Ultrastructural evidence for synaptic scaling across the wake/sleep cycle

Luisa de Vivo,1 Michele Bellesi,1,2 William Marshall,1 Eric A. Bushong,3 Mark H. Ellisman,3–6 Giulio Tononi,1,6 Chiara Cirelli1,6

It is assumed that synaptic strengthening and weakening balance throughout learning to avoid runaway potentiation and memory interference. However, energetic and informational considerations suggest that potentiation should occur primarily during wake, when animals learn, and depression should occur during sleep. We measured 6920 synapses in mouse motor and sensory cortices using three-dimensional electron microscopy. The axon-spine interface (ASI) decreased ~18% after sleep compared with wake. This decrease was proportional to ASI size, which is indicative of scaling. Scaling was selective, sparing synapses that were large and lacked recycling endosomes. Similar scaling occurred for spine head volume, suggesting a distinction between weaker, more plastic synapses (~80%) and stronger, more stable synapses. These results support the hypothesis that a core function of sleep is to renormalize overall synaptic strength increased by wake.

The cerebral cortex in humans contains 16 billion neurons and in mice 14 million neurons (I), and each neuron harbors thousands of synapses (2). Of the billions of cortical synapses of adult mice, ~80% are excitatory, and the majority of these are on dendritic spines (3). Spine size is tightly correlated with synaptic strength (3, 4); the area of the post-synaptic density (PSD), the area of the axon-spine interface (ASI), and the volume of the spine head (HV) are strongly correlated among themselves and with the number of vesicles in the presynapse (5–8), the number of synaptic AMPA receptors (AMPArs (9)), and the amplitude of AMPAR-mediated synaptic currents (10, II) (also called “spines” (3)) were annotated, including spines forming synapses and a minority that lacked synapses (~13% of all protrusions) (table S1). Across all mice, 168 dendritic segments were segmented (101 in M1 and 67 in S1) (Fig. 1D and fig. S1), for a total of 8427 spines, of which 7149 formed a synapse. Synapses were defined by the presence of a presynaptic bouton with at least two synaptic vesicles within a 50-nm distance from the cellular membrane facing the spine, a visible synaptic cleft, and a PSD. In spines forming a synapse, ASI, HV—as well as vesicles, tubules, and multivesicular bodies (MVBS) that together form the nonsmooth endoplasmic reticulum (non- SER) compartment (20)—and the spine apparatus were segmented (Fig. 1E and F) (supplementary materials, materials and methods). After excluding incomplete synapses, 6920 spines with a synapse contributed to the final analysis (tables S1 and S2).

ASI and PSD are strongly correlated with each other, and both become larger after synaptic potentiation (6–8). We focused on ASI—the surface of direct contact between axonal bouton and spine—as a structural measure of synaptic strength because in SBEM images, its exact borders are easier to identify than those of the PSD (21). First, we asked whether ASI sizes change as a function of wake and sleep using a linear mixed-effects (LME) model that included mouse and dendrite as random effects, condition (SW, EW, and S), and brain region (S1 and M1) as categorical fixed effects, and dendrite diameter as a linear fixed effect. Condition had a strong effect on ASI ($\phi^2 = 10.159, df = 2, P = 0.0062$), which did not interact with either brain region or dendrite diameter. Post hoc analysis (adjusted for multiple comparisons) found that ASI sizes after sleep were reduced on average by 18.9% relative to spontaneous wake ($P = 0.001$ and by 17.5% compared with enforced wake ($P = 0.046$) (Fig. 2A and supplementary materials, materials and Methods, LME model for ASI). Spontaneous and enforced wake did not differ (SW versus EW, −1.7%; $P = 0.957$). Thus, ASI sizes decrease with sleep on average by ~18% relative to both spontaneous and enforced wake, independent of time of day. There was instead no difference across groups in the distribution of dendrite ($P = 0.262$) and mitochondrial ($P = 0.445$) diameters, ruling out overall tissue shrinkage after sleep (fig. S2).

Consistent with the range of PSD and spine sizes in mouse somatosensory and auditory cortex (22, 23), the distribution of ASI sizes in our S1 and M1 samples was log-normal (Fig. 2B), a feature

---

1Department of Psychiatry, University of Wisconsin—Madison, 6001 Research Park Boulevard, Madison, WI 53719, USA.
2Department of Experimental and Clinical Medicine, Section of Neuroscience and Cell Biology, Università Politecnica delle Marche, Ancona, Italy.
3National Center for Microscopy and Imaging Research, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92039, USA.
4Department of Neurosciences, School of Medicine, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92039, USA.
*Corresponding author. Email: ccirelli@wisc.edu (C.C.); gtononi@wisc.edu (G.T.)
thought to emerge from multiplicative dynamics (22). On the log scale, the S group showed an overall shift to the left relative to the SW and EW groups, suggesting that the decrease in ASI during sleep obeyed a scaling relationship (Fig. 2B, inset and C). Formal testing (supplementary materials, materials, and methods) confirmed scaling, when sleep was compared with either spontaneous wake (average scaling ~20.1%, \( P = 0.784 \)) or enforced wake (average scaling ~19.1%, \( P = 0.648 \)). Monte Carlo simulations on bootstrapped data (supplementary materials, materials, and methods) suggested that the change in ASI sizes between wake and sleep is not consistent with uniform scaling across all synapses but rather with selective scaling, in which a fraction of all synapses scales and the remaining portion does not. Of the models tested, the best fit was provided when the likelihood of scaling decreased quadratically with increasing ASI size (Fig. 2D).

This model fitted the actual data best, assuming that a majority of all synapses (>80%) would scale and that a minority (<20%) would be less likely to do so (Fig. 2D).

Do morphological features of synapses predict the likelihood of scaling? Given the results in Fig. 2D, we asked whether distinguishing between small to medium synapses (bottom 80%) versus large synapses would predict scaling versus no scaling. This distinction based on size was significant (\( P = 0.009 \); small ASI: S versus SW = –11.9%, \( P = 0.0002 \); S versus EW = –12.5%, \( P = 0.0001 \); large ASI: S versus SW = +0.7%, \( P = 0.999 \); S versus EW = +2.0%, \( P = 0.994 \)) (Fig. 3A) and robust for scaling fractions around 80% (supplementary materials, materials and methods). These results indicate that the ASIs of most synapses decrease during sleep in a manner proportional to their size, and that the largest 20% of spines are less likely to scale.

Plastic changes may preferentially occur in spines that contain recycling endosomes (24), whose presence reflects increased turnover of membranes, glutamate receptors, and other proteins that are essential to support activity-dependent structural changes (13, 24, 25). Indeed, only spines containing vesicles, tubules, and multivesicular bodies (MVBs), most of which are considered of endosomal origin (20), showed significant scaling (\( P = 0.00003 \); vesicles/tubules, +: S versus SW = –25.0%, \( P = 0.00001 \); S versus EW = –20.9%, \( P = 0.0008 \); vesicles/tubules, -: S versus SW = –2.9%, \( P = 0.985 \); S versus EW = –2.8%, \( P = 0.989 \)) (Fig. 3, B and C).

A spine’s structural plasticity may be constrained by the overall spine density of its dendritic branch (26). Although synaptic plasticity by itself was unaffected by wake and sleep (\( P = 0.761 \)), it interacted with the effect of sleep on ASI (\( P = 0.038 \)); the ASI decrease with sleep was largest in less spiny dendrites (S versus SW = –36.4%; S versus EW = –25.3%) and smallest in dendrites with higher synaptic density (S versus SW = 7.8%; S versus EW = –8.2%) (Fig. 3D).

In contrast, ASI decreased with sleep both in the spines with a spine apparatus (27)—a specialization of SER involved in calcium regulation and synthesis of transmembrane proteins—and in those without it (Fig. 3E) (28). Although spines facing an axonal bouton with one or more mitochondria were larger than spines lacking an axonal mitochondrion, scaling again occurred in both groups of spines (Fig. 3F). ASI size scales down between wake and sleep in small- and medium-sized synapses (~80% of the total population) but is less likely to do so in synapses that are large (~20%) or in spines that contain no endosomes and is less marked in highly spiny dendrites.

Because HV is also strongly correlated with synaptic strength, we investigated changes in HV as a function of wake and sleep using a linear model that included the same random and fixed effects as for ASI (supplementary materials, materials, and methods, LME model for HV). Results were consistent with those with ASI (\( \chi^2 = 6.942 \), de Vivo et al., Science 355, 507–510 (2017) 3 February 2017
Fig. 2. ASI size declines in sleep according to a scaling relationship. (A) (Left) Visualization of ASIs in one dendrite. Scale bar, 2.5 μm. (Right) Effect of condition. ASI size decreases in sleep (blue) relative to both spontaneous wake (orange) and enforced wake (red). ASI size is shown for all synapses, each represented by one dot. **P < 0.01. (B) Log-normal distribution of ASI sizes in the three experimental groups. (Inset) Same on a log scale. (C) The decrease in ASI size during sleep is due to scaling. (D) Monte Carlo simulations comparing different models of scaling. Size-dependent selective scaling (green) fits the actual data better than uniform scaling (asterisk) or selective scaling independent of size (brown) (supplementary materials, materials and methods).

Fig. 3. Scaling of ASI size is selective. (A) The effect of sleep is present in small to medium synapses (80% of all synapses) but not in the largest ones (20% of all synapses). (B) The effect of sleep is present in spines with non-SER elements (vesicles, tubules, and multivesicular bodies, labeled “vesicles/tubules”). (Top right) A multivesicular body (arrowhead) and a coated vesicle (asterisk). (Bottom right) A non-SER tubule (arrowhead). (C) the ASI decrease during sleep in spines with vesicles/tubules is due to scaling. (D) The decline of ASI size in sleep is greatest in the dendrites with the lowest synaptic density (range, 0.17 to 1.24/μm²). At the average value of synaptic density (vertical line; 0.70/μm²), the mean overall decrease is ~17.3% (S versus SW =17.4%, P = 0.002; S versus EW =17.3%, P = 0.002). (E and F) ASI size declines in sleep independently of the presence of spine apparatus (asterisk) or mitochondrial in the axonal bouton (arrowheads). Scale bars, 500 nm. In all experimental groups, spines containing a spine apparatus or facing an axonal bouton with mitochondria are larger than spines lacking these elements. **P < 0.01; ***P < 0.001.
The scaling of synaptic size is not uniform, which is consistent with the requirement that learning during wake must potentiate synapses selectively and with the hypothesis that selective renormalization during sleep favors memory consolidation, integration, and “smart” forgetting (16). We do not know how scaling is apportioned between wake and sleep. During wake, there may be a selective up-scaling of a smaller proportion of synapses because learning is limited to a particular environment (37), whereas down-scaling during sleep may be broader because the brain can sample its memories comprehensively and fairly when it is offline (16). We also cannot rule out that a few synapses may up-scale in sleep (16, 17). Future studies labeling individual plastic events in the same synapses over wake and sleep may shed light on this issue. It will also be important to assess which molecular mechanisms are involved in the selective scaling of excitatory synapses in wake and sleep and to evaluate possible changes in inhibitory synapses (32).

We found that the synapses that most likely escape scaling are those that are large, those that lack endosomes, as well as those in crowded dendritic branches. These features may represent structural markers (besides molecular markers (33)) of synapses and associated memory circuits that are either committed or relatively stable despite the profound daily remodeling. We do not know, however, to what extent and over which time scale synapses may switch between this smaller pool of stronger, more stable synapses and the larger pool of weaker, more plastic synapses. An intriguing question is whether the subset of strong and stable synapses may originate preferentially from neurons at the top of the log-normal distribution of firing rates (34), whose level of activity seems to remain stable when the environment changes (35), or perhaps from neurons located in a specific layer (2/3 or 5).

REFERENCES AND NOTES


ACKNOWLEDGMENTS

We thank E. Christensen, P. Horvath, S. Koebe, S. Loschky, R. Massopust, M. Nagai, and A. Schroder for their contribution to the manual segmentation of SBEM images. This work was supported by NIH grants DP 1OD579/1 (G.T.), 1R01MH091326 (G.T.), 1RC1MH092931 (G.T. and C.C.), 1P01NS083514 (G.T. and C.C.), and P41GM103412 for support of the National Center for Microscopy and Imaging research (M.H.E.). The other authors have no competing interests. Data (ASI and HV measures) are available at http://centersforleapconsciousness.med.wisc.edu.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/355/6324/507/suppl/DC1
Materials and Methods.
Fig. S1 to S3
Tables S1 and S2
References (36–53)
19 July 2016; accepted 20 October 2016
10.1126/science.aah5982

Ultrastructural evidence for synaptic scaling across the wake/sleep cycle
Luisa de Vivo, Michele Bellesi, William Marshall, Eric A. Bushong, Mark H. Ellisman, Giulio Tononi and Chiara Cirelli

Science 355 (6324), 507-510.
DOI: 10.1126/science.aah5982

Synapse remodeling during sleep
General activity and information processing while an animal is awake drive synapse strengthening. This is counterbalanced by weakening of synapses during sleep (see the Perspective by Acsády). De Vivo et al. used serial scanning electron microscopy to reconstruct axon-spine interface and spine head volume in the mouse brain. They observed a substantial decrease in interface size after sleep. The largest relative changes occurred among weak synapses, whereas strong ones remained stable. Diering et al. found that synapses undergo changes in synaptic glutamate receptors during the sleep-wake cycle, driven by the immediate early gene Homer1a. In awake animals, Homer1a accumulates in neurons but is excluded from synapses by high levels of noradrenaline. At the onset of sleep, noradrenaline levels decline, allowing Homer1a to move to excitatory synapses and drive synapse weakening. Science, this issue p. 457, p. 507; see also p. 511