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The Biochemistry of Memory: The Twenty-Six Year Journey of a 'New and Specific Hypothesis'

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Abstract

This Special Issue of Neurobiology of Learning and Memory dedicated to Dr. Richard Thompson to celebrate his 80th birthday and his numerous contributions to the field of learning and memory gave us the opportunity to revisit the hypothesis we proposed more than 25 years ago regarding the biochemistry of learning and memory. This review summarizes our early 1980s hypothesis and then describes how it was tested and modified over the years following its introduction. We then discuss the current status of the hypothesis and provide some examples of how it has led to unexpected insights into the memory problems that accompany a broad range of neuropsychiatric disorders.

Introduction

The work of Richard Thompson is foundational to contemporary ideas regarding the neurobiology of memory and, not surprisingly, has been a continuing stimulus to hypothesis building by other investigators. Our own initial and, seen in retrospect, confused thoughts about memory substrates were ultimately shaped into a specific model (Lynch&Baudry, 1984) during daily interactions with Thompson in the early 1980s. We were also influenced by a landmark paper from his lab showing that hippocampal pyramidal neurons shape their firing rates so as to model and predict key elements of a complex conditioning problem (Berger&Thompson, 1978). The authors proposed that repetitive activation of hippocampal afferents by the cues to be learned triggered something like the then recently discovered long-term potentiation (LTP) effect, and in this way produced novel, stable responses by hippocampus. We knew that LTP is easily induced and quite stable in the hippocampal subdivision in which Berger and Thompson had obtained their recordings, so we set out to

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identify a biochemical mechanism that would respond to very brief bursts of afferent activity and produce extremely persistent increases in fast, excitatory transmission. The hypothesis that resulted from this effort has received a great deal of experimental support and it continues to guide our research today.

This review will first summarize our early 1980s hypothesis and then describe how it was tested and modified during the explosion of LTP research that occurred in the years following its introduction. We will then consider the current status of the hypothesis and provide some examples of how it has led to unexpected insights into the memory problems that accompany a broad range of neuropsychiatric disorders. Thoughts about a next generation model that integrates what has been learned from the nearly 30-year old version with recent discoveries from emerging technologies are advanced in a concluding section.

1. The original hypothesis for LTP and memory (1984)

Calpain-induced spectrin degradation and increased number of glutamate receptors

Our initial work on the biochemistry of memory in the early 80s was guided by what we took to be two fundamental requirements for an adequate hypothesis: (i) the critical cellular processes had to produce “functionally meaningful, extremely persistent neurobiological changes of a type that can account for the behavioral manifestations of memory, and (ii) the mechanism must be amenable to selective manipulations”. We further constrained the candidate mechanism by assuming that it, like memory, is triggered by brief physiological events and, despite the brevity of input, produces extremely long lasting synaptic changes (weeks, months).

It was already known that brief bursts of high frequency stimulation to monosynaptic glutamatergic inputs to field CA1 of hippocampus resulted in synapse-specific LTP (Andersen, 1977; Bliss&Lomo, 1973; Dunwiddie&Lynch, 1978; McNaughton, Douglas, & Goddard, 1978) and that calcium is critical for the induction of this effect (Dunwiddie&Lynch, 1979). Subsequent work using intracellular applications of a chelating agent demonstrated that the locus of action for calcium lies in the postsynaptic compartment (Lynch, Larson, Kelso, Barrionuevo, & Schottler, 1983). This meant that an increase in calcium in dendritic spines is the likely trigger for postsynaptic modifications underlying the observed potentiation of fast EPSPs. This idea dovetailed with electron microscopic work suggesting that induction of LTP in field CA1 is accompanied by rapid changes in the anatomy of spines and synapses (Chang&Greenough, 1984; Lee, Dunwiddie, Deitrich, Lynch, & Hoffer, 1981; Lee, Schottler, Oliver, & Lynch, 1980). With this background, we set out to identify a calcium-dependent process that could lead to post-synaptic structural modifications compatible with increased glutamatergic transmission. Using a variety of approaches, and a fruitful collaboration with the RFT laboratory (then at UC Irvine), we discovered the presence of calcium-dependent proteases, calpains, along with a preferred substrate, brain spectrin (at the time known as ‘fodrin’) in synaptic membranes (Baudry, Bundman, Smith, & Lynch, 1981; Baudry&Lynch, 1979; 1980b). There was no evidence at this point that calpain digests spectrin but a series of biochemical studies confirmed the point and further showed that cleavage results in an unusual and very stable breakdown product (Siman, Baudry, and Lynch, 1984); the latter subsequently became a standard marker for in situ activation of calpain. These were exciting results because spectrin cross-links the submembrane cytoskeleton, and its cleavage thus provided a plausible link between calcium influx, calpain activation, and lasting changes to spine and synapse morphology.

But does calpain activation affect the glutamate receptors responsible for mediating the post-synaptic current? Tests of this using binding of ^3H -glutamate to synaptic membranes provided evidence for increased numbers of receptors. We interpreted the findings as

evidence that calpain activation “exposes” occluded glutamate receptors in postsynaptic densities, an effect that could account for the LTP effect (Baudry&Lynch, 1980a). Other studies suggested that the sequence of calpain activation, spectrin degradation, increased ^3H -glutamate binding is activated by the high frequency stimulation bursts used to induce LTP (Lynch, Halpain, & Baudry, 1982), and by eyelid conditioning in rabbit hippocampus (Mamounas, Thompson, Lynch, & Baudry, 1984). Consonant with this last result, intraventricular infusions of calpain inhibitors impaired some but not all forms of learning in adult rats (Staubli, Baudry, & Lynch, 1985).

All of these results culminated in “a new and specific hypothesis” concerning the biochemistry of memory (Lynch&Baudry, 1984) in which calpain plays a central role. This assumed that learning related patterns of repetitive afferent activity causes an unusually deep depolarization of spines and thereby elevates local calcium concentrations to a degree sufficient for activation of calpain-1, the high sensitivity isoform of the enzyme. Calpain then degrades spectrin, resulting in an expansion of the glutamate receptor pool in postsynaptic densities. Repetition of this process would modify the structure of the dendritic spines and their embedded synapses, an event that provides for the long-term stabilization of the increased number of receptors. As we pointed out at the time, the involvement of a protease as a critical regulatory step had several implications. Because proteases produce irreversible modifications of their substrate proteins, and in the case of calpain, functional modifications resulting from partial truncation (Murachi, 1984), their activation results in alterations of cell functions that last for the duration of the lifetime of these proteins. It was thus conceivable that calpain activation could result in several functional changes that could exhibit different time-courses depending on the turn-over of the various proteins truncated by calpain. This feature could therefore account for various stages in memory formation, as truncation of proteins with fast turn-over would be responsible for short-term functional modifications, whereas truncation of proteins with long half-lives would produce longer lasting changes. Beyond these changes directly related to protein half-lives, we postulated that calpain activation could result in very long-lasting functional modifications of synaptic function by indirectly contributing to structural modifications that could be resistant to protein turn-over, such as changing the size or shape of dendritic spines. As we concluded our 1984 paper “if calpain activation is triggered by modest levels of calcium and produces irreversible changes in synaptic chemistry, then its activation would seem to be both a likely event and one that should produce lasting changes in the operation of neuronal circuitries”; as discussed below, evidence collected since then accords with this conclusion and has provided a wealth of details that were missing at the time.

2. The revised LTP hypothesis (2001)

Almost 20 years after the formulation of our original hypothesis, we revisited it on the occasion of another event in the honor of RFT, a Festschrift for his 70th birthday (Baudry&Lynch, 2001). One of the key features of the original hypothesis was that the number of synaptic glutamate receptors was increased with LTP, resulting in increased efficacy of potentiated synapses. Numerous laboratories subsequently described evidence that excitatory synapses can rapidly increase their complement of AMPA-type glutamate receptors, and the assumption that this effect is the substrate of LTP expression has become “core knowledge” in the field (Liao, Scannevin, & Huganir, 2001; Malenka&Nicoll, 1999; Malinow&Malenka, 2002; Maren, Tocco, Standley, Baudry, & Thompson, 1993). The initial discussion of the postsynaptic density focused on the spectrin network and the idea that calpain-mediated disruption of this network was important for modifying the structure of the dendritic spines, something that secondarily increased the size of synapses and their receptor pools. It later became clear that the postsynaptic density (psd) contains an elaborate machinery for the insertion, endocytosis and regulation of receptor number and functions

(Husi&Grant, 2001; Kennedy, 1997; Sala, Piech, Wilson, Passafaro, Liu, & Sheng, 2001; Wyszynski, Valtschanoff, Naisbitt, Dunah, Kim, Standaert, Weinberg, & Sheng, 1999). This includes several proteins that anchor the various types of glutamate receptors, in particular the AMPA and NMDA receptors, in psds. Moreover, numerous studies indicated that AMPA receptors continuously cycle in and out of synaptic membranes under the influence of protein kinases, CaMKII in particular, and phosphatases (Lisman&Zhabotinsky, 2001; Morishita, Connor, Xia, Quinlan, Shenolikar, & Malenka, 2001; Soderling&Derkach, 2000). We discovered that several of the scaffolding proteins (PSD-95, GRIP, SAP-97), as well as particular subunits of AMPA and NMDA receptors themselves, are calpain substrates (Bi, Standley, & Baudry, 1998; Lu, Rong, & Baudry, 2000; Lu, Wyszynski, Sheng, & Baudry, 2001). Combined, these observations raised the possibility that up-regulation of synaptic glutamate receptors reflects calcium-mediated insertion from the cytoplasmic pool, whereas down-regulation involves increases in the rate of internalization (Broutman&Baudry, 2001; Massicotte&Baudry, 2004).

Our 2001 review also discussed the relationship between the original hypothesis and the emerging evidence for ‘silent synapses’ --- contacts containing NMDA but not AMPA receptors and therefore would not produce synaptic responses when the postsynaptic voltage was close to resting membrane potential (~ -70 mV) (Isaac, Nicoll, & Malenka, 1995; Liao et al., 2001; Voronin, Volgushev, Chistiakova, Kuhnt, & Singer, 1996). This idea was initially suggested by physiological experiments and then received strong support from electron microscopic studies linking the size of psds to the number of AMPA and NMDA receptors. Takumi et al. (1999), for example, found that the ratio of AMPA to NMDA receptors decreased with decreasing psd length, and that, at psd length less than 180 nm, AMPA receptors are no longer detected. Conversion of silent synapses into active contacts through the insertion of AMPA receptors, as suggested in our original hypothesis, could thus be a contributor to LTP (although AMPAR-free synapses are too few in number to solely account for the 50-100% increase in EPSC size observed in typical LTP studies). This argument, while attractive, does not address the question of the primary mechanism responsible for psd enlargement presumably needed to provide docking sites for the added AMPARs. In particular, is psd enlargement the result of the active fusion of AMPA receptor-containing vesicles or, conversely, is the increased number of AMPA receptors the consequence of an enlarged psd allowing more receptors to diffuse from extra-synaptic sites into the psds? As discussed below, studies directed at this question, included in a continuing effort to relate mechanisms of LTP expression to those responsible for maintenance of potentiation, played a major role in the further evolution of our working hypothesis.

3. The current version of the LTP and memory hypothesis (2010)

New insights into calpain activation

Recent conceptual and technological advances have opened the way to new tests of the hypothesis that calpain plays an important role in synaptic plasticity and in LTP induction. A critical step came with the discovery that the neurotrophin BDNF, which contributes importantly to LTP induction and memory encoding (Bramham, 2008; Chen, Rex, Babayan, Kramar, Lynch, Gall, & Lauterborn; Lynch, Rex, & Gall, 2007b; Rex, Lin, Kramar, Chen, Gall, & Lynch, 2007), stimulates calpain-2 activation through ERK-mediated phosphorylation (Zadran, Jourdi, Rostamiani, Qin, Bi, & Baudry, 2010). Interestingly, PKA-mediated calpain-2 phosphorylation negatively regulates calpain-2 activation both in fibroblasts (Shiraha, Glading, Chou, Jia, & Wells, 2002) and in neurons (Zadran et al., 2010). These observations are of particular interest in light of evidence that calpain-2, by partially truncating a variety of cytoskeletal proteins, plays a critical role in the regulation of shape and motility in numerous cell types (see (Carragher&Frame, 2002; Perrin&Huttenlocher, 2002) for recent reviews). For example, calpain-2 appears to function

as a molecular switch to control cell spreading and retraction in Chinese hamster ovary cells (Flevaris, Stojanovic, Gong, Chishti, Welch, & Du, 2007). In this system activation of cell adhesion receptors belonging to the integrin family results in calpain activation and, depending on the state of phosphorylation of the integrin cytoplasmic domain, leads to either inhibition of RhoA and spreading or activation of RhoA and cell retraction. It is therefore tempting to envision a similar type of molecular switch in dendritic spines and to equate spreading to potentiation and retraction to depression. Other work has described routes whereby calpain could affect synaptic plasticity via effects on well-characterized signaling cascades. Shimizu et al. reported that calpain, by degrading a Ras inhibitor called the SCN circadian oscillatory protein (SCOP), regulates activation of ERK (Shimizu, Phan, Mansuy, & Storm, 2007), a kinase with numerous links to LTP. A second study reported that calpain, by truncating β -catenin, provides a critical link between synaptic events and transcriptional regulation, as the truncated β -catenin functioned as a transcription factor (Abe&Takeichi, 2007).

Mechanisms for the cytoskeletal component of the original hypothesis

A great deal of progress has also been made over the past five years towards understanding the mechanisms involved in LTP stabilization and in linking them to calpain activation. First it was found that the theta burst afferent stimulation (TBS) used to elicit LTP triggers polymerization of spine actin in adult hippocampal slices. Diverse lines of evidence support the long-held idea that stable LTP requires reorganization of the subsynaptic actin cytoskeleton but only recent technical advances have made it possible to provide a quantitative method to analyze levels of actin polymerization within individual spines. We have now shown that TBS, applied at or above the threshold for LTP induction, rapidly (<2 min) increases the number of spines containing high concentrations of filamentous (F)-actin in the dendritic lamina with potentiated synapses (Kramar, Lin, Rex, Gall, & Lynch, 2006; Lin, Kramar, Bi, Brucher, Gall, & Lynch, 2005) (Fig. 1A,B). Blocking this increase thoroughly eliminates LTP consolidation. The pathways connecting theta burst afferent stimulation to LTP-related actin polymerization in target spines involve three classes of synaptic membrane receptors: (i) glutamate receptors and in particular NMDA receptors (NMDARs), (ii) the above mentioned integrin receptors, and (iii) a group of receptors that we refer to as 'synaptic modifiers' (Rex, Chen, Sharma, Liu, Babayan, Gall, & Lynch, 2009). This last group includes the adenosine A1 receptor (Rex, Kramar, Colgin, Lin, Gall, & Lynch, 2005), the estrogen beta-receptor (Kramar, Chen, Brandon, Rex, Liu, Gall, & Lynch, 2009a; Kramar, Chen, Rex, Gall, & Lynch, 2009b), and, perhaps most importantly, the TrkB receptor for BDNF (Lynch, Kramar, Rex, Jia, Chappas, Gall, & Simmons, 2007a; Rex et al., 2005). Two of the modifiers (BDNF, estrogen) promote theta-driven actin polymerization while the third (adenosine) blocks it (Fig. 2).

The above triad of synaptic receptors regulates two actin-signaling pathways at individual synapses, both of which involve the Rho family of small GTPases (Chen, Rex, Casale, Gall, & Lynch, 2007; Rex et al., 2009) (see Fig. 2). These enzymes are central to virtually all cell biological descriptions of how actin networks are assembled, disassembled, or stabilized (Burridge&Wennerberg, 2004) and it is thus not surprising that they should figure prominently in the cytoskeletal changes triggered by LTP induction. The first pathway consists of RhoA and its effectors, RhoA kinase (ROCK), LIM-kinase, and cofilin (Fig. 2). Cofilin is a constitutively active actin severing protein; its phosphorylation, and thus inactivation, by theta burst activity (Chen et al, 2007) (Fig. 1C,D) provides a straightforward explanation for the actin polymerization triggered by TBS (Lin et al, 2005; Kramar et al, 2006). The second cascade involves Rac and Cdc42, close relatives of RhoA, and their effector p21-activated kinase (PAK). PAK plays a pivotal role in reorganizing the cytoskeleton across cell types and experimental paradigms (Szczepanowska, 2009).

Estrogen, which promotes actin polymerization and LTP, selectively activates RhoA while adenosine, which blocks actin polymerization, suppresses it. Neither treatment significantly increases Cdc42 or Rac activity. What then are the contributions of Rac, Cdc42, and PAK in activity-driven reorganization of the spine cytoskeleton? This question was addressed in hippocampal slice experiments using latrunculin A, a toxin that selective prevents the addition of actin monomers to dynamic ('treadmilling') actin polymers. First, we confirmed that the actin filaments, like LTP, gradually transition into a stable state over several minutes post-TBS. We then established that blocking TBS-induced activation of Rac or PAK prevents both the normal actin filament stabilization (i.e., progressive resistance to latrunculin A) and the consolidation of LTP. It thus appears that LTP induction sets in motion two pathways, one (RhoA >> ROCK, etc) that initiates actin polymerization and a second (Rac>>PAK, etc) that, through routes yet to be identified, stabilizes the new F-actin (Fig. 2). The latter event appears to be the rate-limiting event for the rapid (10-15 minutes) phase of LTP consolidation.

BDNF, which is released by theta bursts, stimulates both of the actin pathways just described, and TBS activates synaptic TrkB receptors for the neurotrophin. In all, joint activation of integrin and TrkB signaling provides a likely route whereby theta bursts initiate GTPase activity and the actin signaling that follows from it. Finally, this conclusion, along with a new set of results, helps explain the essential role of NMDARs in LTP. Specifically, following tetanic stimulation, activation of BDNF's TrkB receptor requires both extracellular BDNF- and NMDAR-driven stimulation of Src family kinases (Chen, Rex, Sanaïha, Lynch, & Gall, 2010b), while others report that the receptors trigger the release of unknown factors that, through matrix proteases, produce ligands for synaptic integrins (Nagy, Bozdagi, Matynia, Balcerzyk, Okulski, Dzwonek, Costa, Silva, Kaczmarek, & Huntley, 2006; Wang, Bozdagi, Nikitczuk, Zhai, Zhou, & Huntley, 2008).

Linking calpain to the mechanisms for cytoskeletal reorganization

Our original hypothesis postulated that calpain, via digestion of subsynaptic structural proteins including spectrin, served to disassemble extant cytoskeleton, and thereby cleared the way for the construction of new actin networks and morphological changes to the synapse. The complex processes described in the preceding sections describe routes for the latter events and thus the stabilization of LTP. Questions now arise as to whether the protease plays a broader role than we originally envisioned and, in particular, if it helps orchestrate the sequence and timing of signaling cascades that assemble and then stabilize newly formed actin filaments in the minutes following LTP induction.

One possibility is suggested by the discovery that BDNF stimulates calpain activation through ERK-mediated phosphorylation. This event could terminate the stabilization sequences that occur during the first 10 minutes post-TBS and, in addition, set in motion activities required for later stages of consolidation. Specifically, calpain degrades integrins and adaptor proteins needed for their activation and signaling (Du, Saïdo, Tsubuki, Indig, Williams, & Ginsberg, 1995; Flevaris et al., 2007; Sawhney, Cookson, Omar, Hauser, & Brattain, 2006). This could explain why the GTPase-initiated signaling lasts for only 10-15 minutes. Furthermore, calpain contributes, via known mechanisms, to mTOR-dependent local protein synthesis (Shimizu et al., 2007). Calpain could accordingly be a molecular switch that drives the shift from early to late phases of consolidation (Fig. 3).

Tests of the synapse expansion component of the original hypothesis

The work on consolidation mechanisms summarized immediately above found high concentrations of several actin-signaling, phosphorylated (p)-proteins (pPAK, pCofilin, pTrkB, etc.) at synapses in the first minutes after TBS. We have used these as markers for

synapses that recently experienced LTP induction to test a key ingredient of our 1984 hypothesis; namely, that LTP is associated with an increase in synapse size. Electron microscopic studies, as noted, have provided evidence for changes in synaptic morphology occurring in conjunction with the potentiation effect, but differ greatly in their conclusions about the nature of the transformation. This likely reflects the relatively small numbers of contacts sampled in EM work and the low percentage of the synaptic population activated in conventional LTP experiments. We re-examined the question using newly developed light microscopic methods and automated counting/measurement techniques that allow for sampling of tens of thousands of synapses from a small dendritic zone innervated by two potentiated inputs. Two separate studies of this type showed that synapses co-localized with any one of three LTP markers were 50-70% larger than their much more numerous neighbors lacking the markers (Chen et al., 2007; Chen et al., 2010c). The size/frequency distributions of double-labeled psds differed from that of control contacts in a manner suggesting that the former had shifted from an ellipsoid to an ovoid configuration, an effect that would be expected to produce an increase in area (Chen et al., 2007). Work from other groups using live imaging obtained similar results for spine morphology (Wang et al 2008). Combined, these findings provide strong support for the calpain/structural change/ LTP hypothesis.

Relationship of the hypothesized LTP mechanism to memory

The original hypothesis assumed that the proposed LTP mechanism served as a substrate for commonplace forms of memory (Lynch&Baudry, 1984). The idea that LTP plays a central role in memory was controversial in 1984 and for some years afterwards, but gradually won widespread acceptance in large part because a large number of papers showing that manipulations of LTP have predictable effects on learning. The discovery that LTP has deep connections to the learning-related theta rhythm likely also contributed to its acceptance as a primary factor in memory encoding. Implicit to work in this area was the expectation (hope) that the analysis of LTP would eventually lead to tools for mapping the location of synapses responsible for the storage of particular types of memory. The idea of memory maps ('engrams') dates back to the beginning of the 20th century [(Lynch, Rex, Chen, & Gall, 2009; Schacter, 1982) for reviews] and no one has played a larger role in its evolution than Richard Thompson. Among other discoveries pertinent to the concept, he was the first to describe a memory circuit in mammals that includes the location of the synapses responsible for long-term storage. Our work on memory sites in the forebrain is a continuation of Thompson's revolutionary analysis of the brainstem and cerebellum.

We first asked if a 30-minute episode of unsupervised learning by control animals increases the number of synapses containing the same actin signaling events induced by LTP in slices. Learning, in our initial study, produced a robust increase in spines double-labeled for pCofilin within the same circumscribed portion of hippocampus (proximal dendrites of field CA1b) used in the slice studies (total synapse counts not increased). While the effect involved only a small percentage of the synaptic population (<3%), it was large compared to the very few double-labeled contacts found in home cage controls. Importantly, the increases were fully blocked by a centrally active inhibitor of NMDA receptors at amnesic doses that did not markedly affect exploratory behavior in the learning environment (Fedulov, Rex, Simmons, Palmer, Gall, & Lynch, 2007). Moreover, synapses associated with pCofilin in the learning experiments were 50-70% larger than their more numerous single-labeled neighbors. In all, then, LTP and learning produce effects of a type expected to yield potentiated synaptic responses.

Subsequent experiments using activated (phosphorylated) TrkB (BDNF receptor) as a marker for LTP-related synaptic changes and obtained results comparable to those found with pCofilin; i.e., an NMDAR-dependent increase in double-labeled synapses that were

larger than other contacts (Chen et al., 2010c). Follow on studies using more rapid data acquisition and processing methods then showed that the increases in pTrk+ contacts were not homogeneously distributed across hippocampal subfields. Most striking, the effects were absent outside of a restricted septo-temporal segment located in the rostral hippocampus (Chen, Rex, Pham, Lynch, & Gall, 2010b). It thus appears that a form of learning that is ubiquitous in humans and animals uses a surprisingly restricted portion of the hippocampus for storage. Continuing advances in technology will shortly make it possible to create maps that cover the entire septo-temporal extent of the hippocampus and, somewhat later, of entire cortical regions. Success in this would go some distance towards the realization of a goal first specified 100 years ago; we think that it would also remove a fundamental barrier to the development of neurobiological theories of memory retrieval and sequencing.

4. Using the hypothesis to uncover causes of memory disorders

Clearly, a great deal has changed since 1984 and, to our surprise, much of this, reflecting findings originating from many laboratories including our own, by and large confirms the ideas discussed in the initial version of the hypothesis for the biochemistry of memory. The overall postulate that patterns of electrical activity occurring during learning result in the activation of calpain, remodeling of spines and synapses, and increased numbers of synaptic AMPA receptors appears to be correct. Links between these patterns of afferent activity and the mechanisms underlying structural modifications have been identified and there is now evidence that learning triggers the proposed LTP machinery at discrete populations of synapses. Our field has not yet arrived at 'standard model' for memory formation but we are drawing much closer.

The how and where of memory are intellectual questions that are basic to a biological understanding of the human experience; this point alone accounts for the intensity with which scientists pursue these issues. Beyond this lies the assumption that progress in this area will eventually provide tools for dealing with learning disorders and for enhancing human cognitive capabilities. As Thompson put it in a 1986 review "Likely applications of this new understanding of the neural bases of learning and memory range from education to the treatment of learning disabilities to the design of new artificial intelligence systems" (Thompson, 1986). The following sections describe a few examples of how this understanding can be applied to human diseases associated with learning impairment.

Fragile-X Mental Retardation Syndrome (FXS)

FXS arises from an expansion of CGG triplet repeats in the 5' UTR of the X-linked *Fmr1* gene, a change resulting in transcriptional silencing of the *Fmr1* gene encoding Fragile X mental retardation protein (FMRP) (Bell, Hirst, Nakahori, MacKinnon, Roche, Flint, Jacobs, Tommerup, Tranenjaerg, Froster-Iskenius, & et al., 1991; Sutcliffe, Nelson, Zhang, Pieretti, Caskey, Saxe, & Warren, 1992), an RNA binding protein (Ashley, Wilkinson, Reines, & Warren, 1993). Spines with abnormal, immature anatomy are a defining characteristic of the condition. LTP defects have been reported for various cortical areas in the FXS model *Fmr1*-KO mice but were thought not to occur in hippocampus (Larson, Jessen, Kim, Fine, & du Hoffmann, 2005; Meredith, Holmgren, Weidum, Burnashev, & Mansvelder, 2007). We found, however, that the threshold for long term potentiation is measurably higher in that structure in the mutants (Lauterborn, Rex, Kramar, Chen, Pandeyarajan, Lynch, & Gall, 2007). Our first tests for errors in activity-driven actin signaling proved negative: TBS produced marked increases in pCofilin+synapses and F-actin dense spines indicating that the RhoA>> ROCK>> LIM-K>> cofilin pathway (Fig. 2, left) is functional in the mutants. The recognition of the second pathway through Rac (see above) caused us to reopen the search for signaling defects and led to the discovery that TBS fails to activate Rac or its effector PAK in hippocampal slices from *Fmr1*-KO mice (Chen et al., 2010a). While other studies

have provided good pharmacological evidence for abnormalities at Fmr1-KO synapses, our results constitute the first direct demonstration of an error in the cellular responses to afferent activity.

As discussed, the Rac>PAK pathway serves to stabilize the newly assembled actin networks (Fig. 2). We therefore tested the explicit prediction that both F-actin stabilization and LTP consolidation would be abnormal in the mutants. Latrunculin A, which disassociates dynamic filaments, was infused into slices at 10 min post-TBS, a time point at which it has no longer affects filamentous actin or LTP in slices from wild type mice. The same treatment in slices from Fmr1-KOs eliminated TBS-induced increases in spines with high levels of F-actin and caused LTP to gradually decay back to baseline. The FXS impairment is not complete: latrunculin applied at 40 min post-TBS had no effect in mutant slices indicating that the essential problem lies in the speed of consolidation (Chen et al., 2010a). In all, the signaling problem in the FXS mouse is surprisingly discrete; the question now becomes how to correct it.

Aging

There is a sizable literature describing the emergence of memory impairments in the transition from young adulthood to middle age. This suggests that LTP deficits should be present in hippocampus well in advance of old age. Tests of this led to the discovery that potentiation is impaired in the basal dendrites of hippocampal field CA1 in 8-10 month-old rats relative to young adults (Rex et al., 2005); work by other groups found comparable deficits in the dentate gyrus *in vivo*. Adenosine signaling changes with age and some find the onset of these disturbances in middle age. As described, adenosine is a synaptic modifier that depresses the RhoA path and we found antagonists of its A1 receptor rescues LTP in middle-aged hippocampus, even when applied shortly after TBS. Adenosine itself produced greater and longer lasting depressions of synaptic responses in middle-aged versus young adult slices. These findings suggest that clearance of extracellular adenosine is slower in the older animals, resulting in a suppression of one of the signaling cascades needed for consolidation. Nevertheless, other work showed that BDNF infusion, or increases in BDNF expression, can offset these problems and restore stabilization of LTP (Rex et al., 2005; Rex, Lauterborn, Lin, Kramar, Rogers, Gall, & Lynch, 2006).

Low estrogen

Changes in cognitive functioning, particularly with regard to memory, are among the behavioral changes associated with menopause. Studies using ovariectomized (OVX) animals to mimic postmenopausal conditions found memory impairments in rats and monkeys across a wide variety of test procedures. That estrogen potently enhances both the RhoA, actin filament assembly pathway in the general model and LTP consolidation, and provides a reasonable explanation for why subnormal concentrations of the steroid would negatively affect memory. In agreement with these ideas, we found that TBS-induced actin polymerization and consolidation are both severely impaired in OVX rats and that both effects are fully restored by 40 minute infusions of estrogen or BDNF (Kramar et al., 2009a; Kramar et al., 2009b). Moreover, estrogen, like BDNF, was found to activate signaling through the RhoA cascade via effects on estrogen receptor beta (Kramar et al., 2009a), and to activate calpain-2 and actin polymerization through stimulation of the ERK pathway (Zadran, Qin, Bi, Zadran, Kim, Foy, Thompson, & Baudry, 2009).

Early stage HD

Cognitive deficits are evident in HD patients and generally worsen with age, ultimately resulting in dementia (Lynch et al., 2007a; Murphy, Carter, Lione, Mangiarini, Mahal, Bates, Dunnett, & Morton, 2000; Van Raamsdonk, Pearson, Slow, Hossain, Leavitt, &

Hayden, 2005). While some debate exists about when the cognition problems first appear, several indicators, especially those involving memory, can be discerned in otherwise asymptomatic gene carriers. Learning deficits occurring before severe motor symptoms or neuron loss have also been reported in different mouse models of HD. These impairments are accompanied by disruption of LTP. We built on these results to test if our general model of LTP consolidation accounts for the plasticity deficits in HD mice. Those studies showed that TBS-induced cofilin phosphorylation and actin polymerization are greatly reduced in three different mouse models of late onset HD (Lynch et al., 2007a; Simmons, Rex, Palmer, Pandeyarajan, Fedulov, Gall, & Lynch, 2009). We confirmed earlier studies indicating that the mice have reduced levels of BDNF, a releasable factor that promotes activation by TBS of the proposed actin assembly and stabilization paths. We then showed that brief infusions (Lynch et al., 2007a) or increases in the endogenous levels (Simmons et al., 2009) of the neurotrophin restore actin polymerization and LTP consolidation in the HD model mice. In all, the plasticity defects in HD appear to be due to disturbances with one of the synaptic modifiers that occupy a central place in the general model.

5. Concluding remarks

Having survived for 26 years, what will the hypothesis look like at the next celebration of Thompson's continuing contributions to learning and memory? For one, we expect that it will be connected to later stages of memory consolidation. There is a large and contentious literature concerning delayed events that serve to anchor memory, which is not surprising given that the cellular substrates of the proposed late phases are typically described in very broad terms (e.g., protein synthesis). But recent work by Sachtor and colleagues (Sacktor, 2008; Yao, Kelly, Sajikumar, Serrano, Tian, Bergold, Frey, & Sacktor, 2008) has identified very detailed mechanisms that are argued to provide a late phase of LTP and memory consolidation. As work of this type evolves, it will be fascinating to test if the rapid stages described in our hypothesis lead naturally to more delayed events (an idea along these lines was noted briefly above). We also anticipate that the extensions of the model to neuropsychiatric problems will yield novel treatment strategies by the time of the next Thompson meeting. An intriguing feature of the studies so far conducted is that they point to defects in the actin signaling cascades underlying spine/synapse reorganization as final common pathways for multiple disorders with distinct etiologies. Hopefully, then, treatments directed at those pathways will produce broad-spectrum improvements. Beyond this lies the possibility of using the contemporary version of the model to design cognition-enhancing drugs (Lynch, 2006). Ideas suggested by the original hypothesis have already resulted in a new class of compounds that is currently in clinical trials (Lynch&Gall, 2006); the 2010 version of the model is much richer and pregnant with possibilities in this direction. Finally, we anticipate that researchers at the next Thompson conference will have begun using the mapping tools, which emerged almost as byproducts of efforts to test and advance the hypothesis, to move from memory to the neurobiology of cognition. The journey from 1984 to the present has been long, and filled with unexpected turns and surprising twists, but seems to be leading to extraordinary new vistas.

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Figure 1.

Theta stimulation increases dendritic spine actin polymerization, and activities of actin regulatory proteins, in adult hippocampus.

Photomicrographs (A-C) show in situ phalloidin labeling of filamentous (F) actin (A,B) and immunolabeling for cofilin (C) and phosphorylated-cofilin (D) in the apical CA1 dendritic field of an adult hippocampal slice. As shown in panels A and B, the incidence of dendritic spines (seen as small white punctae) containing dense F-actin, is low in control tissue (con) and increased by theta burst stimulation (TBS) of afferents to this field: Quantification shows this increase is rapid (<2 min) and lasts for over an hour in acute hippocampal slice tissue (Kramar et al., 2006; Rex et al., 2009). (C,D) Immunofluorescence images show that the actin regulatory protein cofilin (both phosphorylated and unphosphorylated forms detected in C) is abundant in the great proportion of dendritic spines (small punctae) within hippocampal field CA1, whereas in control tissue, the phosphorylated (inactivated) form (detected in D) is present in a small minority of the spine-like profiles in a similar sized sample field; arrows indicate representative labeled spines(deconvolved with field immunofluorescence images shown in C and D). E) Numbers of spines immunopositive for phospho (p)-cofilin are significantly increased within 2 and decline by 15 min after TBS (results in C-E are from Chen et al. 2007).

Figure 2.

Signaling pathways regulating dendritic spine actin remodeling with the induction of LTP by theta burst stimulation (TBS).

Schematic of signaling pathways linking three classes of synaptic to regulation of the actin cytoskeleton. Neurotransmitter receptors (glutamate-receptors), integrins, and modulatory receptors including the A1 adenosine receptor, estrogen receptor beta (ERB) and the TrkB receptor for BDNF) are all activated by LTP-inducing theta burst stimulation and, in turn influence signaling cascades that influence the state of filamentous (F) actin within dendritic spines. As illustrated our studies have shown that the modulatory and integrin receptors regulate signaling through two pathways. The first, through the RhoA GTPase drives new actin polymerization whereas the second, through Cdc42/Rac>PAK, mediates stabilization of the new actin filaments. The downstream effectors of the latter pathway are not known but are likely to involve actin nucleating factors such as cortactin and Arp2/3, both of these are concentrated within dendritic spines and are influenced by TBS in adult tissue (grey lettering denotes elements which may influence these processes but have not been tested).

Figure 3.

Schematic representation of the links between BDNF, calpain, and synaptic modifications underlying long-term potentiation.

Patterns of electrical activity producing LTP trigger the release of BDNF, resulting in activation of TrkB tyrosine kinase, followed by stimulation of ERK. ERK phosphorylates m-calpain, facilitating its activation possibly through binding to membrane-associated PIP2. Activated calpain can produce various modifications of synaptic structure and function by several mechanisms: i) by truncating FAK, calpain can modify adhesion properties of dendritic spines, possibly influencing presynaptic terminals; ii) by regulating elements of the actin cytoskeleton, calpain can participate in the enlargement of dendritic spines; iii) by truncating spectrin and disrupting the accumulation machinery, calpain can facilitate the insertion of AMPA receptors in postsynaptic densities; iv) finally, by regulating local protein synthesis, calpain can participate in the stabilization of the synaptic reorganization taking place after LTP induction.