Importantly, after considering how the recruitment of inter-trial MAPK signaling was affected by the disease state, the authors hypothesized that training with longer inter-trial intervals could restore the natural inter-trial behavior of MAPK activation observed in wild type flies. This proved to be an effective strategy. In the absence of any drug treatment to correct the genetic mutation in SHP2, simply increasing the inter-trial interval was sufficient to rescue the LTM deficit. The consideration of alternative spaced training strategies has additionally benefitted LTM formation in mice with genetic mutations of CREB (Kogan et al., 1997) and of the retinoic acid receptor (Nomoto et al., 2012). Finally, a loss of function of the CREB transcriptional co-activator, CBP, as occurs in many instances of Rubinstein-Taybi syndrome in humans (Roelfsema & Peters, 2007), is associated with learning deficits. Using a computational modeling approach, Liu et al. (2013) recently reported the identification of a novel training pattern that was sufficient to rescue the deficit in long-term synaptic facilitation of cultured *Aplysia* neurons that was observed following an siRNA-mediated knockdown of CBP levels. The authors suggest that similar considerations might be used to identify effective learning strategies in patients suffering from Rubinstein-Taybi and related syndromes (Liu et al., 2013).

Although still highly speculative at this point, the studies above strongly support the notion that there is potential therapeutic benefit in considering the notion that alternative learning strategies may be sufficient to correct, or at least mitigate, the overt learning deficits in a broad spectrum of learning disorders in which knowledge of the molecular signaling revealed in animal studies could be extended into the human arena. In these cases time might just be the best medicine.

Acknowledgments

We would like to thank Dr. Xiaojing Ye for helpful comments on an earlier version of this manuscript. The work included in this review was supported by the NIMH (R01MH041083 and R01MH094792 to TJC, and R01MH081151 to TJC and KC Martin).

References

Abel, T., Martin, K. C., Bartsch, D., & Kandel, E. R. (1998). Memory suppressor genes: Inhibitory constraints on the storage of long-term memory. *Science, 279*, 338–341.

Abraham, W. C., Logan, B., Greenwood, J. M., & Dragunow, M. (2002). Induction and experience-dependent consolidation of stable long-term potentiation lasting months in the hippocampus. *Journal of Neuroscience, 22*, 9626–9634.

Abrahams, J. P., & Camp, C. J. (1993). Maintenance and generalization of object naming training in anomia associated with degenerative dementia. *Clinical Gerontologist, 12*, 57–72.

Ajay, S. M., & Bhalla, U. S. (2004). A role for ERKII in synaptic pattern selectivity on the time-scale of minutes. *European Journal of Neuroscience, 20*, 2671–2680.

Alberini, C. M., Ghirardi, M., Metz, R., & Kandel, E. R. (1994). C/EBP is an immediate-early gene required for the consolidation of long-term facilitation in Aplysia. *Cell, 76*, 1099–1114.

Alkon, D. L., & Naito, S. (1986). Biochemical mechanisms of memory storage. *Journal de physiologie (Paris), 81*, 252–260.

Amano, H., & Maruyama, I. N. (2011). Aversive olfactory learning and associative long-term memory in Caenorhabditis elegans. *Learning & Memory, 18*, 654–665.

Atkins, C. M., Selcher, J. C., Petraitis, J. J., Trzaskos, J. M., & Sweatt, J. D. (1998). The MAPK cascade is required for mammalian associative learning. *Nature Neuroscience, 1*, 602–609.

Bailey, C. H. (1999). Structural changes and the storage of long-term memory in Aplysia. *Canadian Journal of Physiology and Pharmacology, 77*, 738–747.

Bailey, C. H., Bartsch, D., & Kandel, E. R. (1996). Toward a molecular definition of long-term memory storage. *Proceedings of National Academic Science USA, 93*, 13445–13452.

Balota, D. A., Duchek, J. M., Sergent-Marshall, S. D., & Roediger, H. L. (2006). Does expanded retrieval produce benefits over equal-interval spacing? Explorations of spacing effects in healthy aging and early stage Alzheimer's disease. *Psychology and Aging, 21*, 19–31.

Barad, M., Bourtchouladze, R., Winder, D. G., Golan, H., & Kandel, E. (1998). Rolipram, a type IV-specific phosphodiesterase inhibitor, facilitates the establishment of long-lasting long-term potentiation and improves memory. *Proceedings National Academic Science USA, 95*, 15020–15025.

Barco, A., Bailey, C. H., & Kandel, E. R. (2006). Common molecular mechanisms in explicit and implicit memory. *Journal of Neurochemistry, 97*, 1520–1533.

Barnes, C. A. (1995). Involvement of LTP in memory: Are we "searching under the street light"? *Neuron, 15*, 751–754.

Barondes, S. H. (1970). Cerebral protein synthesis inhibitors block long-term memory. *International Review of Neurobiology, 12*, 177–205.

Bartsch, D., Casadio, A., Karl, K. A., Serodio, P., & Kandel, E. R. (1998). CREB1 encodes a nuclear activator, a repressor, and a cytoplasmic modulator that form a regulatory unit critical for long-term facilitation. *Cell, 95*, 211–223.

Bartsch, D., Ghirardi, M., Skehel, P. A., Karl, K. A., Herder, S. P., Chen, M., et al. (1995). Aplysia CREB2 represses long-term facilitation: Relief of repression converts transient facilitation into long-term functional and structural change. *Cell, 83*, 979–992.

Beck, C. D., Schroeder, B., & Davis, R. L. (2000). Learning performance of normal and mutant Drosophila after repeated conditioning trials with discrete stimuli. *Journal of Neuroscience, 20*, 2944–2953.

Benito, E., & Barco, A. (2010). CREB's control of intrinsic and synaptic plasticity: Implications for CREB-dependent memory models. *Trends in Neurosciences, 33*, 230–240.

Bito, H., Deisseroth, K., & Tsien, R. W. (1996). CREB phosphorylation and dephosphorylation: A Ca(2+)- and stimulus duration-dependent switch for hippocampal gene expression. *Cell, 87*, 1203–1214.

Bliss, T. V., & Collingridge, G. L. (1993). A synaptic model of memory: Long-term potentiation in the hippocampus. *Nature, 361*, 31–39.

Bolding, K., & Rudy, J. W. (2006). Place learning in the Morris water task: Making the memory stick. *Learning & Memory, 13*, 278–286.

Brush, J. A., & Camp, C. J. (1998). Using spaced retrieval as an intervention during speech-language therapy. *Clinical Gerontologist, 19*, 51–64.

Cammarota, M., Bevilaqua, L. R., Ardenghi, P., Paratcha, G., Levi de Stein, M., Izquierdo, I., et al. (2000). Learning-associated activation of nuclear MAPK, CREB and Elk-1, along with Fos production, in the rat hippocampus after a one-trial avoidance learning: Abolition by NMDA receptor blockade. *Brain Research. Molecular Brain Research, 76*, 36–46.

Camp, C. J., Foss, J. W., O'Hanlon, A. M., & Stevens, A. B. (1996). Memory interventions for persons with dementia. *Applied Cognitive Psychology, 10*, 193–210.

Carew, T. J., Pinsker, H. M., & Kandel, E. R. (1972). Long-term habituation of a defensive withdrawal reflex in Aplysia. *Science, 175*, 451–454.

Castellucci, V. F., Blumenfeld, H., Goelet, P., & Kandel, E. R. (1989). Inhibitor of protein synthesis blocks long-term behavioral sensitization in the isolated gill-withdrawal reflex of Aplysia. *Journal of Neurobiology, 20*, 1–9.

Cepeda, N. J., Pashler, H., Vul, E., Wixted, J. T., & Rohrer, D. (2006). Distributed practice in verbal recall tasks: A review and quantitative synthesis. *Psychological Bulletin, 132*, 354–380.

Cermak, L. S., Verfaellie, M., Lanzoni, S., Mather, M., & Chase, K. A. (1996). Effect of spaced repetitions on amnesia patients' recall and recognition performance. *Neuropsychology, 10*, 219–227.

Chen, C. C., Wu, J. K., Lin, H. W., Pai, T. P., Fu, T. F., Wu, C. L., et al. (2012). Visualizing long-term memory formation in two neurons of the Drosophila brain. *Science, 335*, 678–685.

Ch'ng, T. H., Uzgil, B., Lin, P., Avliyakulov, N. K., O'Dell, T. J., & Martin, K. C. (2012). Activity-dependent transport of the transcriptional coactivator CRTC1 from synapse to nucleus. *Cell, 150*, 207–221.

Colomb, J., Kaiser, L., Chabaud, M. A., & Preat, T. (2009). Parametric and genetic analysis of Drosophila appetitive long-term memory and sugar motivation. *Genes, Brain and Behavior, 8*, 407–415.

Coomber, C. J. (1998). Site-selective autophosphorylation of Ca2+/calmodulin-dependent protein kinase II as a synaptic encoding mechanism. *Neural Computation, 10*, 1653–1678.

Dash, P. K., Hochner, B., & Kandel, E. R. (1990). Injection of the cAMP-responsive element into the nucleus of Aplysia sensory neurons blocks long-term facilitation. *Nature, 345*, 718–721.

de Salles, B. F., Simao, R., Miranda, F., Novaes, J. D., Lemos, A., & Willardson, J. M. (2009). Rest interval between sets in strength training. *Sports Medicine, 39*, 765–777.

Deutsch, J. A., & Rocklin, K. (1972). Anticholinesterase amnesia as a function of massed or spaced retest. *Journal of Comparative and Physiological Psychology, 81*, 64.

Diamond, D. M., Campbell, A. M., Park, C. R., Halonen, J., & Zoladz, P. R. (2007). The temporal dynamics model of emotional memory processing: A synthesis on the neurobiological basis of stress-induced amnesia, flashbulb and traumatic memories, and the Yerkes-Dodson law. *Neural Plasticity, 2007*, 60803.

Dunlap, G., & Koegel, R. L. (1980). Motivating autistic children through stimulus variation. *Journal of Applied Behavior Analysis, 13*, 619–627.

Ebbinghaus, H. E. (1885/1913). *Memory: A Contribution to Experimental Psychology*. New York: Dover.

Farah, C. A., Weatherill, D., Dunn, T. W., & Sossin, W. S. (2009). PKC differentially translocates during spaced and massed training in Aplysia. *Journal of Neuroscience, 29*, 10281–10286.

Forsberg, A., Engler, H., Almkvist, O., Blomquist, G., Hagman, G., Wall, A., et al. (2008). PET imaging of amyloid deposition in patients with mild cognitive impairment. *Neurobiology of Aging, 29*, 1456–1465.

Galluccio, L., & Rovee-Collier, C. (2006). Nonuniform effects of reinstatement within the time window. *Learning and Motivation, 37*, 1–17.

Genoux, D., Haditsch, U., Knobloch, M., Michalon, A., Storm, D., & Mansuy, I. M. (2002). Protein phosphatase 1 is a molecular constraint on learning and memory. *Nature, 418*, 970–975.

Gerber, B., Wustenberg, D., Schutz, A., & Menzel, R. (1998). Temporal determinants of olfactory long-term retention in honeybee classical conditioning: Nonmonotonous effects of the training trial interval. *Neurobiology of Learning and Memory, 69*, 71–78.

Gettinger, M., Bryant, N. D., & Fayne, H. R. (1982). Designing spelling instruction for learning-disabled children - An emphasis on unit size, distributed practice, and training for transfer. *Journal of Special Education, 16*, 439–448.

Gibbon, J., Baldock, M. D., Locurto, C., Gold, L., & Terrace, H. S. (1977). Trial and intertrial durations in autoshaping. *Journal of Experimental Psychology-Animal Behavior Processes, 3*, 264–284.

Gonzalez, G. A., & Montminy, M. R. (1989). Cyclic AMP stimulates somatostatin gene transcription by phosphorylation of CREB at serine 133. *Cell, 59*, 675–680.

Goverover, Y., Arango-Lasprilla, J. C., Hillary, F. G., Chiaravalloti, N., & DeLuca, J. (2009a). Application of the spacing effect to improve learning and memory for functional tasks in traumatic brain injury: A pilot study. *American Journal of Occupational Therapy, 63*, 543–548.

Goverover, Y., Hillary, F. G., Chiaravalloti, N., Arango-Lasprilla, J. C., & DeLuca, J. (2009b). A functional application of the spacing effect to improve learning and memory in persons with multiple sclerosis. *Journal of Clinical and Experimental Neuropsychology, 31*, 513–522.

Grassi, J. R. (1971). Effects of massed and spaced practice on learning in brain-damaged, behavior-disordered, and normal children. *Journal of Learning Disabilities, 4*, 237–242.

Greene, R. L. (1990). Spacing effects on implicit memory tests. *Journal of Experimental Psychology-Learning Memory and Cognition, 16*, 1004–1011.

Guzowski, J. F. (2002). Insights into immediate-early gene function in hippocampal memory consolidation using antisense oligonucleotide and fluorescent imaging approaches. *Hippocampus, 12*, 86–104.

Hart, A. K., Fioravante, D., Liu, R. Y., Phares, G. A., Cleary, L. J., & Byrne, J. H. (2011). Serotonin-mediated synapsin expression is necessary for long-term facilitation of the Aplysia sensorimotor synapse. *Journal of Neuroscience, 31*, 18401–18411.

Haslam, C., Hodder, K. I., & Yates, P. J. (2011). Errorless learning and spaced retrieval: How do these methods fare in healthy and clinical populations? *Journal of Clinical and Experimental Neuropsychology, 33*, 432–447.

Hirano, Y., Masuda, T., Naganos, S., Matsuno, M., Ueno, K., Miyashita, T., et al. (2013). Fasting launches CRTC to facilitate long-term memory formation in Drosophila. *Science, 339*, 443–446.

Hopper, T., Drefs, S. J., Bayles, K. A., Tomoeda, C. K., & Dinu, I. (2010). The effects of modified spaced-retrieval training on learning and retention of face–name associations by individuals with dementia. *Neuropsychological Rehabilitation, 20*, 81–102.

Hopper, T., Mahendra, N., Kim, E., Axuma, T., Bayles, K. A., Cleary, S. J., et al. (2005). Evidence-based practice recommendations for working with individuals with dementia: Spaced-retrieval training. *Journal of Medical Speech-Language Pathology, 13*.

Huang, Y. Y., & Kandel, E. R. (1994). Recruitment of long-lasting and protein kinase A-dependent long-term potentiation in the CA1 region of hippocampus requires repeated tetanization. *Learning & Memory, 1*, 74–82.

Irvine, E. E., Vernon, J., & Giese, K. P. (2005). AlphaCaMKII autophosphorylation contributes to rapid learning but is not necessary for memory. *Nature Neuroscience, 8*, 411–412.

Irvine, E. E., von Hertzen, L. S., Plattner, F., & Giese, K. P. (2006). AlphaCaMKII autophosphorylation: A fast track to memory. *Trends in Neurosciences, 29*, 459–465.

Jackson, C. E., Maruff, P. T., & Snyder, P. J. (2013). Massed versus spaced visuospatial memory in cognitively healthy young and older adults. *Alzheimer's & Dementia, 9*, S32–S38.

Karpicke, J. D., & Bauernschmidt, A. (2011). Spaced retrieval: Absolute spacing enhances learning regardless of relative spacing. *Journal of Experimental Psychology-Learning Memory and Cognition, 37*, 1250–1257.

Kim, M., Huang, T., Abel, T., & Blackwell, K. T. (2010). Temporal sensitivity of protein kinase a activation in late-phase long term potentiation. *PLoS Computational Biology, 6*, e1000691.

Klapdor, K., & Van Der Staay, F. J. (1998). Repeated acquisition of a spatial navigation task in mice: Effects of spacing of trials and of unilateral middle cerebral artery occlusion. *Physiology & Behavior, 63*, 903–909.

Klunk, W. E., Engler, H., Nordberg, A., Wang, Y., Blomqvist, G., Holt, D. P., et al. (2004). Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B. *Annual Neurol, 55*, 306–319.

Kogan, J. H., Frankland, P. W., Blendy, J. A., Coblentz, J., Marowitz, Z., Schutz, G., et al. (1997). Spaced training induces normal long-term memory in CREB mutant mice. *Current Biology, 7*, 1–11.

Kornell, N., & Bjork, R. A. (2008). Learning concepts and categories: Is spacing the "Enemy of Induction"? *Psychological Science, 19*, 585–592.

Kornell, N., Castel, A. D., Eich, T. S., & Bjork, R. A. (2010). Spacing as the Friend of Both Memory and Induction in Young and Older Adults. *Psychology and Aging, 25*, 498–503.

Koshibu, K., Graff, J., Beullens, M., Heitz, F. D., Berchtold, D., Russig, H., et al. (2009). Protein phosphatase 1 regulates the histone code for long-term memory. *Journal of Neuroscience, 29*, 13079–13089.

Kovacs, K. A., Steullet, P., Steinmann, M., Do, K. Q., Magistretti, P. J., Halfon, O., et al. (2007). TORC1 is a calcium- and cAMP-sensitive coincidence detector involved in hippocampal long-term synaptic plasticity. *Proceedings National Academic Science USA, 104*, 4700–4705.

Kramar, E. A., Babayan, A. H., Gavin, C. F., Cox, C. D., Jafari, M., Gall, C. M., et al. (2012). Synaptic evidence for the efficacy of spaced learning. *Proceedings National Academic Science USA, 109*, 5121–5126.

Landauer, T. K., & Bjork, R. A. (1978). *Optimum rehearsal patterns and name learning*. London: Academic Press.

Liu, R. Y., Cleary, L. J., & Byrne, J. H. (2011). The requirement for enhanced CREB1 expression in consolidation of long-term synaptic facilitation and long-term excitability in sensory neurons of Aplysia. *Journal of Neuroscience, 31*, 6871–6879.

Liu, R. Y., Zhang, Y., Baxter, D. A., Smolen, P., Cleary, L. J., & Byrne, J. H. (2013). Deficit in long-term synaptic plasticity is rescued by a computationally predicted stimulus protocol. *Journal of Neuroscience, 33*, 6944–6949.

Lukowiak, K., Adatia, N., Krygier, D., & Syed, N. (2000). Operant conditioning in Lymnaea: Evidence for intermediate- and long-term memory. *Learning & Memory, 7*, 140–150.

Lukowiak, K., Cotter, R., Westly, J., Ringseis, E., & Spencer, G. (1998). Long-term memory of an operantly conditioned respiratory behaviour pattern in lymnaea stagnalis. *Journal of Experimental Biology, 201*(Pt 6), 877–882.

Lynch, G., Kramar, E. A., Babayan, A. H., Rumbaugh, G., & Gall, C. M. (2013). Differences between synaptic plasticity thresholds result in new timing rules for maximizing long-term potentiation. *Neuropharmacology, 64*, 27–36.

Lyons, L. C., Green, C. L., & Eskin, A. (2008). Intermediate-term memory is modulated by the circadian clock. *Journal of Biological Rhythms, 23*, 538–542.

Malenka, R. C. (1994). Synaptic plasticity in the hippocampus: LTP and LTD. *Cell, 78*, 535–538.

Malenka, R. C., & Bear, M. F. (2004). LTP and LTD: An embarrassment of riches. *Neuron, 44*, 5–21.

Malleret, G., Haditsch, U., Genoux, D., Jones, M. W., Bliss, T. V., Vanhoose, A. M., et al. (2001). Inducible and reversible enhancement of learning, memory, and long-term potentiation by genetic inhibition of calcineurin. *Cell, 104*, 675–686.

Martin, K. C., Michael, D., Rose, J. C., Barad, M., Casadio, A., Zhu, H., et al. (1997). MAP kinase translocates into the nucleus of the presynaptic cell and is required for long-term facilitation in Aplysia. *Neuron, 18*, 899–912.

Mauelshagen, J., Sherff, C. M., & Carew, T. J. (1998). Differential induction of long-term synaptic facilitation by spaced and massed applications of serotonin at sensory neuron synapses of Aplysia californica. *Learning & Memory, 5*, 246–256.

Mayford, M., Siegelbaum, S. A., & Kandel, E. R. (2012). Synapses and memory storage. *Cold Spring Harbor Perspectives in Biology, 4*.

Morrow, K. L., & Fridriksson, J. (2006). Comparing fixed- and randomized-interval spaced retrieval in anomia treatment. *Journal of Communication Disorders, 39*, 2–11.

Mozzachiodi, R., & Byrne, J. H. (2010). More than synaptic plasticity: Role of nonsynaptic plasticity in learning and memory. *Trends in Neurosciences, 33*, 17–26.

Mozzachiodi, R., Lorenzetti, F. D., Baxter, D. A., & Byrne, J. H. (2008). Changes in neuronal excitability serve as a mechanism of long-term memory for operant conditioning. *Nature Neuroscience, 11*, 1146–1148.

Muller, U., & Carew, T. J. (1998). Serotonin induces temporally and mechanistically distinct phases of persistent PKA activity in Aplysia sensory neurons. *Neuron, 21*, 1423–1434.

Naqib, F., Sossin, W. S., & Farah, C. A. (2012). Molecular determinants of the spacing effect. *Neural Plasticity, 2012*, 581291.
Nomoto, M., Takeda, Y., Uchida, S., Mitsuda, K., Enomoto, H., Saito, K., et al. (2012). Dysfunction of the RAR/RXR signaling pathway in the forebrain impairs hippocampal memory and synaptic plasticity. *Molecular, Brain, 5*.
Pagani, M. R., Oishi, K., Gelb, B. D., & Zhong, Y. (2009). The phosphatase SHP2 regulates the spacing effect for long-term memory induction. *Cell, 139*, 186–198.
Parsons, R. G., & Davis, M. (2012). A metaplasticity-like mechanism supports the selection of fear memories: Role of protein kinase a in the amygdala. *Journal of Neuroscience, 32*, 7843–7851.
Philips, G. T., Sherff, C. M., Menges, S. A., & Carew, T. J. (2011). The tail-elicited tail withdrawal reflex of Aplysia is mediated centrally at tail sensory-motor synapses and exhibits sensitization across multiple temporal domains. *Learning & Memory, 18*, 272–282.
Philips, G. T., Tzvetkova, E. I., & Carew, T. J. (2007). Transient mitogen-activated protein kinase activation is confined to a narrow temporal window required for the induction of two-trial long-term memory in Aplysia. *Journal of Neuroscience, 27*, 13701–13705.
Philips, G. T., Ye, X., Kopec, A. M., & Carew, T. J. (2013). MAPK establishes a molecular context that defines effective training patterns for long-term memory formation. *Journal of Neuroscience, 24*, 7565–7573.
Pineda, V. V., Athos, J. I., Wang, H., Celver, J., Ippolito, D., Boulay, G., et al. (2004). Removal of G(ialpha1) constraints on adenylyl cyclase in the hippocampus enhances LTP and impairs memory formation. *Neuron, 41*, 153–163.
Pinsker, H., Carew, T. J., Hening, W., & Kandel, E. R. (1973). Long-term sensitization of a defensive withdrawal reflex in Aplysia californica. *Science, 182*, 1039–1042.
Pittenger, C., Huang, Y. Y., Paletzki, R. F., Bourtchouladze, R., Scanlin, H., Vronskaya, S., et al. (2002). Reversible inhibition of CREB/ATF transcription factors in region CA1 of the dorsal hippocampus disrupts hippocampus-dependent spatial memory. *Neuron, 34*, 447–462.
Placais, P. Y., Trannoy, S., Isabel, G., Aso, Y., Siwanowicz, I., Belliart-Guerin, G., et al. (2012). Slow oscillations in two pairs of dopaminergic neurons gate long-term memory formation in Drosophila. *Nature Neuroscience, 15*, 592–599.
Radwanska, K., Medvedev, N. I., Pereira, G. S., Engmann, O., Thiede, N., Moraes, M. F., et al. (2011). Mechanism for long-term memory formation when synaptic strengthening is impaired. *Proceedings National Academic Science USA, 108*, 18471–18475.
Rahimi, R., Qaderi, M., Faraji, H., & Boroujerdi, S. S. (2010). Effects of very short rest periods on hormonal responses to resistance exercise in men. *Journal of Strength and Conditioning Research, 24*, 1851–1859.
Raman, M., McLaughlin, K., Violato, C., Rostom, A., Allard, J. P., & Coderre, S. (2010). Teaching in small portions dispersed over time enhances long-term knowledge retention. *Medical Teacher, 32*, 250–255.
Riches, N. G., Tomasello, M., & Conti-Ramsden, G. (2005). Verb learning in children with SLI: Frequency and spacing effects. *Journal of Speech, Language and Hearing Research, 48*, 1397–1411.
Roberson, E. D., & Sweatt, J. D. (1996). Transient activation of cyclic AMP-dependent protein kinase during hippocampal long-term potentiation. *Journal of Biological Chemistry, 271*, 30436–30441.
Roelfsema, J. H., & Peters, D. J. (2007). Rubinstein-Taybi syndrome: Clinical and molecular overview. *Expert Reviews in Molecular Medicine, 9*, 1–16.
Rogers, R. F., Talk, A. C., & Matzel, L. D. (1994). Trial-spacing effects in Hermissenda suggest contributions of associative and nonassociative cellular mechanisms. *Behavioral Neuroscience, 108*, 1030–1042.
Rosenegger, D., Parvez, K., & Lukowiak, K. (2008). Enhancing memory formation by altering protein phosphorylation balance. *Neurobiology of Learning and Memory, 90*, 544–552.
Rovee-collier, C., Evancio, S., & Earley, L. A. (1995). The time window hypothesis – Spacing effects. *Infant Behavior & Development, 18*, 69–78.
Sangha, S., Scheibenstock, A., McComb, C., & Lukowiak, K. (2003). Intermediate and long-term memories of associative learning are differentially affected by transcription versus translation blockers in Lymnaea. *Journal of Experimental Biology, 206*, 1605–1613.
Scheibenstock, A., Krygier, D., Haque, Z., Syed, N., & Lukowiak, K. (2002). The Soma of RPeD1 must be present for long-term memory formation of associative learning in Lymnaea. *Journal of Neurophysiology, 88*, 1584–1591.
Sharma, S. K., Bagnall, M. W., Sutton, M. A., & Carew, T. J. (2003a). Inhibition of calcineurin facilitates the induction of memory for sensitization in Aplysia: Requirement of mitogen-activated protein kinase. *Proceedings National Academic Science USA, 100*, 4861–4866.
Sharma, S. K., Sherff, C. M., Shobe, J., Bagnall, M. W., Sutton, M. A., & Carew, T. J. (2003b). Differential role of mitogen-activated protein kinase in three distinct phases of memory for sensitization in Aplysia. *Journal of Neuroscience, 23*, 3899–3907.
Shea, C. H., Lai, Q., Black, C., & Park, J. H. (2000). Spacing practice sessions across days benefits the learning of motor skills. *Human Movement Science, 19*, 737–760.
Shoephoerster, H. (1962). Research into variations of the test-study plan of teaching spelling. *Elementary English, 39*, 460–462.
Shors, T. J., & Matzel, L. D. (1997). Long-term potentiation: What's learning got to do with it? *Behavioral and Brain Sciences, 20*, 597–614 (discussion 614–555).
Silva, A. J., Kogan, J. H., Frankland, P. W., & Kida, S. (1998). CREB and memory. *Annual Review Neuroscience, 21*, 127–148.
Simone, P. M., Bell, M. C., & Cepeda, N. J. (2012). Diminished but not forgotten: Effects of aging on magnitude of spacing effect benefits. *Journals of Gerontology Series B: Psychological Sciences and Social Sciences*.
Small, J. A. (2012). A new frontier in spaced retrieval memory training for persons with Alzheimer's disease. *Neuropsychological Rehabilitation, 22*, 329–361.
Stough, S., Shobe, J. L., & Carew, T. J. (2006). Intermediate-term processes in memory formation. *Current Opinion in Neurobiology, 16*, 672–678.
Sutton, M. A., Ide, J., Masters, S. E., & Carew, T. J. (2002). Interaction between amount and pattern of training in the induction of intermediate- and long-term memory for sensitization in aplysia. *Learning & Memory, 9*, 29–40.
Sutton, M. A., Masters, S. E., Bagnall, M. W., & Carew, T. J. (2001). Molecular mechanisms underlying a unique intermediate phase of memory in Aplysia. *Neuron, 31*, 143–154.
Taubenfeld, S. M., Milekic, M. H., Monti, B., & Alberini, C. M. (2001). The consolidation of new but not reactivated memory requires hippocampal C/EBPbeta. *Nature Neuroscience, 4*, 813–818.
Tidyman, W. E., & Rauen, K. A. (2009). The RASopathies: Developmental syndromes of Ras/MAPK pathway dysregulation. *Current Opinion in Genetics & Development, 19*, 230–236.
Tsao, J. C. I., & Craske, M. G. (2000). Timing of treatment and return of fear: Effects of massed, uniform-, and expanding-spaced exposure schedules. *Behavior Therapy, 31*, 479–497.
Tully, T., Preat, T., Boynton, S. C., & Del Vecchio, M. (1994). Genetic dissection of consolidated memory in Drosophila. *Cell, 79*, 35–47.
van Giezen, A. E., Arensman, E., Spinhoven, P., & Wolters, G. (2005). Consistency of memory for emotionally arousing events: A review of prospective and experimental studies. *Clinical Psychology Review, 25*, 935–953.
Villemagne, V. L., Pike, K. E., Darby, D., Maruff, P., Savage, G., Ng, S., et al. (2008). Abeta deposits in older non-demented individuals with cognitive decline are indicative of preclinical Alzheimer's disease. *Neuropsychologia, 46*, 1688–1697.
Vlach, H. A., & Sandhofer, C. M. (2012). Distributing Learning Over Time: The Spacing Effect in Children's Acquisition and Generalization of Science Concepts. *Child Development, 83*, 1137–1144.
Wainwright, M. L., Zhang, H., Byrne, J. H., & Cleary, L. J. (2002). Localized neuronal outgrowth induced by long-term sensitization training in Aplysia. *Journal of Neuroscience, 22*, 4132–4141.
Williams, D. A., Frame, K. A., & LoLordo, V. M. (1991). Reexamination of contextual conditioning with massed versus distributed unconditioned stimuli. *Journal of Experimental Psychology: Animal Behavior Processes, 17*, 202–209.
Winder, D. G., Mansuy, I. M., Osman, M., Moallem, T. M., & Kandel, E. R. (1998). Genetic and pharmacological evidence for a novel, intermediate phase of long-term potentiation suppressed by calcineurin. *Cell, 92*, 25–37.
Wittstock, S., Kaatz, H. H., & Menzel, R. (1993). Inhibition of brain protein synthesis by cycloheximide does not affect formation of long-term memory in honeybees after olfactory conditioning. *Journal of Neuroscience, 13*, 1379–1386.
Woo, N. H., Duffy, S. N., Abel, T., & Nguyen, P. V. (2003). Temporal spacing of synaptic stimulation critically modulates the dependence of LTP on cyclic AMP-dependent protein kinase. *Hippocampus, 13*, 293–300.
Wood, M. A., Kaplan, M. P., Park, A., Blanchard, E. J., Oliveira, A. M., Lombardi, T. L., et al. (2005). Transgenic mice expressing a truncated form of CREB-binding protein (CBP) exhibit deficits in hippocampal synaptic plasticity and memory storage. *Learning & Memory, 12*, 111–119.
Wustenberg, D., Gerber, B., & Menzel, R. (1998). Short communication: Long- but not medium-term retention of olfactory memories in honeybees is impaired by actinomycin D and anisomycin. *European Journal of Neuroscience, 10*, 2742–2745.
Xia, S. Z., Feng, C. H., & Guo, A. K. (1998). Multiple-phase model of memory consolidation confirmed by behavioral and pharmacological analyses of operant conditioning in Drosophila. *Pharmacology, Biochemistry and Behavior, 60*, 809–816.
Ye, X., Shobe, J. L., Sharma, S. K., Marina, A., & Carew, T. J. (2008). Small G proteins exhibit pattern sensitivity in MAPK activation during the induction of memory and synaptic facilitation in Aplysia. *Proceedings National Academic Science USA, 105*, 20511–20516.
Yin, J. C., Del Vecchio, M., Zhou, H., & Tully, T. (1995). CREB as a memory modulator: Induced expression of a dCREB2 activator isoform enhances long-term memory in Drosophila. *Cell, 81*, 107–115.
Yin, J. C., & Tully, T. (1996). CREB and the formation of long-term memory. *Current Opinion in Neurobiology, 6*, 264–268.
Yin, J. C., Wallach, J. S., Del Vecchio, M., Wilder, E. L., Zhou, H., Quinn, W. G., et al. (1994). Induction of a dominant negative CREB transgene specifically blocks long-term memory in Drosophila. *Cell, 79*, 49–58.
Zhang, Y. L., Liu, R. Y., Heberton, G. A., Smolen, P., Baxter, D. A., Cleary, L. J., et al. (2012). Computational design of enhanced learning protocols. *Nature Neuroscience, 15*, 294–297.
Zhou, Y., Wu, H., Li, S., Chen, Q., Cheng, X. W., Zheng, J., et al. (2006). Requirement of TORC1 for late-phase long-term potentiation in the hippocampus. *PLoS ONE, 1*, e16.