

# Translation regulation in sleep

## Making experience last

Julie Seibt<sup>1,2</sup> and Marcos G. Frank<sup>1,\*</sup>

<sup>1</sup>Department of Neuroscience; Perelman School of Medicine; University of Pennsylvania; Philadelphia, PA USA; <sup>2</sup>Institute of Physiology; University of Bern; Bern, Switzerland

**S**leep improves cognition and is necessary for normal brain plasticity, but the precise cellular and molecular mechanisms mediating these effects are unknown. At the molecular level, experience-dependent synaptic plasticity triggers new gene and protein expression necessary for long-lasting changes in synaptic strength.<sup>1</sup> In particular, translation of mRNAs at remodeling synapses is emerging as an important mechanism in persistent forms of synaptic plasticity *in vitro* and certain forms of memory consolidation.<sup>2</sup> We have previously shown that sleep is required for the consolidation of a canonical model of *in vivo* plasticity (i.e., ocular dominance plasticity [ODP] in the developing cat).<sup>3</sup> Using this model, we recently showed that protein synthesis during sleep participates in the consolidation process. We demonstrate that activation of the mammalian target of rapamycin [mTOR] pathway, an important regulator of translation initiation,<sup>4</sup> is necessary for sleep-dependent ODP consolidation and that sleep promotes translation (but not transcription) of proteins essential for synaptic plasticity (i.e., ARC and BDNF). Our study thus reveals a previously unknown mechanism operating during sleep that consolidates cortical plasticity *in vivo*.

Experience-dependent plasticity involves gene expression that is highly regulated at both the transcriptional and translational levels. In particular, regulation at the translational step has become an important mechanism allowing spatial fine-tuning of protein expression and input

specific synaptic plasticity, unlike transcription that is confined to the nucleus.<sup>1</sup> Translational machinery (ribosomes, mRNA, translation factors) is present in axons and dendrites and allows neurons to adapt their response to environmental stimulation by changing their proteomic profile locally.<sup>5</sup> The importance of protein synthesis in the consolidation of synaptic plasticity and memory has long been recognized.<sup>6,7</sup> More recently substantial progress has been made in identifying the molecular mechanisms that regulate activity-dependent protein synthesis. Most steps of translation (initiation, elongation and mRNA sequestration [e.g., mRNA binding proteins]) have been implicated in plasticity-dependent translation regulation.<sup>8,9</sup> One critical step involved in this process is the initiation step, mostly controlled by the mTOR pathway.<sup>4,10</sup> mTOR, via its direct downstream target, eukaryotic initiation factor 4E (eIF4E)-binding protein 1 (4E-BP1), regulates the translation initiation of 5' capped mRNA (which comprises most of the mRNA in the cell).<sup>10</sup> Previous studies have shown that sleep promotes transcription of mRNA involved in translation regulation,<sup>11,12</sup> but whether those factors are activated during sleep is not known. It is also known that sleep, especially deep slow-wave sleep,<sup>13,14</sup> is correlated with increased protein synthesis, but to date no specific function has been associated with this phenomenon.

ODP is a classic form of plasticity *in vivo* that refers to physiological and anatomical changes in visual cortical circuits triggered by transiently blocking patterned

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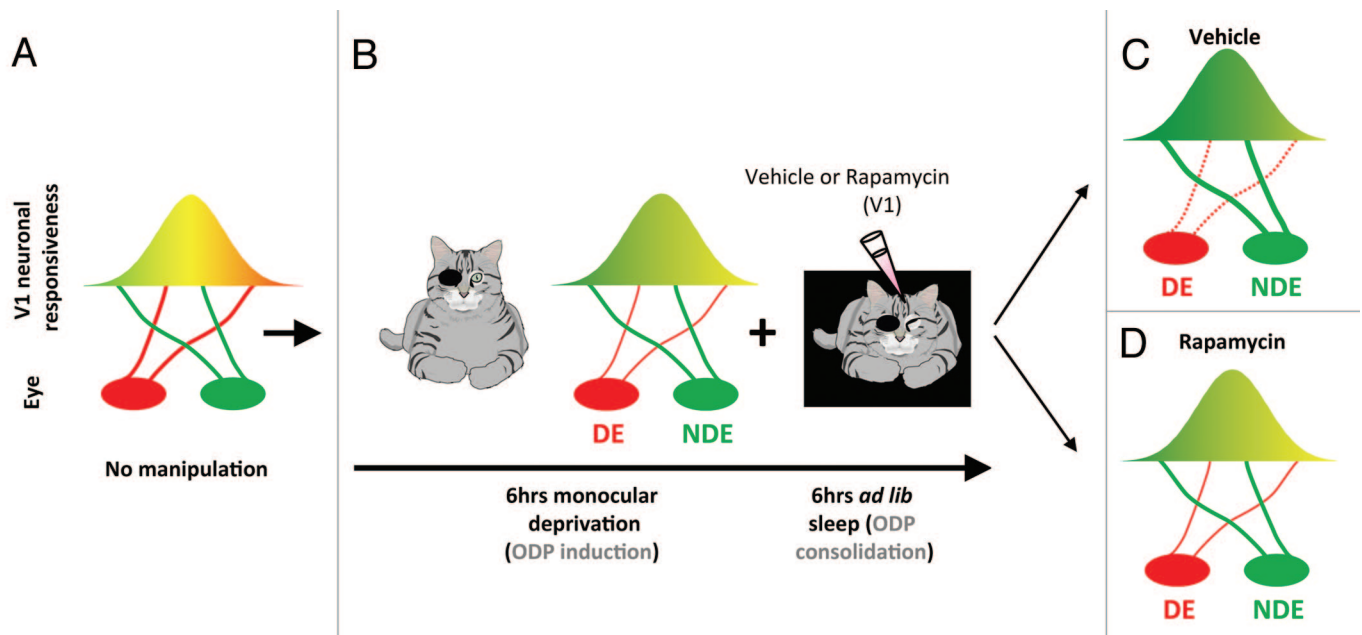
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\*Correspondence to: Marcos G. Frank;  
Email: [mgf@mail.med.upenn.edu](mailto:mgf@mail.med.upenn.edu)

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**Figure 1.** Protein synthesis is required for sleep-dependent ocular dominance plasticity (ODP). (A) In developing cats with normal vision, most neurons in the primary visual cortex (V1) are binocular (*i.e.* equally responsive to inputs from either eye, represented as the yellow area). (B) When animals are deprived of patterned visual input in one eye (*i.e.* monocular deprivation) most neurons in V1 become responsive only to stimulation of the non-deprived eye (NDE). This process is induced very rapidly in awake cats (6 h) and is enhanced/consolidated by subsequent sleep (6 h). To test the role of mTOR in sleep-dependent ODP, visual cortices are infused with vehicle or the selective mTOR inhibitor rapamycin during the post-MD sleep period. (C) Sleep-dependent ODP is intact in the vehicle infused hemispheres and includes a maintenance of depression of the DE visual input (dotted red line) and potentiation of the NDE input (thick red line). (D) Inhibition of protein synthesis in V1 with rapamycin during post-MD sleep blocks sleep-dependent ODP. This reflects inhibition of both plastic changes normally observed after sleep (the weakening of the DE and the strengthening of NDE inputs). This results in a V1 plasticity phenotype that is normally seen after the initial 6 h of monocular deprivation only in awake animal (compare B and D).

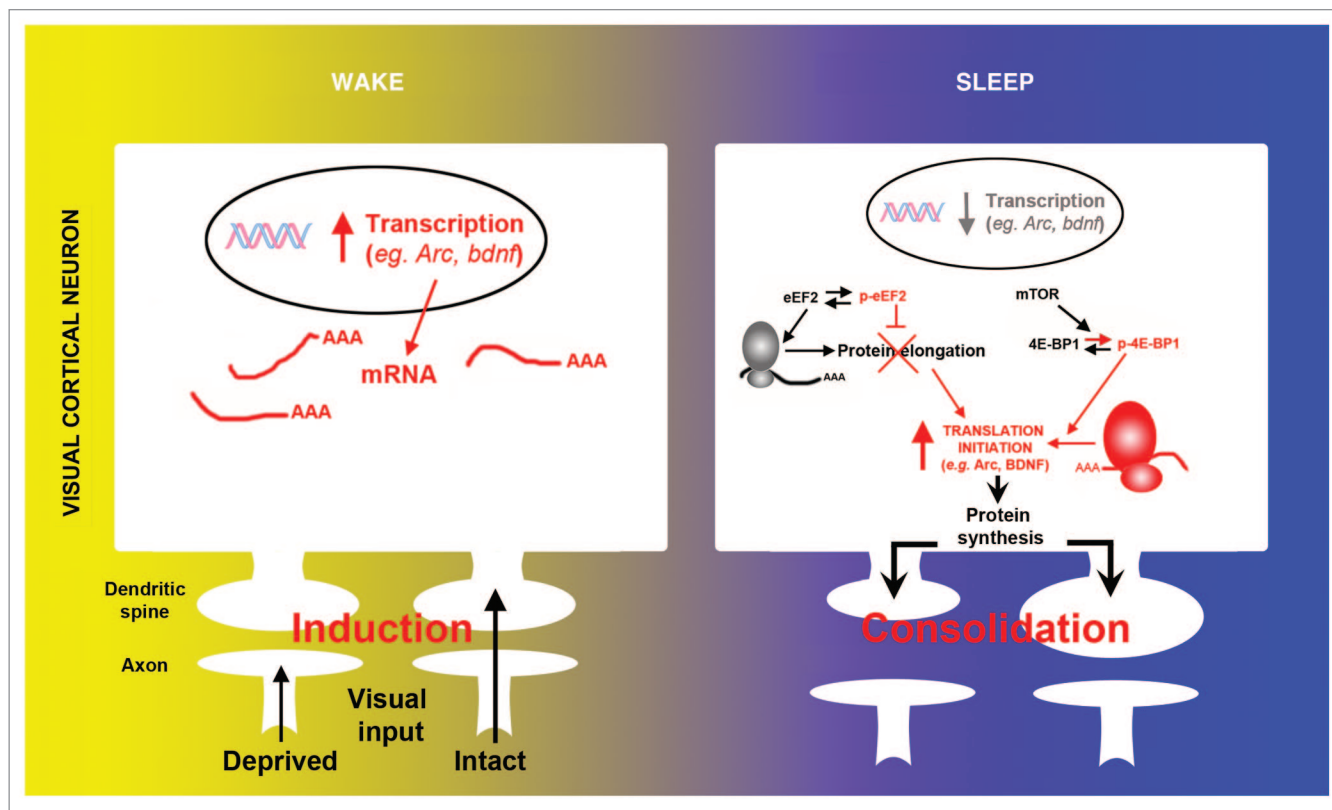
vision in one eye (monocular deprivation, **Figure 1**).<sup>15</sup> This type of brain plasticity is considered physiological because in contrast to electrically evoked potentials or tetanic stimulation, it is triggered by alterations of natural sensory input. It also involves various types of synaptic plasticity (Hebbian and non-Hebbian<sup>16</sup>), many of which require *de novo* protein synthesis to be consolidated. We have previously shown that ODP can be divided temporally into an induction phase (during waking) and a consolidation phase (during sleep) involving cellular mechanisms of depression and potentiation of the deprived and the non-deprived visual pathways, respectively (**Fig. 1**).<sup>3,17</sup> In our recent study,<sup>18</sup> we now show that inhibition of mTOR-dependent protein synthesis during sleep impairs the consolidation of ODP (**Fig. 1**). Remarkably, mTOR inhibition during wake does not affect the induction phase of ODP. This first finding is important because it suggests that, at a functional level, protein synthesis necessary for ODP consolidation

occurs only during sleep. We also find that mTOR inhibition during sleep impairs both potentiation of the open eye and depression of the deprived eye visual pathways (**Fig. 1**). This confirms previous findings in *in vitro* models showing the importance of protein synthesis for stabilization of both weakening (e.g., long-term depression) and strengthening (e.g., long-term potentiation) of synapses and demonstrates the requirement for mTOR during sleep in both processes.<sup>19</sup>

Considerable effort has been focused on identifying mRNAs that undergo rapid translation in response to synaptic activation.<sup>20</sup> Most mRNAs identified in neuronal processes (*i.e.*, dendrites and axons) have important functions in synaptic plasticity. This includes plasticity related genes such as *arc* and *bdnf*. In our recent report we show that sleep differentially affects transcription and translation of these genes. Using quantitative PCR in visual cortical tissue, we show that the mRNA level of both genes decreased during sleep, as described in studies in

adult rodents<sup>12</sup> (**Fig. 2**). However levels of the corresponding proteins transiently increased during the same time period in total protein extracts as well as in synaptic enriched protein fractions (*i.e.*, synaptoneurone) (**Fig. 2**), demonstrating selective activation of protein synthesis during sleep. In the visual cortex, transcription of the immediate early gene (IEG) *arc* is triggered by visual input. This suggested that the decreased level of transcripts observed after sleep might also be an indirect consequence of reduced visual input. This was confirmed as transcription of another important IEG, *c-fos*, decreased in a similar manner during sleep, and both transcripts were similarly reduced by simply blocking binocular vision in an awake animal.

Collectively, these findings are important for the following reasons. First, they demonstrate that transcriptional changes are not necessarily mimicked, at the functional level, by changes of the corresponding proteins. This may be especially true for forms of synaptic plasticity that involve



**Figure 2.** Molecular evidence of protein synthesis regulation during sleep. During wake, the induction of ocular dominance plasticity (monocular deprivation) triggers activity-dependent transcription of selected genes (e.g., *arc*, *bdnf*, *c-fos*) in V1. Subsequent sleep activates a cascade of translational events (increased translation initiation via 4E-BP1 phosphorylation and reduced global elongation via eEF2 phosphorylation) leading to a net increase in translation initiation of subsets of mRNA. *Arc* and *bdnf* are two examples of important plasticity-related genes where transcription is decreased and translation is increased during sleep.

translation in the consolidation process. Second, they suggest that some transcriptional changes ascribed to sleep might be epiphenomena, rather than reflecting an active process. This is important because genome wide screening methods have been extensively used to clarify the role of sleep in brain function<sup>11,12,21</sup> and current hypotheses on sleep function are based on changes in transcriptomes.<sup>11,22</sup> Of course, more comprehensive studies examining a wider-array of genes, their proteins and activity-dependent transcription mechanisms (e.g., CREB-dependent transcription) are needed. Several studies have shown that sleep (especially REM sleep) may promote rapid gene transcription<sup>23,24</sup> and that several important gene groups are transcribed during sleep.<sup>11,12</sup> This indicates that while the transcription of some genes is not actively regulated by sleep, this may not be universally true for others.

Interestingly, sleep following monocular deprivation, not only induced

translation initiation (via increased phosphorylation of the mTOR target 4E-BP1), it also decreased protein elongation (via phosphorylation of the eukaryote elongation factor 2 [eEF2]) (Fig. 2). This may seem paradoxical (i.e., enhanced initiation and decreased elongation rate), but similar events are triggered during synaptic plasticity *in vitro*<sup>25,26</sup> and *in vivo*<sup>27,28</sup> and may promote the translation of specific subsets of mRNAs (e.g., *ARC*<sup>29</sup>) (Fig. 2). This was supported by our results showing that translation of plasticity related genes other than *ARC* and *BDNF*, such as  $\alpha$ CamKII or *GlurI*, was not affected by sleep. We further confirmed that the molecular changes observed at the translational level (i.e., increased *BDNF* and *ARC* protein expression and translation factors phosphorylation) were specific to sleep as they did not occur in animals instead kept awake after monocular deprivation. Therefore an important and exciting future direction

for this research is to discover which genes are actively translated during sleep.

These findings provide a new way to investigate sleep function and raise a number of exciting questions. The first is to determine the exact location of sleep-dependent protein synthesis. Our results suggest that translation mechanisms are activated at synapses because most of the protein changes we detect occur in synaptoneuroosomes. But these results have to be confirmed and extended to a wider panel of candidate proteins that are translated in an activity-dependent manner (e.g., *MAP1b*, tissue-plasminogen activator). Second, our results suggest that specific proteins (e.g., *ARC* and *BDNF*) are produced during sleep and not others (e.g.,  $\alpha$ CaMKII and *GlurI*). This could be explained by the fact that sleep promotes the translation of specific pools of mRNAs. The underlying mechanisms are likely to be complex. For example, RNA-binding proteins are important translation

regulators as they allow mRNA transport while inhibiting their translation. Among them, the cytoplasmic polyadenylation element binding protein (CPEB) and Fragile X mental retardation protein (FMRP) are known to be critical in *in vitro* models of synaptic plasticity, memory formation and proper brain development.<sup>30</sup> Interestingly, it is well known that translation of  $\alpha$ CaMKII and GluRI (that are not regulated in our model) depends on CPEB.<sup>31-33</sup> Other important mRNA-binding mechanisms, such as microRNAs, have also been shown to be regulated by sleep,<sup>34,35</sup> but their function, if any, in sleep-dependent plasticity is unknown. Third, our findings indicate that understanding the synaptic proteomic profile of the sleeping brain will provide key insights into sleep function and neurological disorders. In a wide range of vertebrate species, sleep is maximal during times when the brain is rapidly maturing and highly plastic. Sleep also promotes brain protein synthesis not only postnatally, but prenatally as well.<sup>36</sup> These ontogenetic periods are accompanied by a number of important processes that require protein synthesis (e.g., axon guidance, dendritogenesis, and synaptogenesis). It is therefore likely that sleep-dependent protein synthesis plays critical roles in the initial establishment and refinement of developing synaptic circuitry.<sup>37</sup> Moreover, it is also likely that this function is retained in some fashion across the lifespan.<sup>38</sup>

At the clinical level, deregulation in mRNA transport and translation is responsible for mental retardation symptoms in several developmental psychiatric disorders, such as Fragile X syndrome (FXS; mutation of the *fmrp* gene)<sup>39</sup> and most likely Down and Angelman syndromes and autism spectrum disorders.<sup>40-42</sup> Genomic screening studies provide strong evidence that sleep promotes RNA trafficking and general translational events.<sup>43,44</sup> This in turn suggests that sleep is indeed a preferred time for these processes. Mice with mutations in *fmrp* (FXS) and *Ube3a* (involved in Angelman syndrome) genes, both involved in different aspect of translation regulation, have impaired sleep characterized by increased wake periods for example.<sup>45,46</sup> Therefore a better understanding of how natural

behavioral brain states (sleep vs. wake) participate in general mRNA transport and translation is central to understanding how miscues in translation regulation contribute to neurological diseases.

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