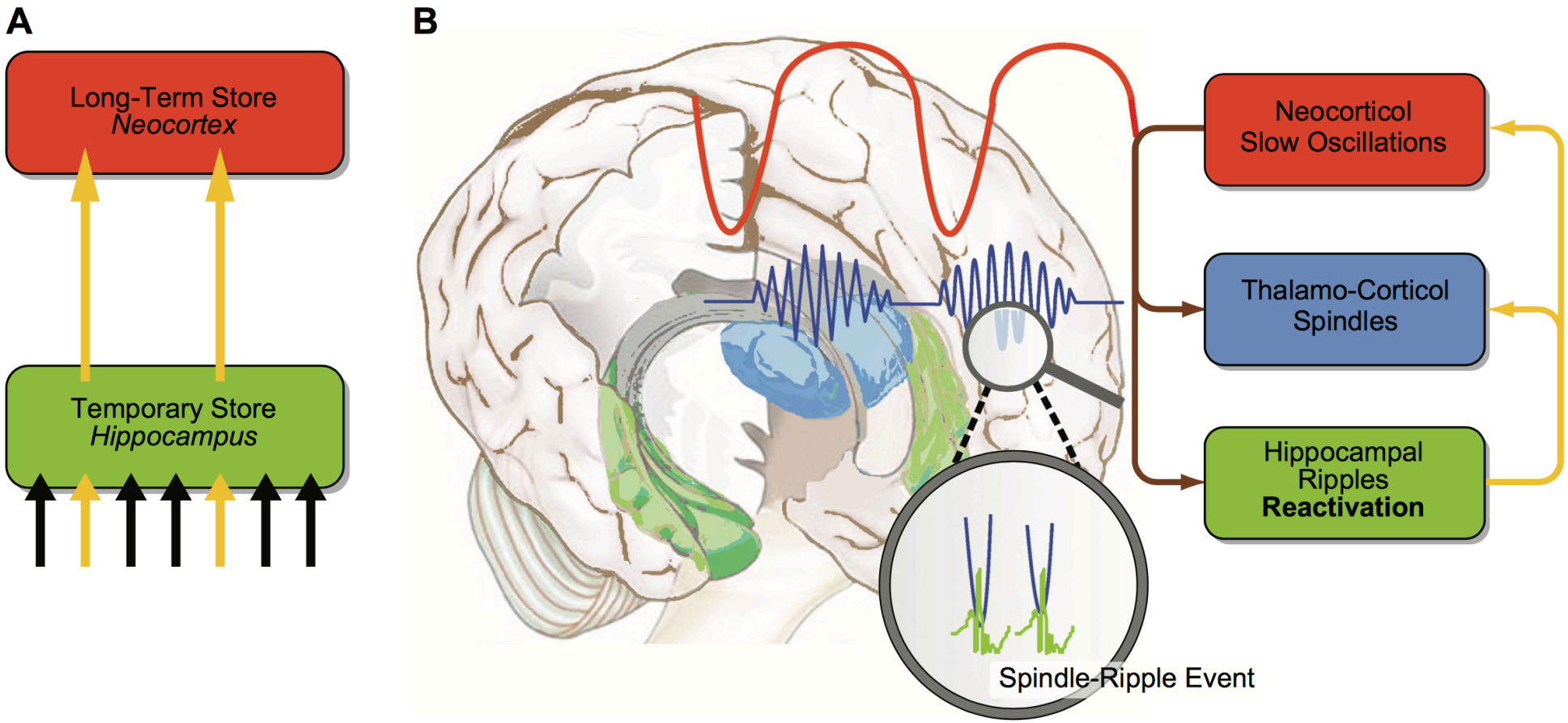


A model of active system consolidation during sleep. *A*: during SWS, memories newly encoded into a temporary store (i.e., the hippocampus in the declarative memory system) are repeatedly reactivated, which drives their gradual redistribution to the long-term store (i.e., the neocortex). *B*: system consolidation during SWS relies on a dialogue between neocortex and hippocampus under top-down control by the neocortical slow oscillations (red). The depolarizing up phases of the slow oscillations drive the repeated reactivation of hippocampal memory representations together with sharp wave-ripples (green) and thalamo-cortical spindles (blue). This synchronous drive allows for the formation of spindle-ripple events where sharp wave-ripples and associated reactivated memory information becomes nested into succeeding troughs of a spindle (shown at larger scale).

Rasch B, Born J. About Sleep's Role in Memory. Physiol Rev 93: 681–766, 2013.

Ultrastructural evidence for synaptic scaling across the wake/sleep cycle

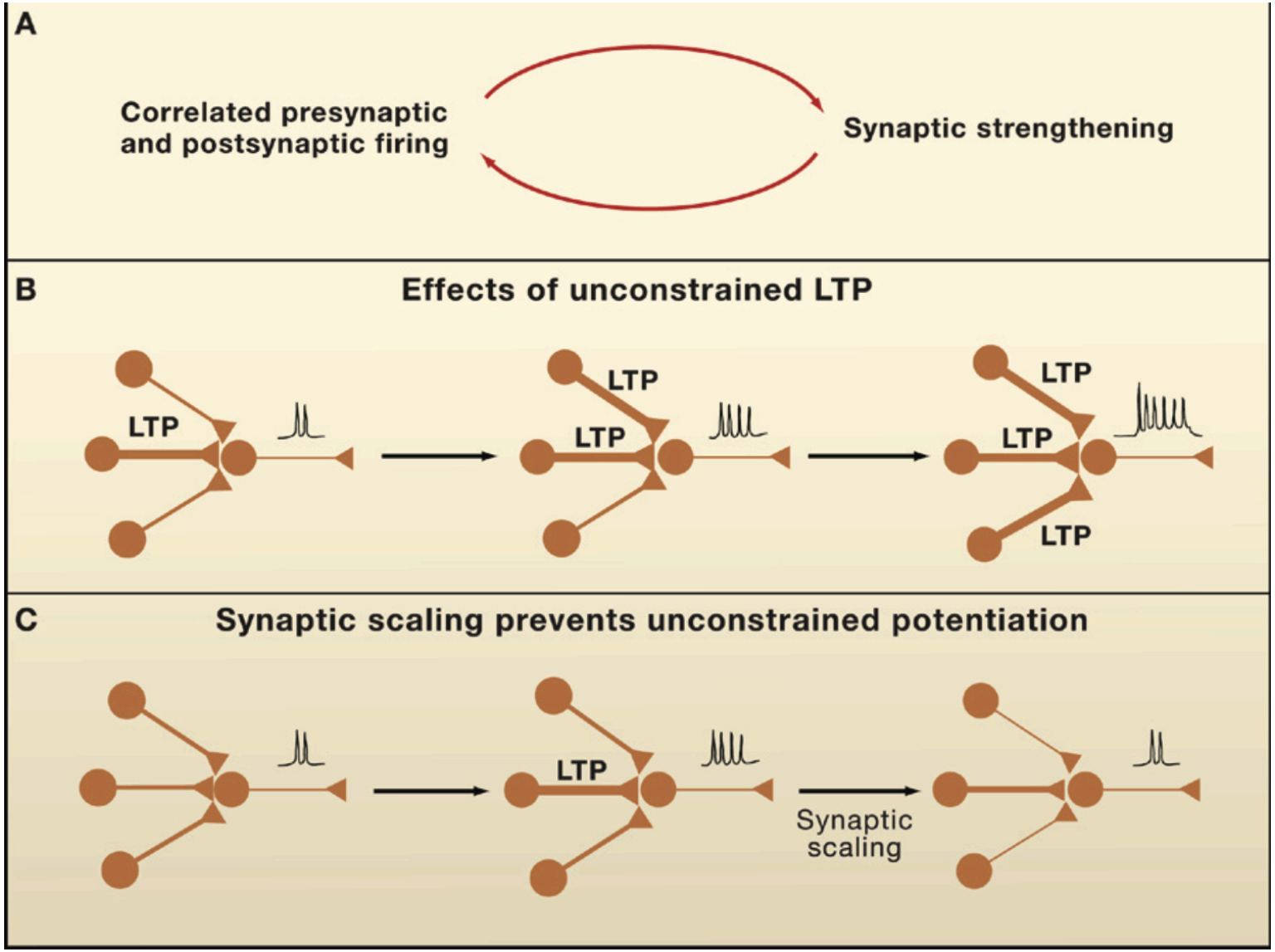
Luisa de Vivo,[1] Michele Bellesi,[1,2] William Marshall,[1] Eric A. Bushong,[3] Mark H. Ellisman,[3,4] Giulio Tononi,[1*] Chiara Cirelli[1*]

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.

Vivo, L., Bellesi, M., Marshall, W., Bushong, E., Ellisman, M., Tononi, G., & Cirelli, C. (2017). Ultrastructural evidence for synaptic scaling across the wake/ sleep cycle. Science (New York, N.Y.), 355(6324), 507–510.

The cerebral cortex in humans contains 16 billion neurons and in mice 14 million neurons (1), 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 postsynaptic 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, 11).

1. S. Herculano-Houzel, Front. Hum. Neurosci. 3, 31 (2009).
2. Y. Tang, J. R. Nyengaard, D. M. De Groot, H. J. Gundersen, Synapse 41, 258–273 (2001).
3. A. Holtmaat, K. Svoboda, Nat. Rev. Neurosci. 10, 647–658 (2009).
4. J. Nishiyama, R. Yasuda, Neuron 87, 63–75 (2015).
5. K. M. Harris, J. K. Stevens, J. Neurosci. 9, 2982–2997 (1989).
6. N. L. Desmond, W. B. Levy, Brain Res. 453, 308–314 (1988).
7. P. A. Buchs, D. Muller, Proc. Natl. Acad. Sci. U.S.A. 93, 8040–8045 (1996).
8. C. E. Cheetham, S. J. Barnes, G. Albieri, G. W. Knott, G. T. Finnerty, Cereb. Cortex 24, 521–531 (2014).
9. Y. Katz et al., Neuron 63, 171–177 (2009).
10. M. Matsuzaki et al., Nat. Neurosci. 4, 1086–1092 (2001).
11. M. Bosch et al., Neuron 82, 444–459 (2014).



Mechanisms of Synaptic Plasticity Are Potentially Destabilizing

(A) Correlated presynaptic and postsynaptic firing induces long-term potentiation (LTP), which then allows the presynaptic neuron to drive the postsynaptic neuron more strongly. This increases the correlation between presynaptic and postsynaptic activation, which drives more LTP, and so on in an unconstrained positive feedback cycle.
(B) Unconstrained LTP will lose synapse specificity, because when one input undergoes LTP and drives the postsynaptic neuron more strongly, it makes it easier for other inputs to make the postsynaptic neuron re, and they begin to undergo LTP as well.
(C) Homeostatic synaptic scaling prevents this runaway potentiation. When LTP of one input in-creases postsynaptic ring, synaptic scaling will reduce the strength of all synaptic inputs until the ring rate returns to control levels. Note that synaptic strengths are reduced proportionally, so that the relative strength of the potentiated synapse remains the same.

Turrigiano, G. (2008). The Self-Tuning Neuron: Synaptic Scaling of Excitatory Synapses. Cell, 135(3), 422–435.

The Synaptic Homeostasis Hypothesis (SHY)

Changes in synaptic strength are the primary mechanisms mediating learning and memory (12, 13). Synaptic potentiation and depression must be balanced to avoid either saturation or obliteration of neural signaling and memory traces (14), and it is usually assumed that overall synaptic strength is regulated throughout learning (15). **The synaptic homeostasis hypothesis (SHY)** (16) argues, however, that owing to energy and signaling requirements, learning should occur primarily through synaptic potentiation during wake, leading to a net increase in synaptic strength. *Overall synaptic renormalization by net weakening should occur during sleep, when animals are disconnected from the environment. The reason is that spontaneous neural activity can sample memories in a comprehensive and fair manner only if the brain is offline, without being at the mercy of current environmental inputs. Sleep can thus promote the acquisition, consolidation, and integration of new information as well as restore cellular function (16).*

12. D. E. Feldman, Annu. Rev. Neurosci. 32, 33–55 (2009).
13. R. L. Huganir, R. A. Nicoll, Neuron 80, 704–717 (2013).
14. C. von der Malsburg, Kybernetik 14, 85–100 (1973).
15. ***Chistiakova, M., Bannon, N., Chen, J.-Y., Bazhenov, M., & Volgushev, M. (2015). Homeostatic role of heterosynaptic plasticity: models and experiments. Frontiers in Computational Neuroscience, 9. doi: 10.3389/fncom.2015.00089***
16. ***Tononi, G., & Cirelli, C. (2014). Sleep and the Price of Plasticity: From Synaptic and Cellular Homeostasis to Memory Consolidation and Integration. Neuron, 81(1), 12–34***

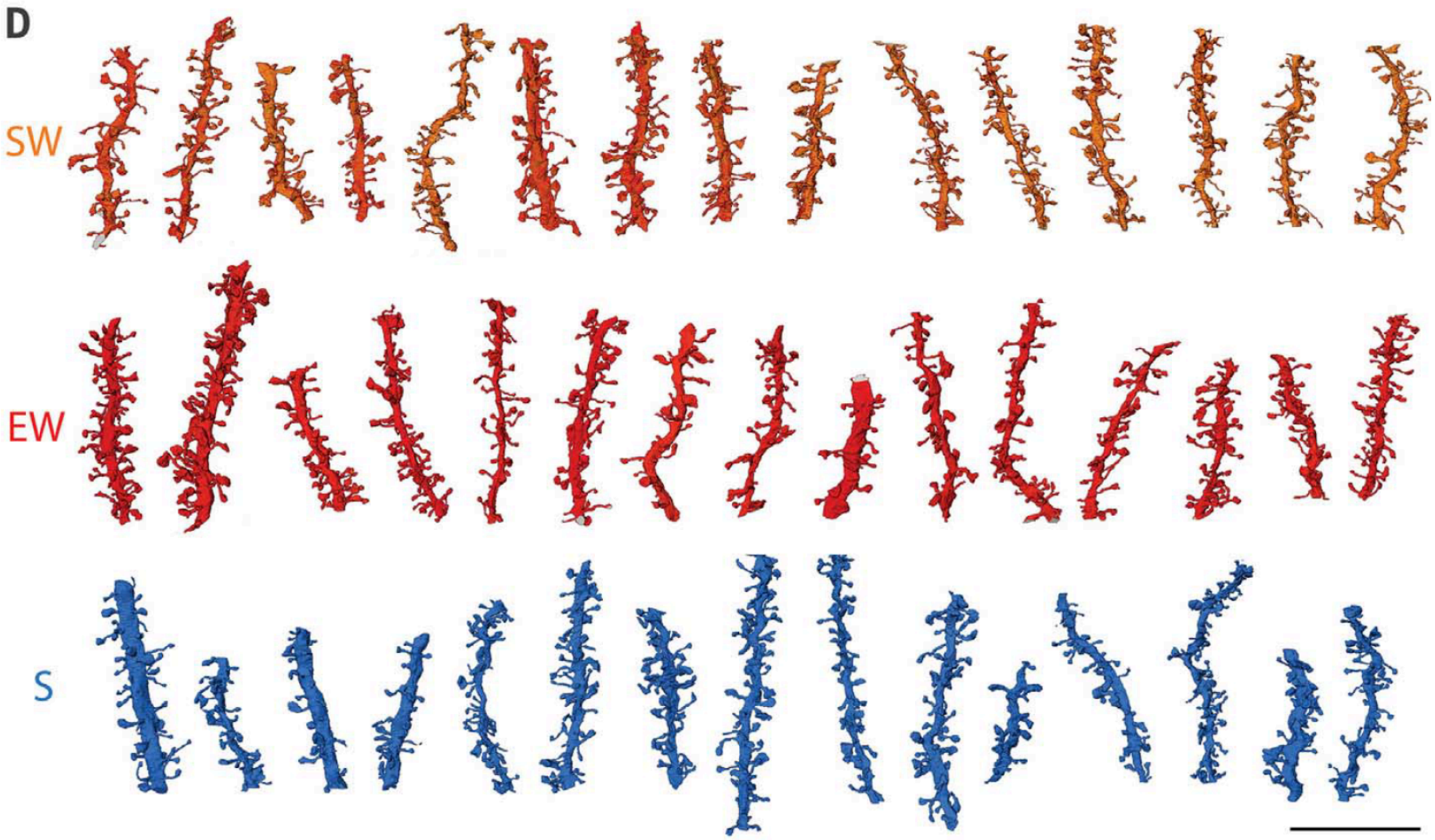
SHY makes an intriguing prediction:
Billions of cortical excitatory synapses should increase in size after wake and decrease after sleep, independent of circadian time. Furthermore, although synaptic renormalization should affect a majority of synapses, it should also be selective, to allow for both stability and plasticity (16–18).

16. *Vivo, L., Bellesi, M., Marshall, W., Bushong, E., Ellisman, M., Tononi, G., & Cirelli, C. (2017). Ultrastructural evidence for synaptic scaling across the wake/sleep cycle. Science (New York, N.Y.),355(6324), 507–510.*

17. ***Rasch B, Born J. About Sleep's Role in Memory. Physiol Rev 93: 681–766, 2013.***

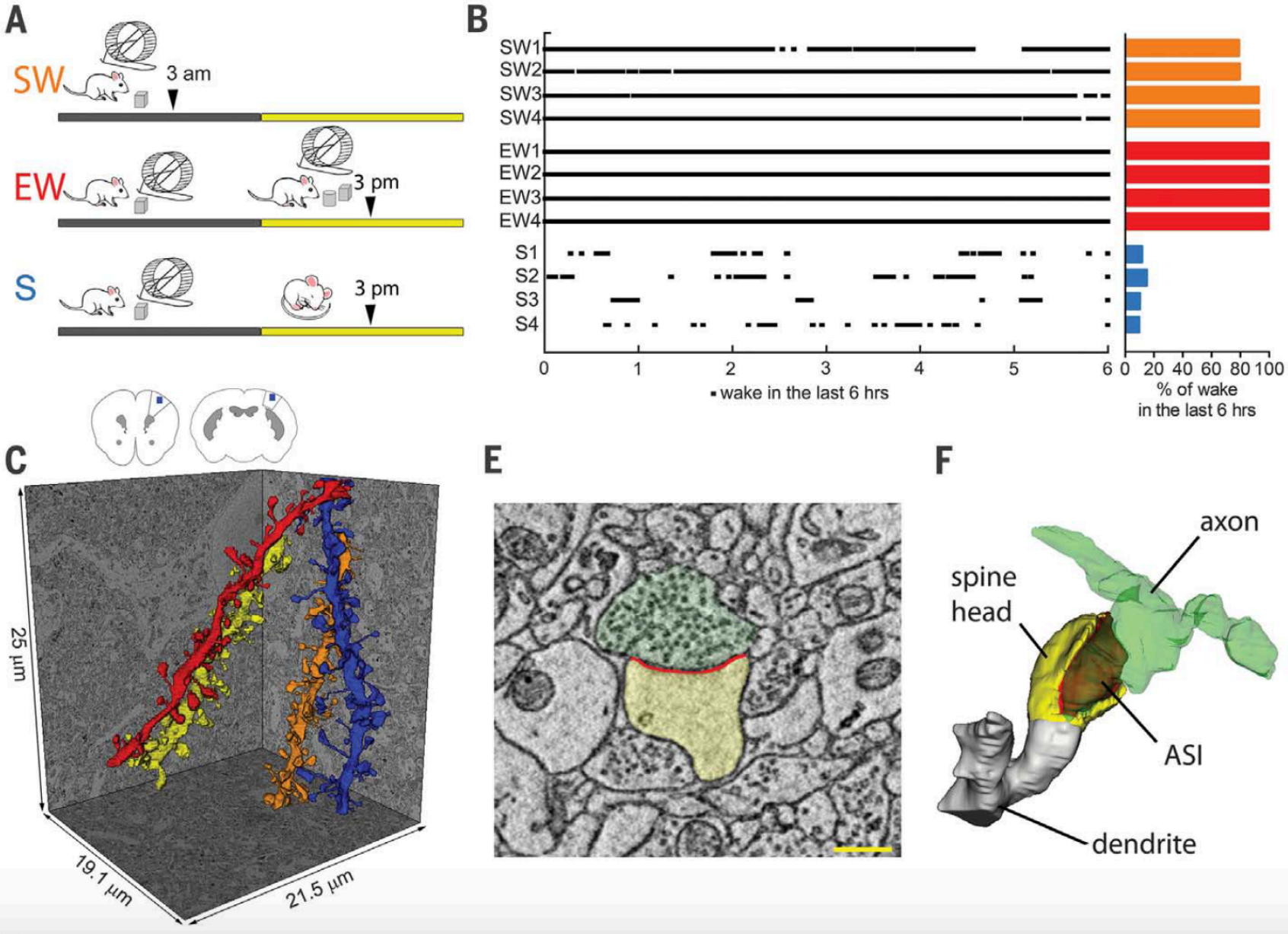
18. W. C. Abraham, A. Robins, Trends Neurosci. 28, 73–78 (2005).

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 (postsynaptic density).



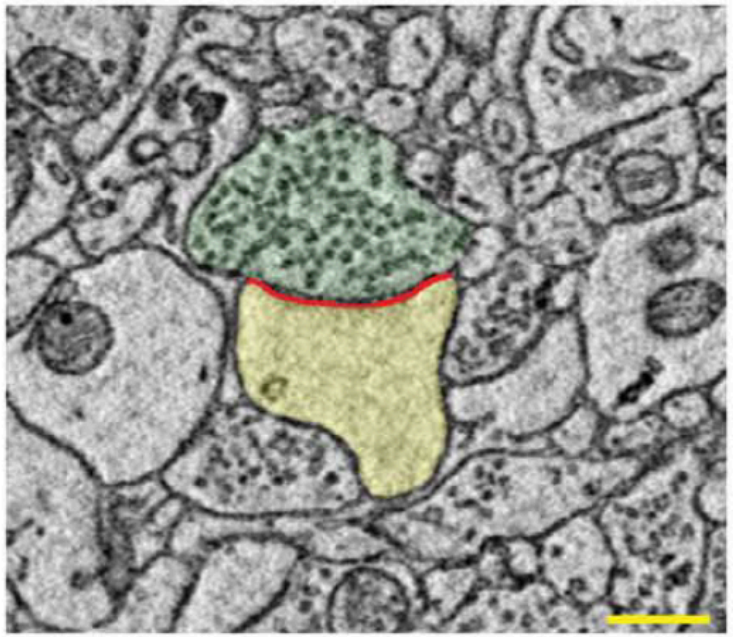
(D) Some of the dendritic segments from SW, EW, and S mice reconstructed in this study .Scale bar, 15 mm

Vivo, L., Bellesi, M., Marshall, W., Bushong, E., Ellisman, M., Tononi, G., & Cirelli, C. (2017). Ultrastructural evidence for synaptic scaling across the wake/sleep cycle. Science 355(6324), 507–510.



Experimental groups and SBEM segmentation of cortical synapses.
(A) The three experimental groups. SW, spontaneous wake at night; EW, wake during the day enforced by exposure to novel objects; S, sleep during the day. Arrowheads indicate time of brain collection.
(B) Percent of wake in each mouse (four mice per group) during the last 6 hours before brain collection
(C) Schematic representation of mouse primary motor (M1, left) and somatosensory (S1, right) cortex, with the region of SBEM data collection indicated in layer 2 (blue box), and reconstruction of four spiny dendritic segments in S1.

E



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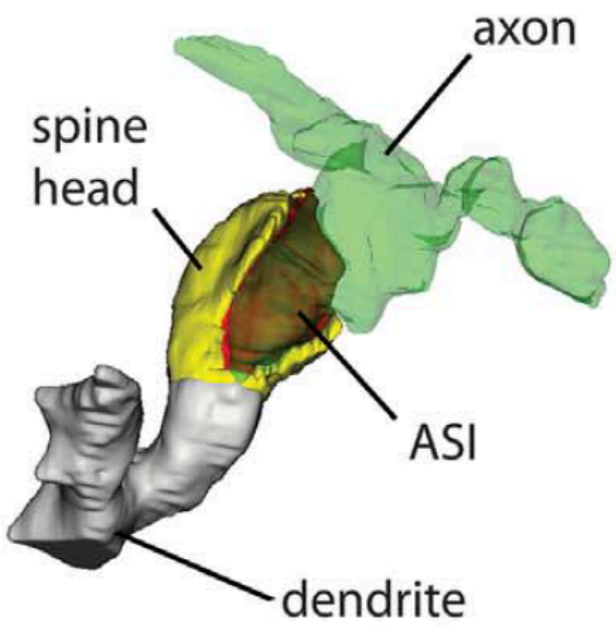
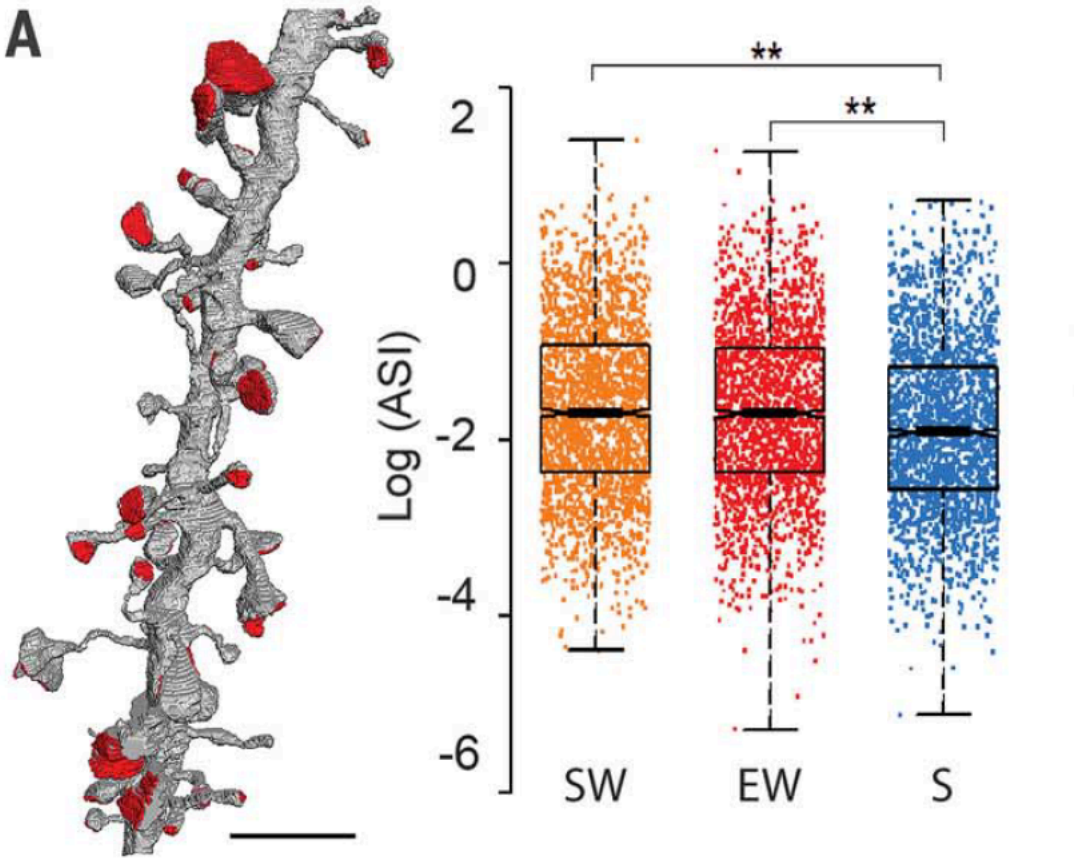


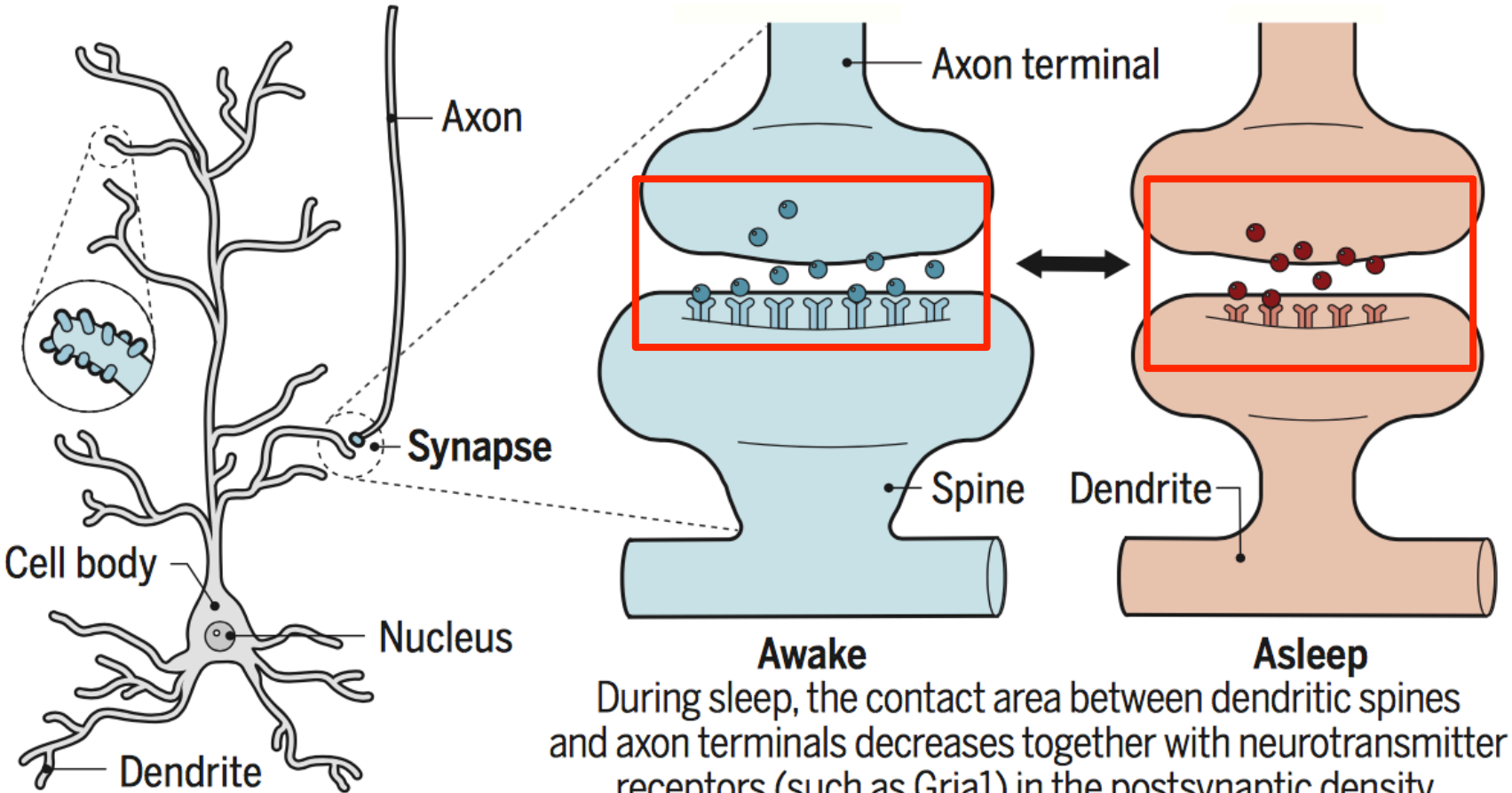
(E and F) Raw image of a cortical spine containing a synapse and its 3D reconstruction.
Spine head is in yellow, ASI (axon-spine interface) is in red, and axonal bouton is in green.
Scale bar, 350 nm.

(A) ASI (axon-spine interface) 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 = 0.003)

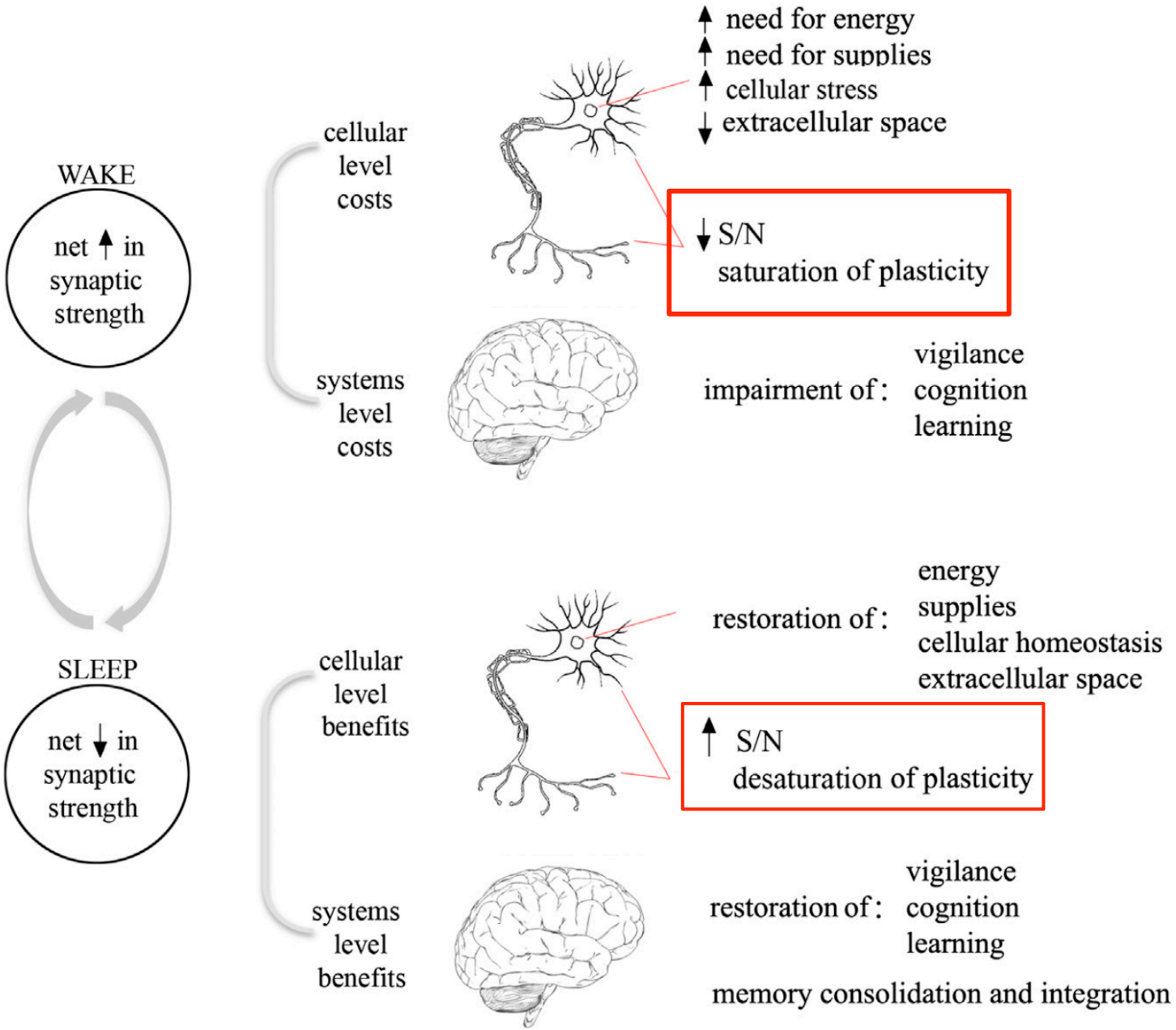


Spine dynamics

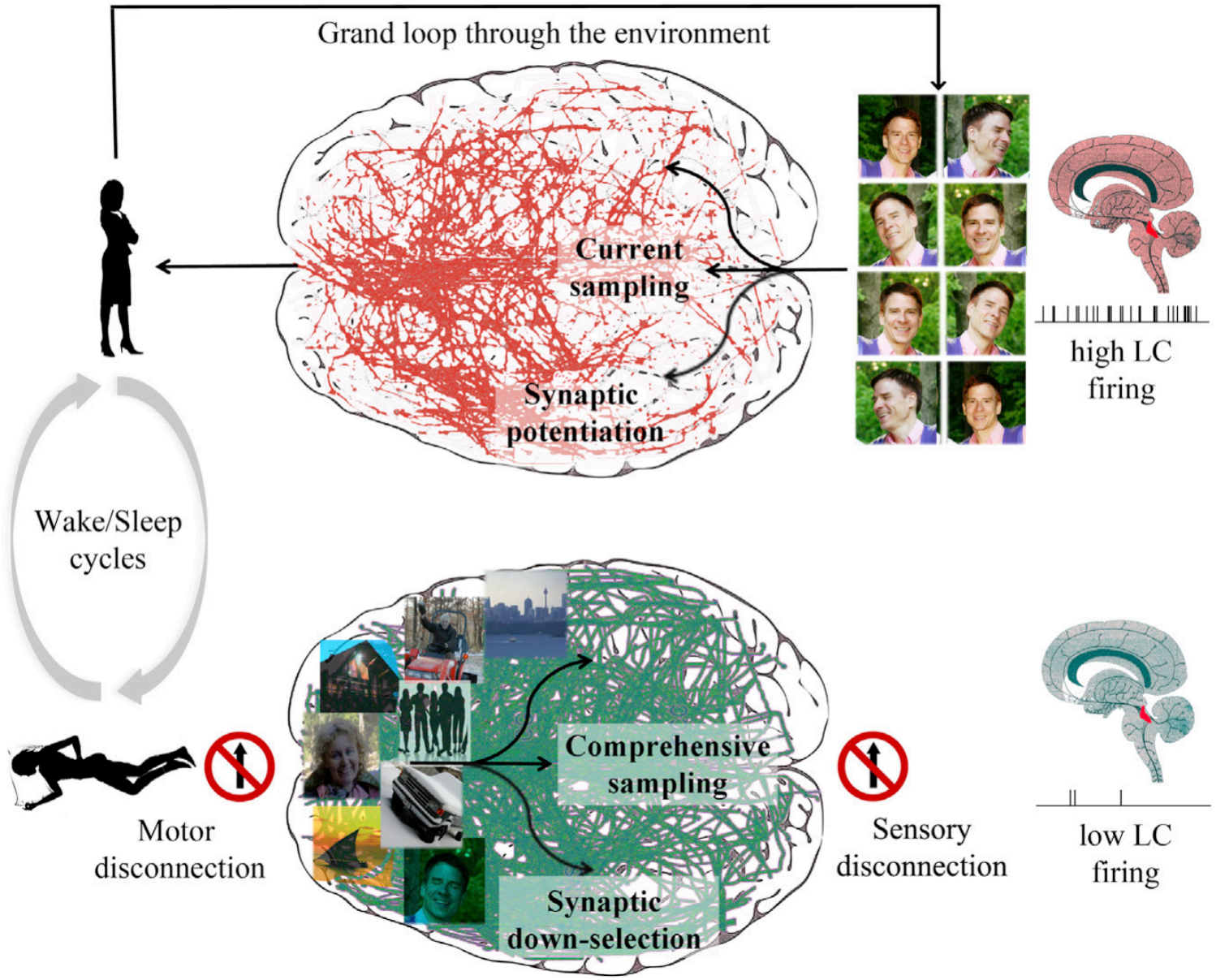
Synaptic connections are altered between sleep and wake states.



During sleep, the contact area between dendritic spines and axon terminals decreases together with neurotransmitter receptors (such as Gria1) in the postsynaptic density.



Tononi, G., & Cirelli, C. (2014). Sleep and the Price of Plasticity: From Synaptic and Cellular Homeostasis to Memory Consolidation and Integration. Neuron, 81(1), 12–34

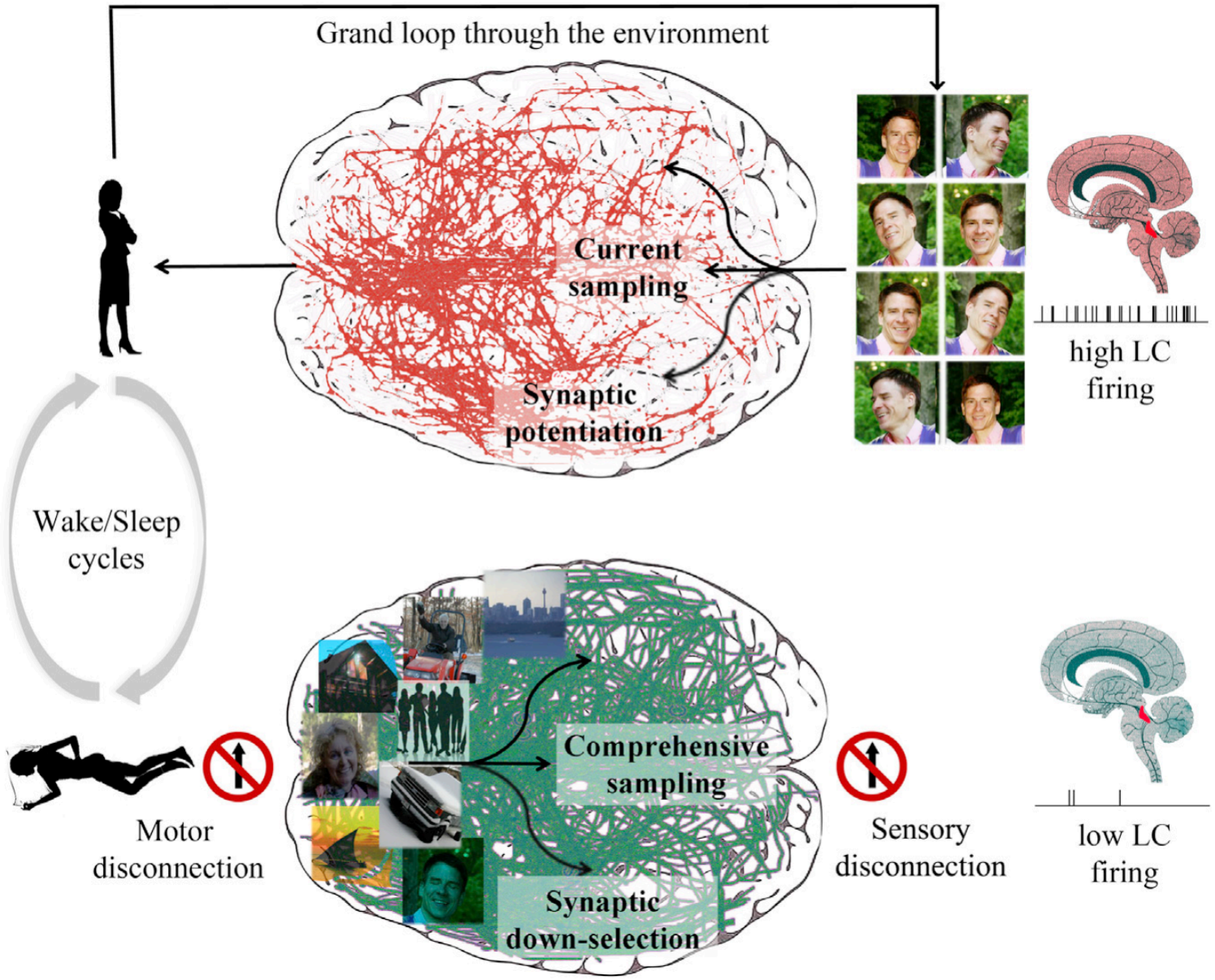


SHY, Wake/Sleep Cycles, and the Plasticity-Stability Dilemma

Top: during wake the brain interacts with the environment (grand loop) and samples a limited number of inputs dictated by current events (current sampling, here represented by a new acquaintance). High levels of neuromodulators, such as noradrenaline released by the locus coeruleus (LC), ensure that suspicious coincidences related to the current sampling percolate through the brain and lead to synaptic potentiation.

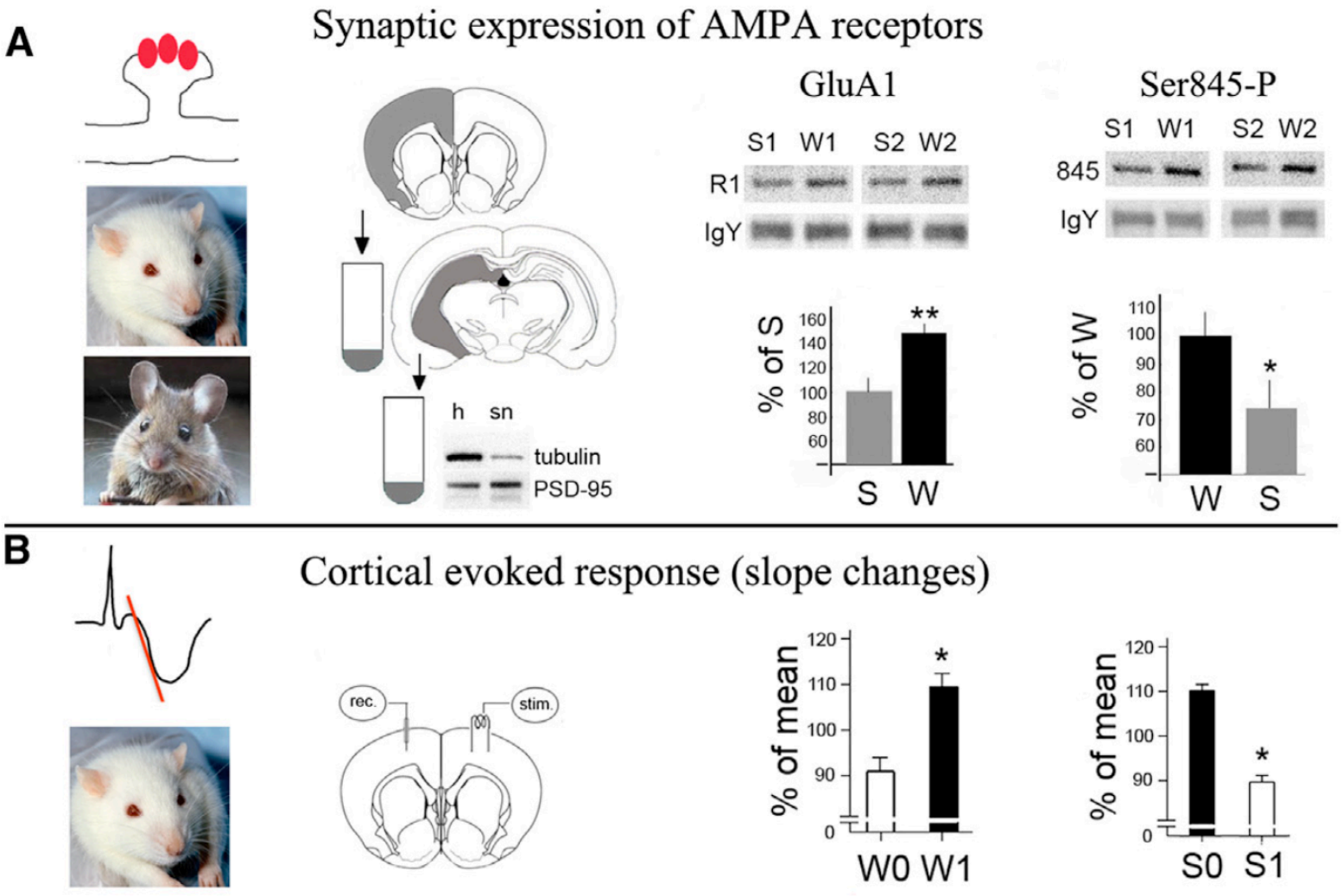
Bottom: during sleep, when the brain is disconnected from the environment on both the sensory and motor sides, spontaneous activity permits a comprehensive sampling of the brain's knowledge of the environment, including old memories about people, places, etc.

Tononi, G., & Cirelli, C. (2014). Sleep and the Price of Plasticity: From Synaptic and Cellular Homeostasis to Memory Consolidation and Integration. Neuron, 81(1), 12–34



Low levels of neuromodulators, combined with the synchronous, ON and OFF firing pattern of many neurons during NREM sleep events such as slow waves, spindles, and sharp- wave ripples, are conducive to synaptic down- selection: synapses belonging to the fittest circuits, those that were strengthened repeatedly during wake and/or are better integrated with older memories, are protected and survive. By contrast, synapses belonging to circuits that were only rarely activated during wake and/or fit less well with old memories, are progressively depressed and eventually eliminated over many wake/sleep cycles. The green lines in the sleeping brain (right), taken from Murphy et al. (2009), illustrate the propagation of slow waves during NREM sleep, as established using high-density EEG and source modeling.

Tononi, G., & Cirelli, C. (2014). Sleep and the Price of Plasticity: From Synaptic and Cellular Homeostasis to Memory Consolidation and Integration. Neuron, 81(1), 12–34



Evidence Supporting SHY

(A) Experiments in rats and mice show that the number and phosphorylation levels of GluA1-AMPARs increase after wake (data from rats are from Vyazovskiy et al., 2008. Electrophysiological analysis of cortical evoked responses using electrical stimulation (in rats, from Vyazovskiy et al., 2008) and TMS (in humans, from Huber et al., 2013) shows increased slope after wake and decreased slope after sleep. In

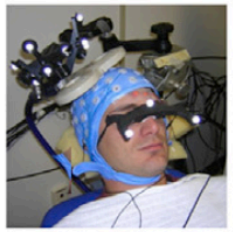
(B) W0 and W1 indicate onset and end of 4 hr of wake; S0 and S1 indicate onset and end of 4 hr of sleep, including at least 2 hr of NREM sleep.

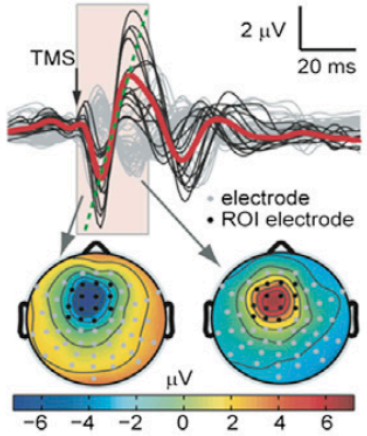
Tononi, G., & Cirelli, C. (2014). Sleep and the Price of Plasticity: From Synaptic and Cellular Homeostasis to Memory Consolidation and Integration. Neuron, 81(1), 12–34

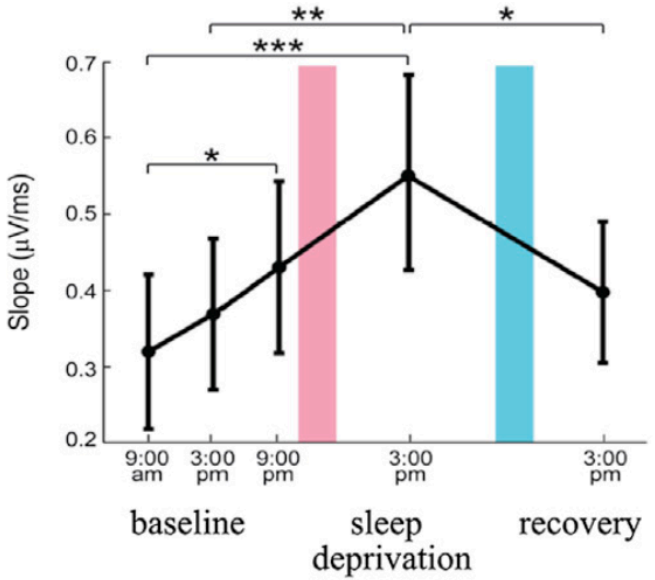
Vyazovskiy, V., Cirelli, C., Pfister-Genskow, M., Faraguna, U., & Tononi, G. (2008). Molecular and electrophysiological evidence for net synaptic potentiation in wake and depression in sleep. Nature Neuroscience, 11(2), 200–208.

Huber, R., Maki, H., Rosanova, M., Casarotto, S., Canali, P., Casali, A.G., Tononi, G., and Massimini, M. (2013). Human cortical excitability increases with time awake. Cereb. Cortex 23, 332–338.

B’

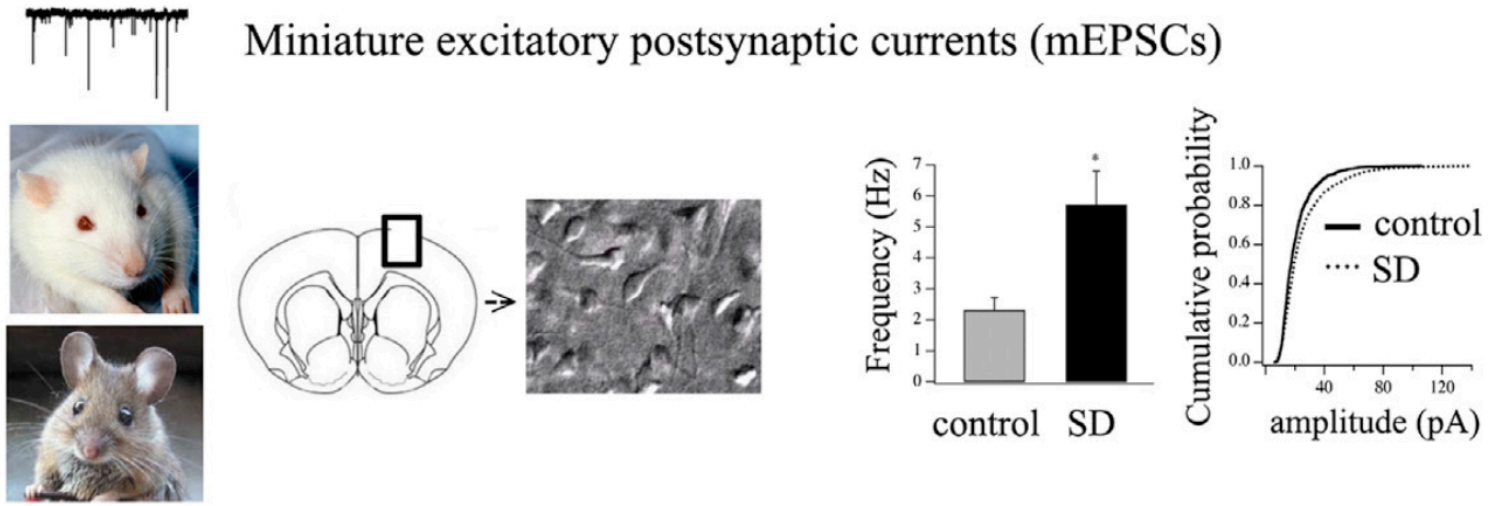






B’, pink and blue bars indicate a night of sleep deprivation and a night of recovery sleep, respectively.
(B’’) In vitro analysis of mEPSCs in rats and mice shows increased frequency and amplitude of mEPSCs after wake and sleep deprivation (SD) relative to sleep (control).

B’’



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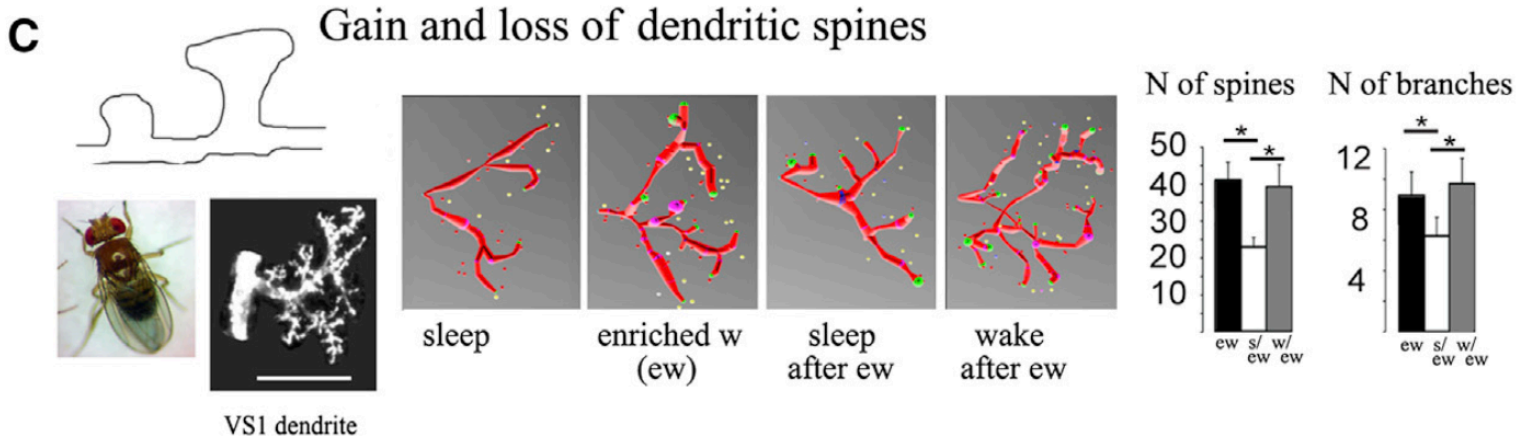
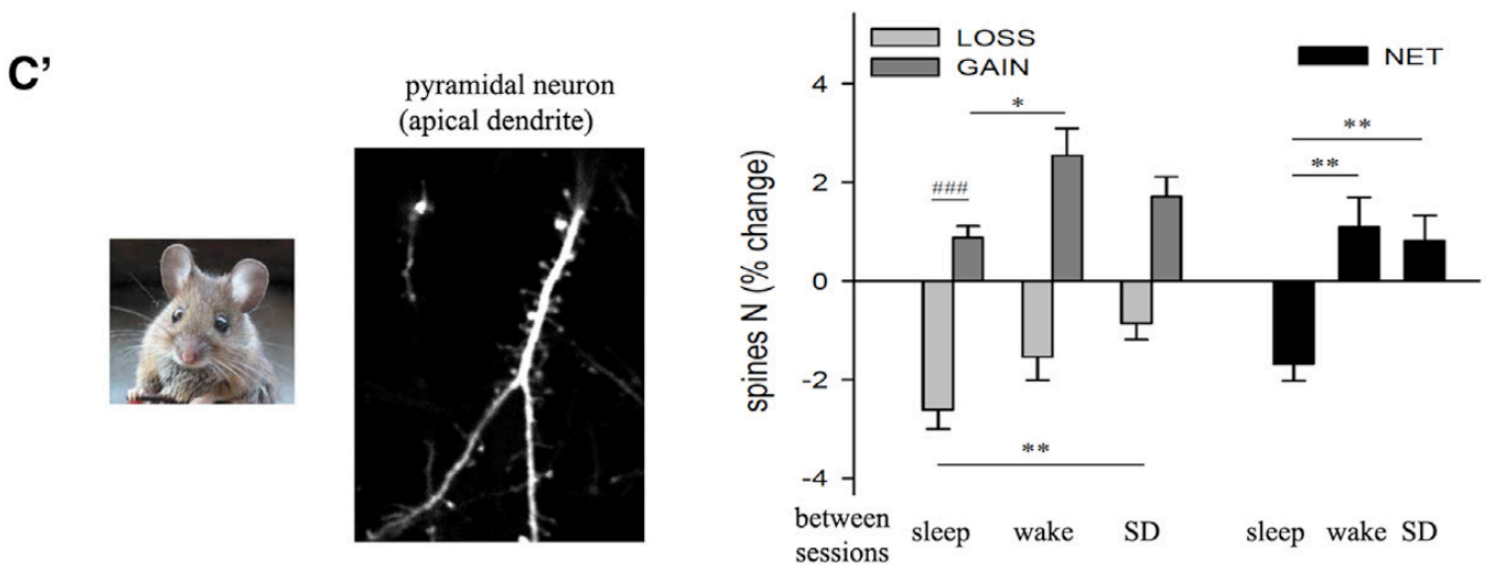




C and C': In flies, the number of spines and dendritic branches in the visual neuron VS1 increase after enriched wake (ew) and decrease only if flies are allowed to sleep (from Bushey et al., 2011).

(C') Structural studies in adolescent mice show a net increase in cortical spine density after wake and sleep deprivation (SD) and a net decrease after sleep (from Maret et al., 2011).

Tononi, G., & Cirelli, C. (2014). Sleep and the Price of Plasticity: From Synaptic and Cellular Homeostasis to Memory Consolidation and Integration. Neuron, 81(1), 12–34

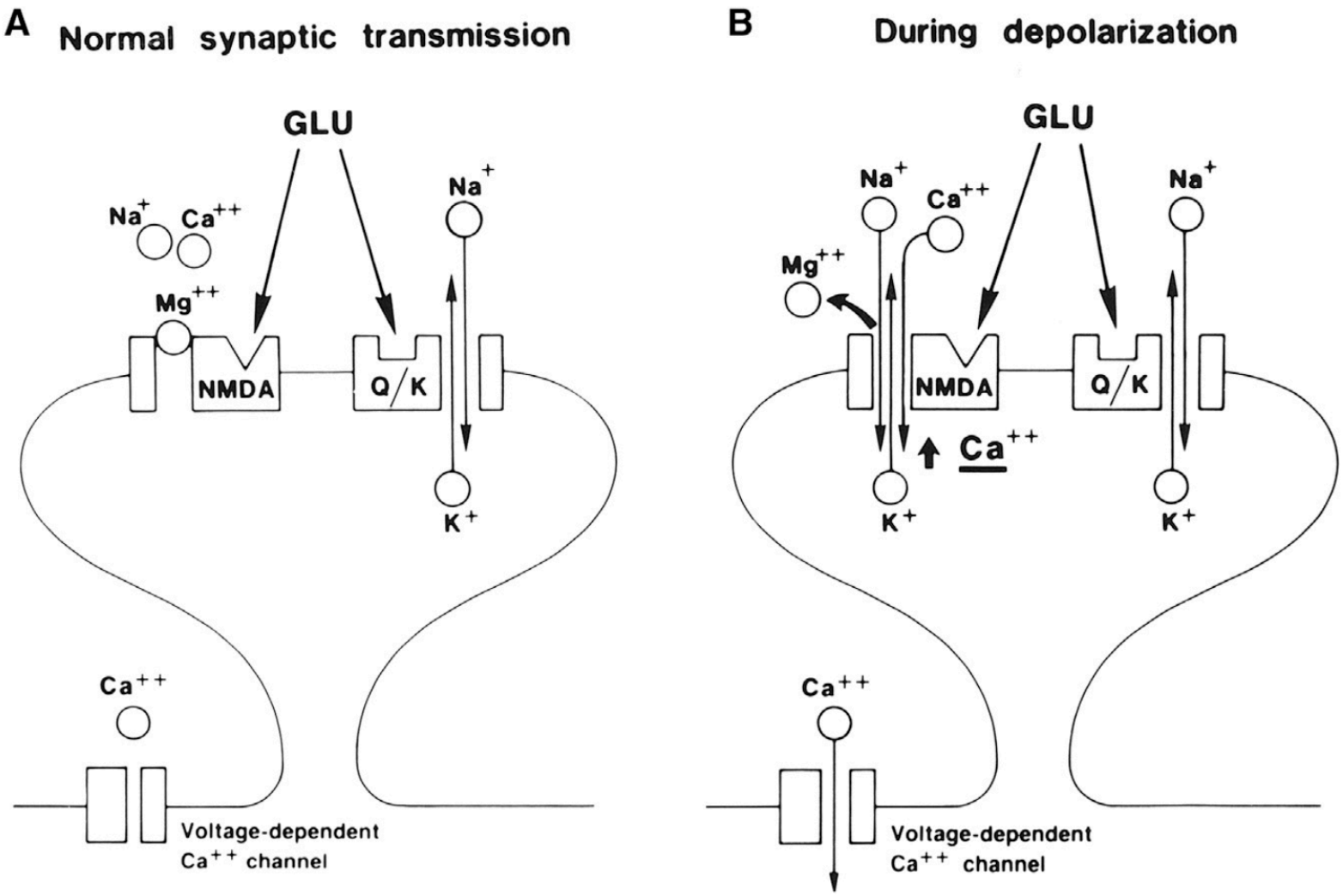
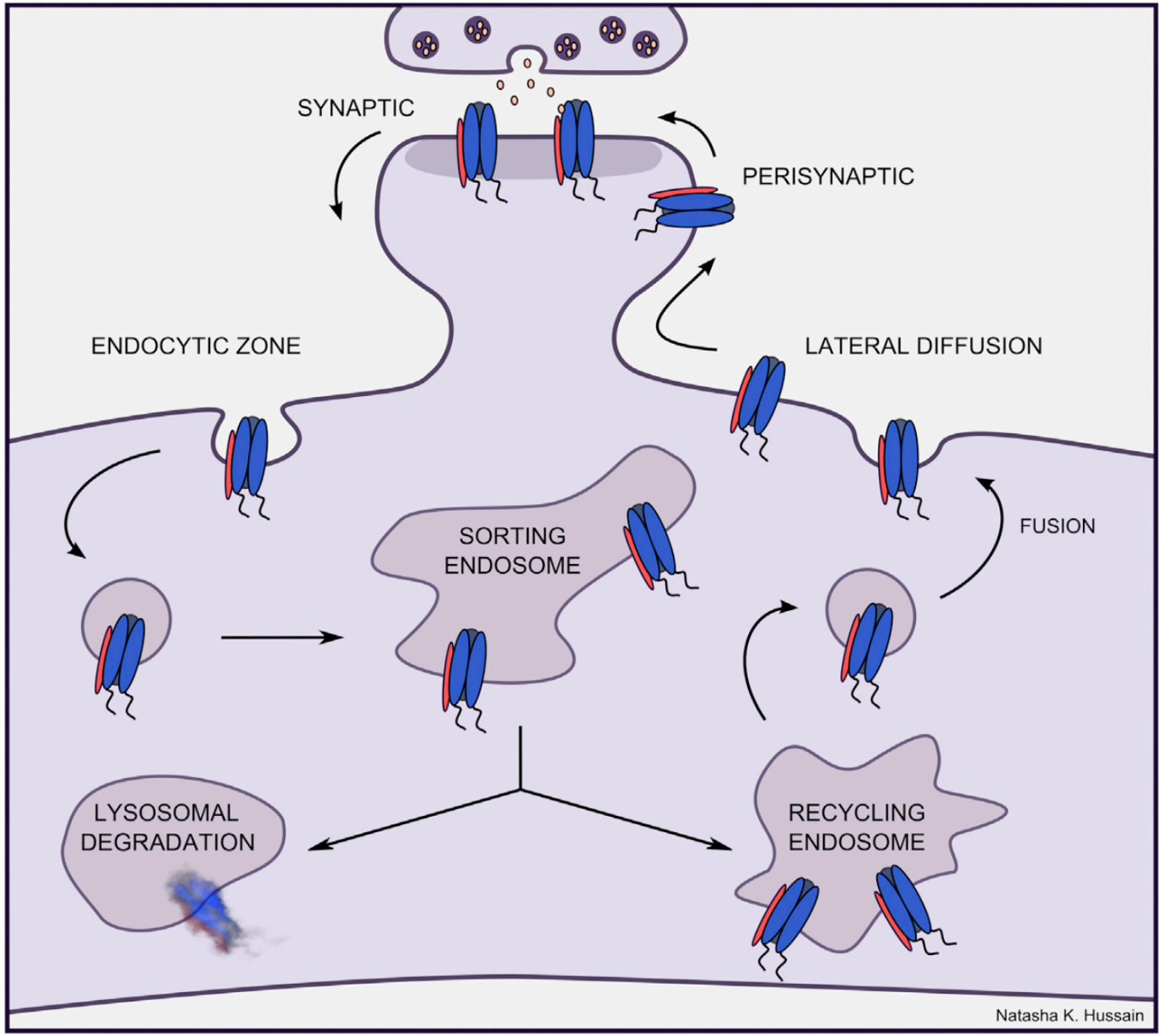


Figure 1. Model Published in 1988 for the Mechanism of Induction of LTP in the CA1 Region of the Hippocampus

(A) The events occurring during low-frequency synaptic transmission. Glutamate is released from the presynaptic terminal and acts on both the NMDA and the Q/K type of receptors (now called AMPA Receptors). Na^+ and K^+ flow through the Q/K receptor channel, but not through the NMDA receptor channel, due to Mg^{+2} block of this channel.

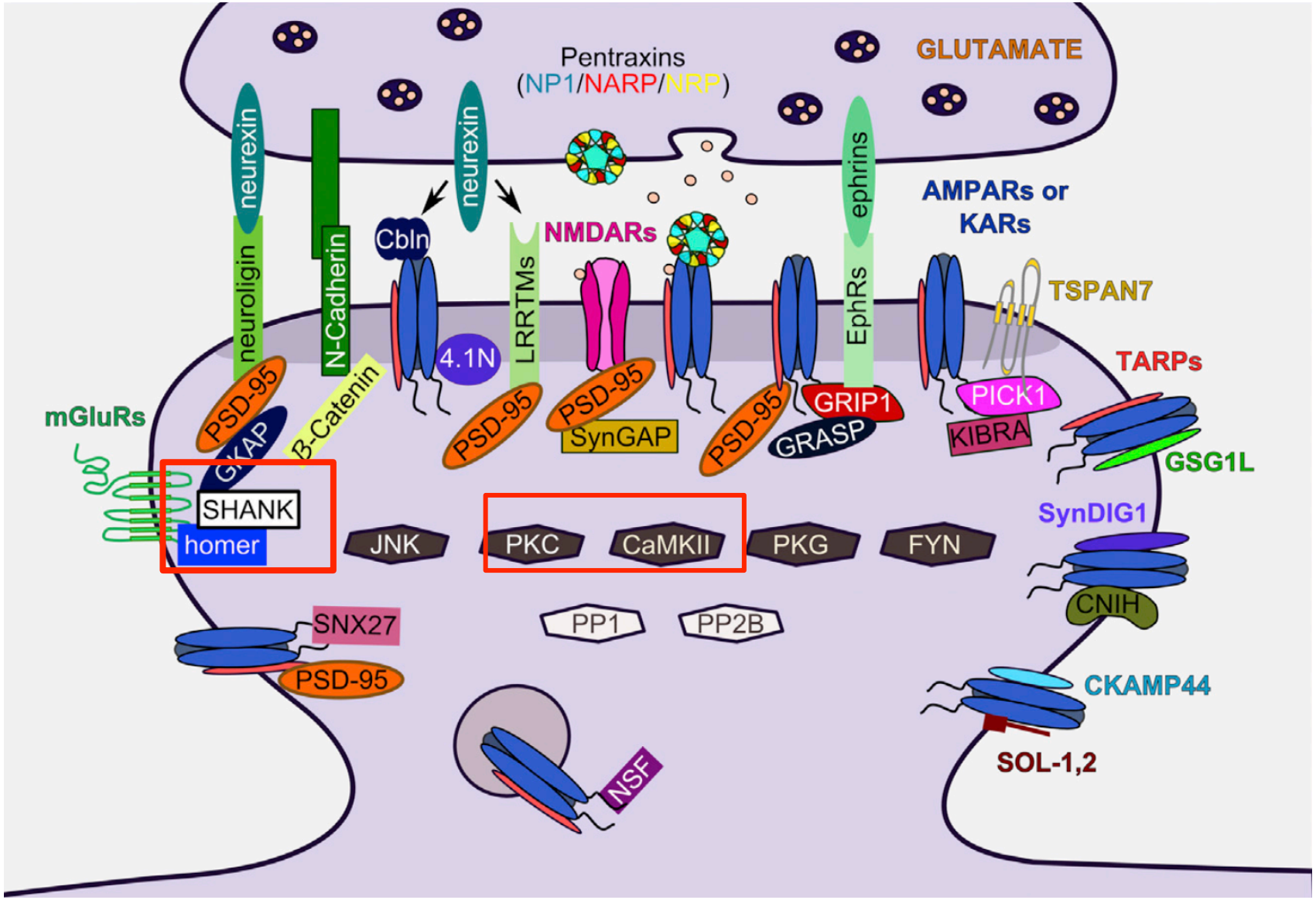
(B) The events occurring when the postsynaptic membrane is depolarized, as would occur during a high-frequency tetanus. The depolarization relieves the Mg^{+2} block of the NMDA channel, allowing Na^+, K^+, and most importantly Ca^{+2} to flow through the channel. The rise in Ca^{+2} in the dendritic spines is proposed to provide a trigger for subsequent events leading to LTP. Depolarization would also open voltage-dependent Ca^{+2} channels on the dendritic shafts, but this source of Ca^{+2} does not have access to the spine. It is important to note that this model includes only events involved in the induction of LTP and not in its maintenance (taken from Nicoll et al., 1988).



Dynamic AMPAR Trafficking during Synaptic Plasticity

AMPARs are now known to rapidly traffic between membrane compartments and to be highly mobile within the plasma membrane. Receptors rapidly move laterally in the extrasynaptic plasma membrane and can enter and exit synapses where they interact with scaffold proteins within the PSD to immobilize them and concentrate them at the synaptic plasma membrane. The receptors can be endocytosed and then move through endosomal compartments to be sorted for degradation or for recycling back to the plasma membrane. This trafficking is highly regulated during LTP and LTD resulting in increases or decreases in the steady state level of receptors at the synapse.

During LTP, receptors from nonsynaptic pools, either from the dendritic shaft plasma membrane or from intracellular pools, are recruited to synapses to potentiate synaptic transmission. In contrast, during LTD, receptors diffuse from the synapse and are then endocytosed and degraded resulting in decreases in synaptic strength.



Scaffolding and Trafficking Proteins Involved in AMPAR Membrane Trafficking and Synaptic Plasticity

Over the last 25 years a molecular machine involved in the structure and function of the excitatory synapse and the regulation of AMPAR membrane trafficking has been revealed. Dozens of proteins have been identified including signaling proteins such as protein kinases (PKA, CaMKII, PKC) and phosphatases (PP2B, PP1) that regulate receptor trafficking as well as proteins that directly or indirectly interact with receptors to immobilize them within the PSD. Central to this PSD structural complex are the MAGUKs, PSD-95, PSD-93, SAP97, and SAP102, which interact with many other proteins to modulate the structure and function of the synapse. Additional proteins, such as NSF, GRIP1/2, and PICK, can couple receptors to the endocytic or exocytic machinery to regulate exocytosis or endocytosis or help escort them through endosomal pathways. Recently, several transsynaptic proteins such as neuroligins, neurexins, and the LRRTMs have been linked not only to synapse formation but also to AMPAR trafficking and synaptic plasticity. For reviews, see Anggono and Huganir (2012), Sheng and Sala (2001), Shepherd and Huganir (2007), Xu (2011), and Zheng

Anggono, V., and Huganir, R.L. (2012). Regulation of AMPA receptor trafficking and synaptic plasticity. Curr. Opin. Neurobiol. *22*, 461–469.

Sheng, M., and Sala, C. (2001). PDZ domains and the organization of supramolecular complexes. Annu. Rev. Neurosci. *24*, 1–29.

Shepherd, J.D., and Huganir, R.L. (2007). The cell biology of synaptic plasticity: AMPA receptor trafficking. Annu. Rev. Cell Dev. Biol. *23*, 613–643.

Xu, W. (2011). PSD-95-like membrane associated guanylate kinases (PSD- MAGUKs) and synaptic plasticity. Curr. Opin. Neurobiol. *21*, 306–312.

Zheng, C.Y., Seabold, G.K., Horak, M., and Petralia, R.S. (2011). MAGUKs, synaptic development, and synaptic plasticity. Neuroscientist *17*, 493–512.

SLEEP RESEARCH

Homer1a drives homeostatic scaling-down of excitatory synapses during sleep

Graham H. Diering,[1] Raja S. Nirujogi,[2]* Richard H. Roth,[1]* Paul F. Worley,[1] Akhilesh Pandey,[2] Richard L. Huganir[1]†

Diering, G., Nirujogi, R., Roth, R., Worley, P., Pandey, A., & Huganir, R. (2017). Homer1a drives homeostatic scaling-down of excitatory synapses during sleep. Science (New York, N.Y.), 355(6324), 511–515.

Homeostatic scaling-down weakens excitatory synapses through removal and dephosphorylation of synaptic AMPA-type glutamate receptors and is mediated by alterations in the signaling of protein kinase A (PKA) and group I metabotropic glutamate receptors (mGluR1/5) (9, 10).

9. G. H. Diering, A. S. Gustina, R. L. Huganir, Neuron 84, 790–805 (2014).
10. J. H. Hu et al., Neuron 68, 1128–1142 (2010).

Using biochemistry, proteomics and imaging in mice, we find that during sleep, synapses undergo widespread alterations in composition and signaling, including weakening of synapses through removal and dephosphorylation of synaptic AMPA-type glutamate receptors. These changes are driven by the immediate early gene Homer1a and signaling from group I metabotropic glutamate receptors mGluR1/5. Homer1a serves as a molecular integrator of arousal and sleep need via the wake- and sleep-promoting neuromodulators, noradrenaline and adenosine, respectively. Our data suggest that homeostatic scaling-down, a global form of synaptic plasticity, is active during sleep to remodel synapses and participates in the consolidation of contextual memory.

Diering, G., Nirujogi, R., Roth, R., Worley, P., Pandey, A., & Huganir, R. (2017). Homer1a drives homeostatic scaling-down of excitatory synapses during sleep. Science (New York, N.Y.), 355(6324), 511–515

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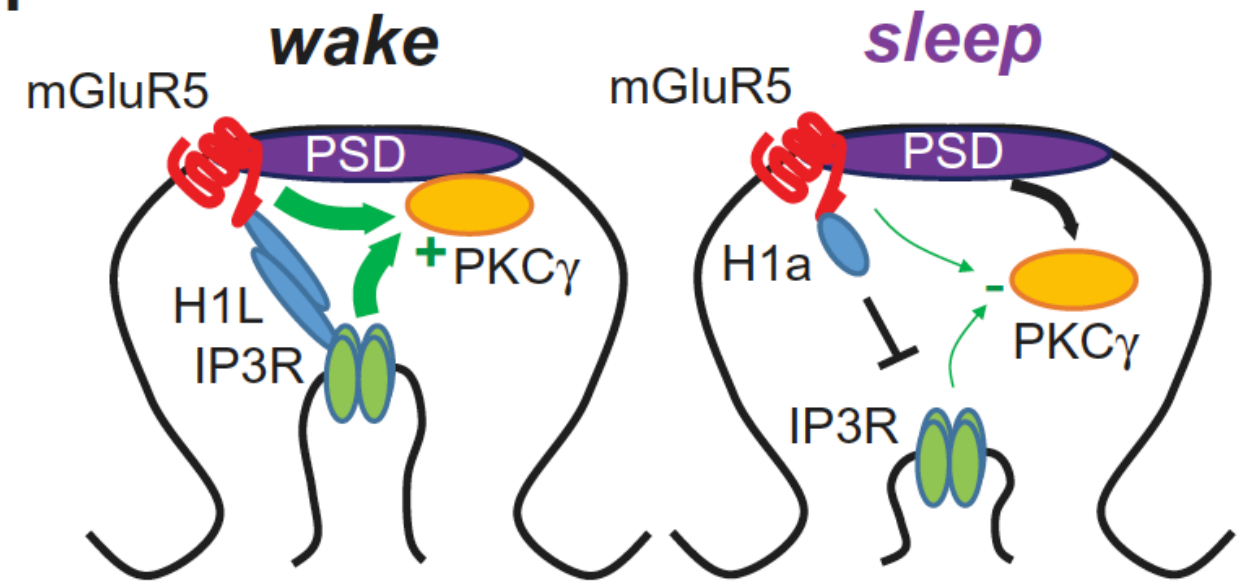
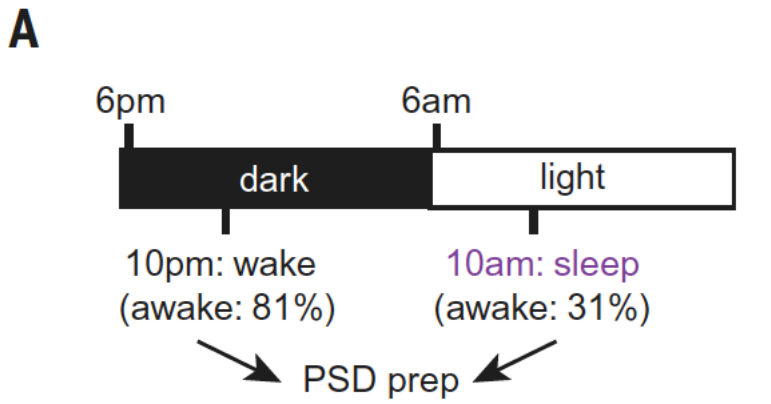
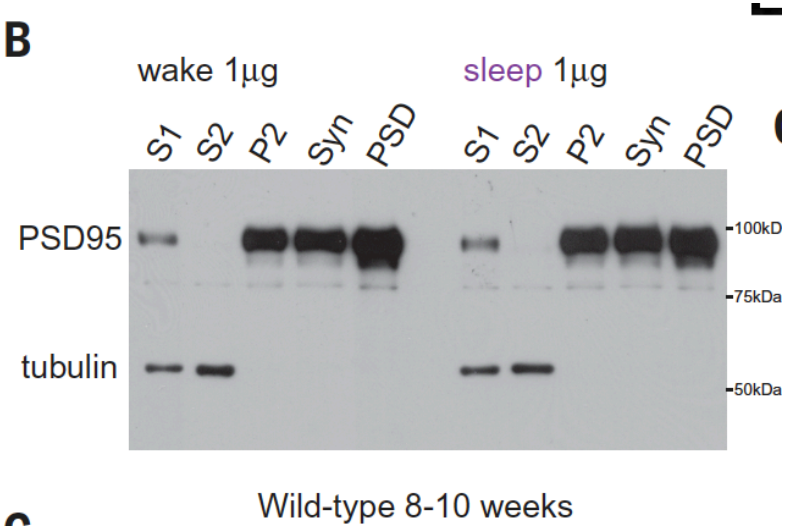


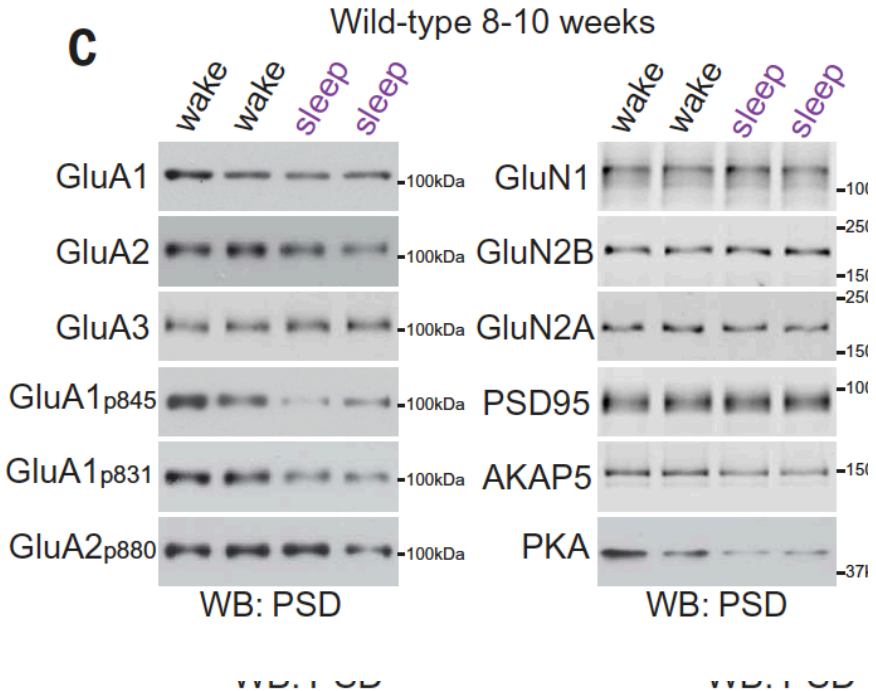
Model of Homer1a dependent remodeling of mGluR5 signaling complex.

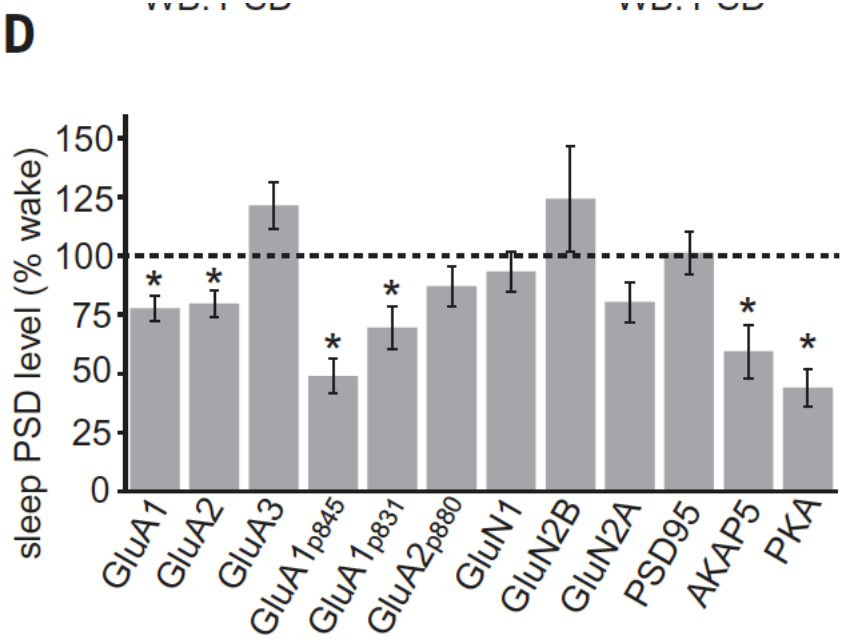
Diering, G., Nirujogi, R., Roth, R., Worley, P., Pandey, A., & Huganir, R. (2017). Homer1a drives homeostatic scaling-down of excitatory synapses during sleep. Science 355(6324), 511–515

Scaling-down was absent in Homer1a KO neurons and suggest that PSD targeting of Homer1a during sleep results in a loss of synaptic mGluR5 and associated signaling molecules, **disassembly of the mGluR5-Homer1L-IP3R complex, and removal of synaptic AMPARs.**

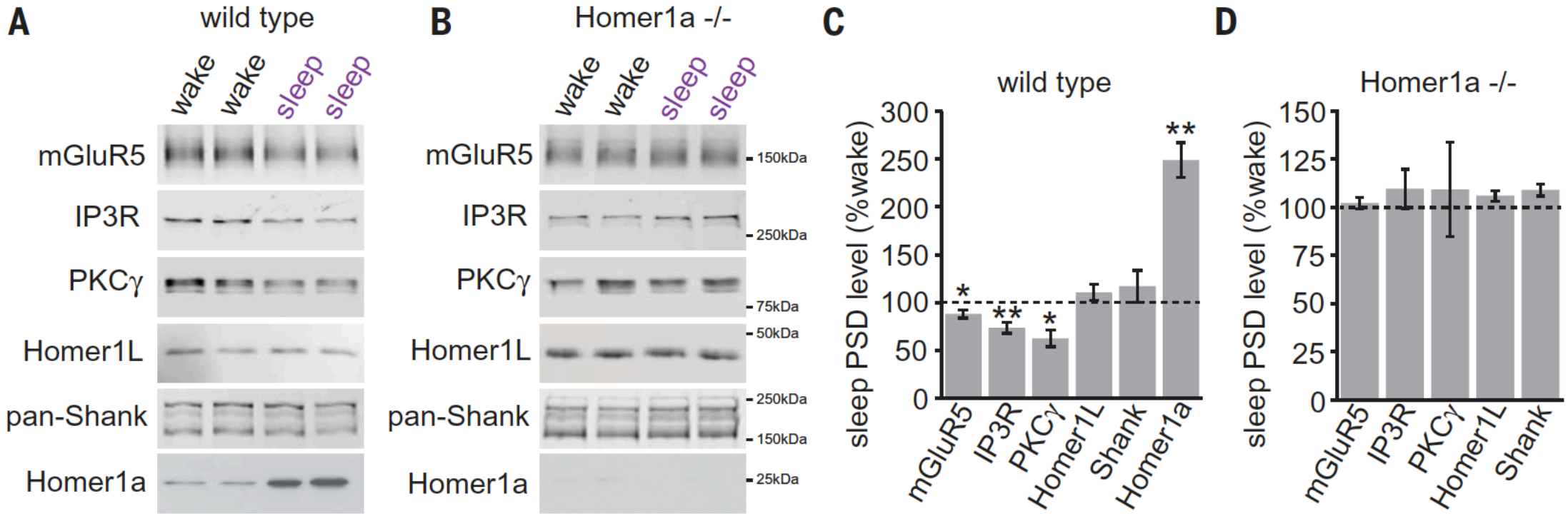




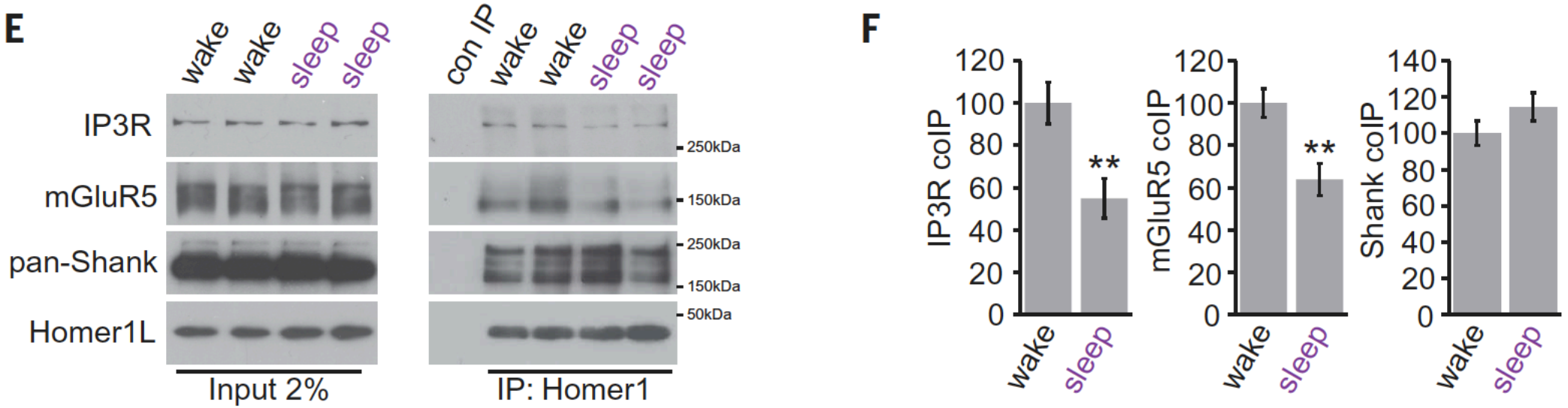




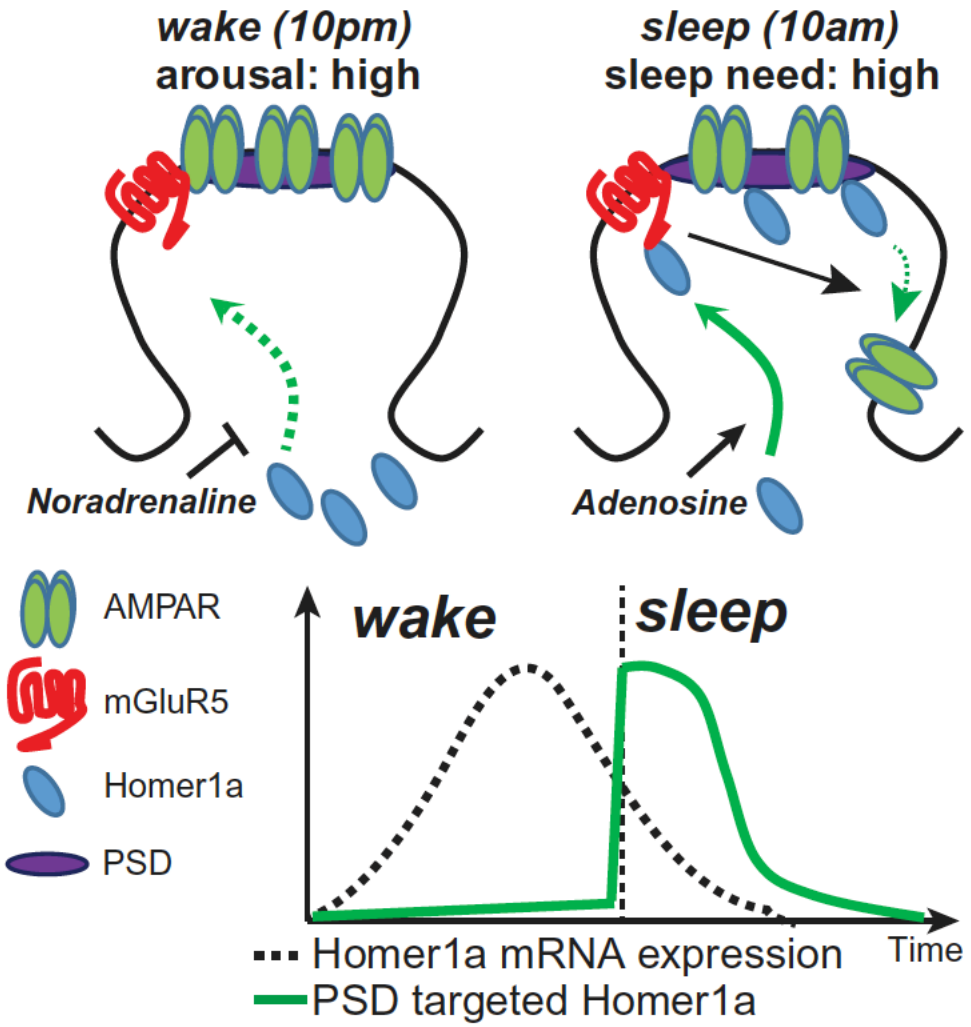
PSD samples collected during sleep contained significantly less GluA1 and GluA2 AMPAR subunits, decreased phosphorylation of GluA1 at S845 and S831, decreased catalytic subunit of PKA and A-Kinase Anchor Protein 5 (AKAP5) compared with PSDs prepared from the wake phase (Fig. 1, C and D).



Whereas mGluR5, PKCg, and IP3R showed a concerted decrease in PSD during sleep, PSD Homer1a levels drastically increased (Fig. 2, A and C).
Wake/sleep regulation of PSD-associated mGluR5, PKCg, and IP3R was absent in Homer1a knock-out (KO) mice (Fig. 2, B and D).



Using coimmunoprecipitation, we **observed significantly less interaction between Homer1L, mGluR5, and IP3R during the sleep phase**, whereas interaction between Homer1L and Shank (21) were unchanged (Fig. 2, E and F).



Acute treatment of cultured neurons with adenosine or A1 receptor–selective agonist CCPA significantly increased Homer1a PSD levels showing that adenosine, through the A1 receptor, can promote Homer1a PSD trafficking. Together, these data suggest that Homer1a serves as a molecular integrator of arousal and sleep need through the opposing actions of NA and adenosine.

During waking life, learning-related synaptic activity drives the expression of Homer1a which is excluded from synapses due to high levels of NA. At the onset of sleep or after prolonged wakefulness, NA levels decline (26) and adenosine increases (27); Homer1a is then targeted to synapses where it binds mGluR1/5 and remodels/activates mGluR1/5 signaling to drive synapse weakening (Fig. 4I). This mechanism links wake-related learning and gene expression with remodeling of synapses during sleep.