

activity in the SCN and monitoring the sleep stages from rats *in vivo*. Although conceptually straightforward, this dual long-term recording in freely moving animals is technically quite difficult, in large part because the SCN is near the bottom surface of the brain. Specifically, the authors asked whether the central circadian pacemaker that regulates the sleep/wake switch also gets feedback about the specific sleep state. Recall that sleep can be recognized as REM (rapid eye movement) or non-REM-sleep (NREM). Within the NREM-stages, deep sleep episodes are marked by low-frequency brain waves and defined as slow-wave sleep (SWS). The amount of SWS increases after sleep deprivation and decreases as a result of sleep. For these reasons and others^{2,3}, SWS is considered a marker for the restorative and homeostatically regulated sleep processes.

Strikingly, Meijer *et al.* found a clear correlation between sleep states and the neuronal activity in the SCN. The frequency of electrical activity in the SCN undergoes a daily rhythm, with higher activity found during the day in both day- and night-active animals (Fig. 1). On top of this circadian modulation of firing rate, the SCN neurons fired at lower rates during NREM sleep, and higher rates during REM sleep. A closer look at the NREM stages revealed a significant negative correlation between the SWS and the SCN activity, but no correlation with NREM sleep containing higher-frequency waves. The transitions between vigilance states were tightly paralleled by changes in SCN firing rate.

Meijer and her colleagues then put this correlation to the test by examining the effect of sleep deprivation on SCN activity. SWS or REM sleep was prevented over a two-hour period by briefly disturbing the animals as they entered these sleep stages. Neuronal activity in the SCN was significantly higher during the SWS deprivation

compared to undisturbed SWS episodes. In contrast, REM deprivation led to a decrease in the mean SCN firing rate compared to controls during REM sleep. These findings are consistent with the suggestion that SWS inhibits the firing rate of neurons in the SCN, whereas REM sleep increases the firing rate. The results provide strong evidence that information about these sleep states is transmitted to the SCN.

Although the current work of Meijer and colleagues provides no direct evidence for the underlying anatomical pathways and functional significance of this feedback loop, it certainly raises some interesting possibilities. A recent study demonstrated that the firing rate of individual SCN neurons is highly correlated with the degree of expression of one of the circadian clock genes, *Period*¹⁴. By altering the firing rates of SCN neurons, information about sleep states can influence the molecular feedback loops that lie at the heart of the circadian timing system. This communication between the sleep homeostat and the circadian oscillator might allow the circadian system to track the amount of SWS and REM sleep during the previous daily sleep/wake cycle. Perhaps the circadian system responds to a night of insufficient sleep by making it easier to go to bed early the following day?

Unraveling the neurobiological mechanisms underlying sleep has broad implications for industrial and post-industrial societies. By some estimates, 50% of the adult population suffers from difficulties sleeping at night and staying awake during the day (2003 Sleep in America poll, National Sleep Foundation: http://www.sleepfoundation.org/NSAW/2003presskit/pk_pollhighlights.html). In older people and in patients with psychiatric and neurological disorders, this percentage is far higher. Although it would be premature to claim that the present study will have an

immediate clinical impact, sleep disorders can arise from dysfunction in the circadian system, the sleep homeostat, or in communications between the two. With the work of Meijer and colleagues, we are a step closer to understanding the neurobiological basis of the coupling between the sleep homeostat and the circadian system. Understanding the basic neurobiology of sleep provides the opportunity to develop treatments that target the pathophysiology of sleep disorders rather than just the symptoms. There is a huge need for such improvements in treatments; sleep dysfunction has been estimated to cost the US economy alone around \$18 billion annually due to lost productivity. Given the scale of this problem, the question is not if we can afford to sleep in this 24/7 society, but rather if we can afford not to sleep. The least we can do is to promote the research that will enable us to get a good night's sleep.

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NCS-1 Stirs Somnolent Synapses

Robert S Zucker

How is synaptic facilitation mediated? New work suggests that a calcium-sensing molecule, neuronal calcium sensor-1 (NCS-1) transduces a residual calcium signal into an enhancement of transmitter release at excitatory synapses in the hippocampus.

Some synapses are sedentary. Left undisturbed, they seem to nod off. Single presynaptic action potentials release vesicles with low probability, having little postsyn-

naptic effect. They have to be stirred from somnolence by a bout of presynaptic spikes. Once so roused, single action potentials can be much more effective, releasing several times as many quanta and taking more postsynaptic control. This form of short-term synaptic plasticity is called homosynaptic facilitation, and is a major factor in shaping the frequency dependence of synaptic transmission.

How synapses do this remains a mystery. The process has long been known to be calcium dependent¹, prompting a search for a calcium binding molecule that might mediate facilitation. In this issue Sippy *et al.*² claim to have found such a molecule, and present evidence that neuronal calcium sensor-1 (NCS-1) mediates synaptic facilitation at excitatory synapses in the mammalian hippocampus.

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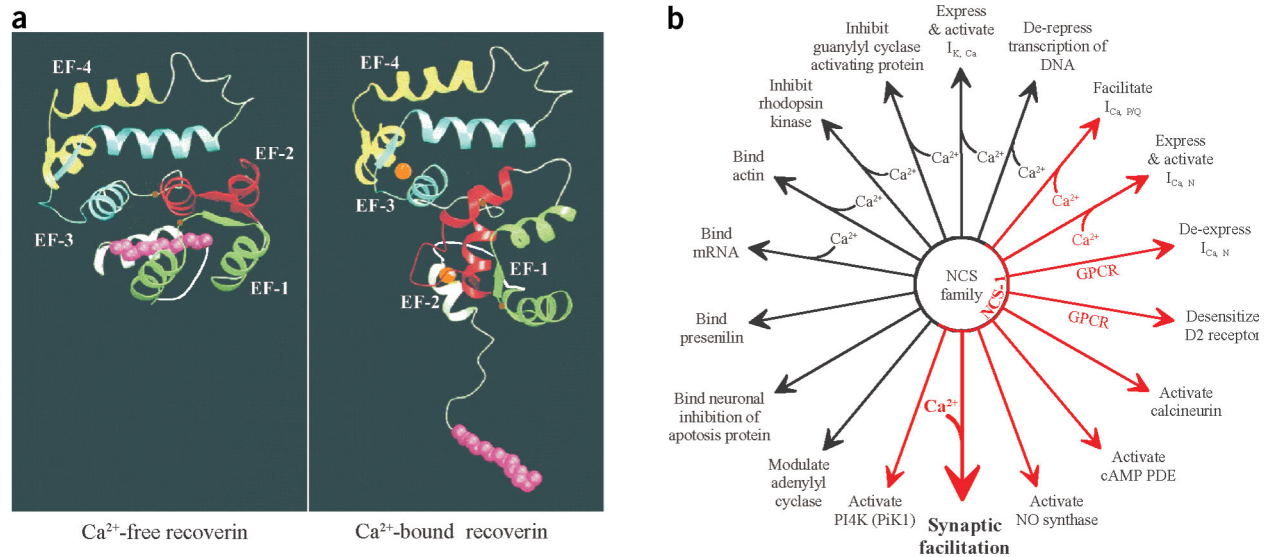


Figure 1 NCS proteins. **(a)** Ribbon structures of the NCS protein recoverin with and without calcium (orange spheres in right panel) bound to EF-hands 2 and 3. The four EF-hands are colored green, red, blue and yellow, the N-terminal myristoyl group is in magenta, and the swivel glycine residues are brown. Modified from Ref. 14. Original figure reproduced with permission from Burgoyne, R.D. & Weiss, J.L. *Biochem. J.* **353**, 1–12, 2001 © the Biochemical Society. **(b)** Some of the roles attributed to NCS proteins. Red lines, mediation by NCS-1; black line, other NCS proteins. GPCR marks effects mediated by activation of G-protein-coupled receptor kinases. I_{K, Ca}, calcium-dependent potassium channels; I_{Ca, N} (I_{Ca, P/Q}), N- (P/Q-) type calcium channels; D2, dopamine type 2; PDE, phosphodiesterase; NO, nitric oxide; PI4K (PIK1), the phosphatidylinositol 4-hydroxykinase PIK1.

NCS-1 is a member of a family of calcium-sensing molecules that undergo large conformational changes upon binding calcium, and regulate a variety of cellular processes (Fig. 1). The overexpression of the *Drosophila* homologue of NCS-1, called frequenin³, increases facilitation at neuromuscular junctions. However, later work suggested that these effects on facilitation were indirect, perhaps caused by changes in potassium channel function or resting [Ca²⁺]_i levels due to altered Na⁺/Ca²⁺ exchange^{4,5}. Moreover, in some synapses facilitation arises in part from the propensity of presynaptic calcium channels to increase their activation by repetitive depolarizations⁶. Therefore effects of NCS-1 on synaptic transmission could simply reflect effects on calcium channels or calcium regulatory processes, which were not measured in these earlier studies. Moreover, at *Xenopus* neuromuscular junctions, NCS-1 also appears to enhance baseline transmission⁷, adding further confusion to the picture.

It is in this context that the study by Sippy *et al.*² sheds new light on the role of NCS-1 at synapses. They transfected dissociated hippocampal neurons with NCS-1 and found that, at glutamatergic synapses, facilitation of evoked transmission to paired pulses and in short trains was specifically enhanced, increasing the

reliability of postsynaptic activation. There was no effect of NCS-1 on basal transmission, confirming that basal transmission and facilitation can be independently regulated. Calcium currents, at least in cell bodies, were unaffected, even to repeated depolarizations. However, synapses between untransfected neurons showed a net depression, raising the concern that an apparent increase in facilitation was really a consequence of reduced depression. But when depression was relieved in a low-calcium medium, facilitation was still enhanced, indicating that the facilitation process itself was specifically altered. The frequency and amplitude of spontaneous miniature EPSPs was also unaffected, except following a train of action potentials, when evoked release was facilitated. Concurrent enhancement of evoked and spontaneous release is a general characteristic of facilitation⁶.

One potential caveat was that the facilitation produced by NCS-1 might be different from the native process occurring in neurons not over-expressing this protein. The authors addressed this question by making use of the curious observation that their calcium-phosphate transfection procedure reduced the normal expression of native NCS-1 in neurons exposed to this reagent but not transfected with any constructs. This down-regulation of native NCS-1,

which *in vivo* is concentrated at hippocampal nerve terminals⁸, was accompanied by a reduction in facilitation in calcium-phosphate treated cells, suggesting that normal facilitation is also produced by the action of native NCS-1.

The question immediately arises: How does NCS-1 facilitate transmitter release? At present, there are two models for synaptic facilitation that are vying for acceptance. Both depend on a residual elevation in resting [Ca²⁺]_i following nerve activity, as required by the experimental findings that rapid reduction in residual [Ca²⁺]_i by photolysis of a caged Ca²⁺ buffer rapidly eliminates facilitation and that facilitation is temporally correlated with residual [Ca²⁺]_i^{6,9}.

In the first of the currently popular models, nerve terminals are endowed with a high-affinity calcium buffer that can be partially saturated by residual calcium. Following prior activity, the saturated buffer is less able to capture Ca²⁺ ions before they bind to the secretory trigger, leading to a supra-linear summation of the local rise in [Ca²⁺]_i during an action potential with the resting [Ca²⁺]_i level. This extra elevation in peak [Ca²⁺]_i at the secretory trigger facilitates release. NCS-1 could act as such a saturable calcium buffer. In this model, lowering bathing [Ca²⁺] usually decreases facilitation by de-saturating the

buffer. Facilitation seems to work this way at mossy fiber terminals¹⁰, cortical multipolar inhibitory synapses¹⁰, and dentate granule terminals¹¹, and perhaps also at the calyx of Held⁹, where a putative saturable buffer has yet to be identified.

In the second model, Ca^{2+} ions remaining from prior activity bind to a presynaptic target that facilitates release by interacting somehow with the secretory machinery. This calcium binding target is quite distinct from the calcium sensor triggering release, where calcium acts cooperatively at low-affinity binding sites, such as a low-affinity synaptotagmin isoform. For this model to work, the calcium binding site onto the facilitation target must occur rapidly, because facilitation is eliminated within milliseconds by sudden $[\text{Ca}^{2+}]_i$ reduction by photolysis of a photosensitive buffer⁶. The facilitation target must be one with high affinity, because very modest (micromolar) $[\text{Ca}^{2+}]_i$ levels can activate substantial facilitation, which may saturate at $[\text{Ca}^{2+}]_i$ levels of just a few micromolar. And the target must be diffusionally isolated from calcium channel mouths, where the local very high $[\text{Ca}^{2+}]_i$ acting on a fast high-affinity target would produce maximal facilitation to every single action potential^{12,13}. Clearly NCS-1 has the necessary attributes. In this model, lowering bathing $[\text{Ca}^{2+}]$ often increases facilitation, by reducing the countervailing effects of depression. Facilitation seems to work this way at crayfish neuromuscular junctions^{12,13}, at hippocampal Schaffer collateral synapses¹⁰, and at hippocampal mossy fiber terminals when calbindin has been genetically knocked-out¹⁰. However, in none of these preparations has the molecule mediating facilitation been identified.

Which of these models explains NCS-1's action at hippocampal synapses? In contemplating this question, it helps to assess what is known of neuronal calcium sensors. NCS-1 is a member of a multifunctional class of proteins that transduce a rise in cytoplasmic calcium concentration ($[\text{Ca}^{2+}]_i$) into a multitude of cellular responses¹⁴. All NCS proteins contain four EF-hand calcium binding domains, and most have an N-terminal myristoyl group. The best understood member of the NCS family is recoverin, a photoreceptor protein that regulates photopigment sensitivity.

Calcium binds at high affinity ($K_D < 0.3 \mu\text{M}$) to two of recoverin's EF-hands, inducing a major rotation in the molecule about two glycine swivels that results in the extrusion of the N-terminal myristoyl group (Fig. 1a). Myristoylation can contribute to membrane association, and conservation of the myristoyl group and glycine swivels in most NCS proteins suggest that a calcium-dependent translocation of NCS proteins into membranes may generally be involved in their subsequent actions¹⁵. The downstream targets of this class of proteins forms a diverse group, including the activation of other kinases, phosphatases, phosphodiesterases, cyclases, synthases, and G-protein-coupled receptors, the regulation of expression and voltage-dependent activation of diverse ion channels, regulation of gene repression, and binding to cytoskeletal components and proteins involved in apoptosis (Fig. 1b).

So how does NCS-1 work at hippocampal synapses? It is likely that NCS-1 acts by binding calcium, as expression with a mutant having a defective EF-hand had a greatly reduced effect². Its myristoyl site was not essential, because a mutant that cannot be myristoylated was fully active². Myristoylation is required for PI4K activation, but is not needed for many of the other actions of NCS proteins¹⁴. It is impossible to say for sure, but several clues suggest that NCS-1 acts as a calcium-binding target modulating release, rather than simply as a saturable buffer: (1) NCS molecules are certainly more complicated than the typical buffer, undergoing large conformational changes mediating many cell responses by protein-protein interactions; (2) the lack of effect of NCS-1 expression on basal transmission in hippocampal synapses argues against its action as a buffer affecting $[\text{Ca}^{2+}]_i$ at the secretory trigger; and (3) the external $[\text{Ca}^{2+}]$ -dependence of facilitation is more consistent with NCS-1 acting to modulate release than to buffer $[\text{Ca}^{2+}]_i$. Moreover, at *Xenopus* neuromuscular junctions, expression of extra NCS-1 at only $0.2 \mu\text{M}$ enhances basal release⁷, which is an effect in the wrong direction and at too low a concentration to attribute to a buffering action. It seems that NCS-1 can affect basal release at some synapses, and mediate facilitation at others, by regulating in different ways the release machinery.

How might NCS-1 affect the secretory apparatus to produce facilitation? We can only speculate, but several possibilities come to mind. NCS-1 might interact with SNARE proteins, or one of their many binding partners, to increase the sensitivity of secretion to a sharp local $[\text{Ca}^{2+}]_i$ elevation as residual $[\text{Ca}^{2+}]_i$ builds up, which remains possible in synapses where buffer saturation appears unlikely. NCS-1 may act to 'prime' docked vesicles, making more of them available for release, perhaps increasing the probability of multivesicular release from active zones. Or it may mobilize additional vesicles to docking sites to increase the size of the readily releasable vesicle pool. It may also activate previously dormant active zones. With careful experiments, one might be able to tease apart some of these possibilities, and we may look forward to efforts to distinguish the alternatives.

Finally, the discovery of a molecule mediating synaptic facilitation opens up the possibility of its operating as a developmental or experiential regulatory site for modification of synaptic properties. Sippy *et al.*² showed that increasing facilitation is an effective mechanism for strengthening one synapse's control of the activity of a postsynaptic neuron. The up- or down-regulation of NCS-1 at selected synapses could remodel the function of a neural network, dramatically altering the way it responds to stimuli, processes information, or generates patterns of activity.

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