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Research Report

Protein synthesis and consolidation of memory-related synaptic changes

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ABSTRACT

Although sometimes disputed, it has been assumed for several decades that new proteins synthesized following a learning event are required for consolidation of subsequent memory. Published findings and new results described here challenge this idea. Protein synthesis inhibitors did not prevent Theta Bust Stimulation (TBS) from producing extremely stable long-term potentiation (LTP) in experiments using standard hippocampal slice protocols. However, the inhibitors were effective under conditions that likely depleted protein levels prior to attempts to induce the potentiation effect. Experiments showed that induction of LTP at one input, and thus a prior episode of protein synthesis, eliminated the effects of inhibitors on potentiation of a second input even in depleted slices. These observations suggest that a primary role of translation and transcription processes initiated by learning events is to prepare neurons to support future learning. Other work has provided support for an alternative theory of consolidation. Specifically, if the synaptic changes that support memory are to endure, learning events/TBS must engage a complex set of signaling processes that reorganize and re-stabilize the spine actin cytoskeleton. This is accomplished in fast (10 min) and slow (50 min) stages with the first requiring integrin activation and the second a recovery of integrin functioning. These results align with, and provide mechanisms for, the long-held view that memories are established and consolidated over a set of temporally distinct phases.

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1. Introduction

For over 50 years thinking about how memories are consolidated has been dominated by the hypothesis that the learning experience must initiate the synthesis of new proteins if the memory is to persist. Early support for this idea emerged from reports that protein synthesis inhibitors delivered around the time of the learning event impaired long-term retention of memories in a variety of behavioral tasks but had little or no effect when the retention interval was brief (Davis and Squire,

Abbreviations: ACSF, artificial cerebrospinal fluid; act-ß1, activated ß1 integrin; ANIS, anisomycin; BDNF, brain derived neurotrophic factor; bref., brefeldin A; LTP, long term potentiation; TBS, theta burst stimulation

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1984; Hernandez and Abel, 2008). Although agents that prevent transcription are highly toxic, their use also led to the related idea that behaviors resulting in enduring memories signal to the nucleus to initiate expression of plasticity-related genes and their protein products (Alberini, 2009; Kandel, 2001; Squire and Barondes, 1970).

The case for the protein synthesis hypothesis was further strengthened by evidence that:

- A learning experience can increase the expression of genes and proteins related to synaptic functioning (Gall et al., 1998; Ganguly et al., 2013; Guzowski et al., 2001; Robles et al., 2003).
- Suppression of these same genes or gene products via knock-outs or regionally targeted treatments (oligonucleotides, AAV transfection) impair retention (Guzowski et al., 2000; Minichiello et al., 1999; Nagy et al., 2006; Plath et al., 2006; Ploski et al., 2008).
- When applied locally to hippocampus or amygdala, highly selective manipulations of transcription have profound effects on long-term retention (Barrett et al., 2011; McQuown et al., 2011; Nonaka et al., 2014).

Growing evidence that long-term potentiation (LTP) is a substrate for many forms of memory prompted new investigations into the role of protein synthesis in the consolidation of learning-related synaptic plasticity. Consistent with the behavioral literature, neither protein-synthesis nor transcription inhibitors impaired the initial, early phase of LTP but both caused potentiation to gradually dissipate (Frey et al., 1996, 1988; Frey and Morris, 1997; Huang and Kandel, 1994; Sacktor, 2008; Tsokas et al., 2005). Evidence also emerged that the induction of LTP stimulates gene expression and translation events associated with learning (Kelleher et al., 2004; Miyashita et al., 2008; Park et al., 2006; Pevzner et al., 2012; Steward and Worley, 2002; Tao et al., 1998; Tsokas et al., 2005). Moreover, manipulations of gene expression and translation produced results that accord well with the above findings (Guzowski et al., 2000; Korte et al., 1998; Minichiello, 2009).

Given this large body of supporting evidence it is surprising that the protein synthesis hypothesis has not been universally accepted (Canal et al., 2007; Gold, 2008a, 2008b; Routtenberg and Rekart, 2005; Rudy, 2008). Opposition to the idea is based on two classes of evidence. One set indicates that at least some of the memory impairments produced by protein synthesis inhibitors may be the result of their off-target effects (e.g., Canal et al., 2007; Sharma et al., 2012). Another body of results indicates that memories and LTP can indeed persist even in the face of severe inhibition of protein synthesis (Abbas, 2013; Abbas et al., 2009, 2011; Abraham and Williams, 2008; Fonseca et al., 2006b; Martinez et al., 1981; Pang et al., 2004; Staubli et al., 1985; Villers et al., 2012).

The second data set is cause for concern because it not only challenges the central argument, it questions the relevance of the well-documented findings that both behavior and LTP-inducing stimulation induce changes in gene expression (Alberini, 2009; Bramham and Messaoudi, 2005; Chen et al., 2010; Guzowski et al., 2001; Taubenfeld et al., 2001). If neither long term memories nor LTP depend on the generation of new proteins induced by the initiating events, then what is the function of activity-regulated changes in gene and protein expression? Moreover, if memory consolidation does not depend on the initiation of new protein synthesis, then what events are critical for consolidation?

The present paper addresses the above issues. We report evidence that reinforces the conclusion that the consolidation of LTP is not blocked by protein synthesis inhibition, then describe circumstances in which the inhibitors are effective, and finally demonstrate that multiple LTP events obviate the negative actions of the inhibitors when such are present. These observations help explain some of the discrepant results in the literature and lead to the conclusion that induced synthesis is not, *under normal circumstances*, important to current encoding but instead paves the way for future memory formation. We also review studies demonstrating that early and delayed phases of LTP and memory consolidation dependent upon activation and subsequent recovery of signaling by integrin-class adhesion proteins, respectively, and that these events are protein synthesis independent. A final section will attempt to integrate the hypothesis that temporally distinct stages of integrin-driven cytoskeletal reorganization underly multiple stages of memory consolidation with evidence that learning and LTP induction trigger the production of proteins necessary for long term storage.

2. Reorganizing the spine actin cytoskeleton is a consolidating event

Our interest in the contribution of newly synthesized proteins to the consolidation of LTP emerged from research directed at understanding mechanisms that regulate the dendritic spine actin cytoskeleton and, thus, spine morphology. Results from our laboratories and elsewhere (Fukazawa et al., 2003; Kramar et al., 2006; Krucker et al., 2000; B. Lin et al., 2005; Okamoto et al., 2009; Wang et al., 2008) led to the conclusion that the enlargement and stabilization of the spine actin cytoskeleton initiated by Theta Burst Simulation (TBS) may be a critical consolidating event. Specifically, potentiation induced with either TBS or high frequency stimulation elicits, and depends upon, new actin polymerization in dendritic spines (Fig. 1). Further analyses demonstrated that these structural events are driven by separate signaling streams that control the assembly (polymerization) and subsequent stabilization of the new actin filaments (Chen et al., 2007; Fedulov et al., 2007; Kramar et al., 2006; Mantzur et al., 2009; Rehberg et al., 2010; Rex et al., 2009, 2010). Integrins, a group of transmembrane adhesion receptors that regulate the cytoskeleton at most types of cellular junctions (Brakebusch and Fassler, 2003), play a central role in these processes, as indicated by results of studies using toxins, small peptides, neutralizing antibodies, or genomic manipulations (Kramar et al., 2006; Nagy et al., 2006; Wang et al., 2008). Downstream intracellular signaling cascades (small GTPases and their effectors) initiated by integrins have also been linked to LTP stabilization (Rex et al., 2009). Notably,