

Special Issue: Brain Aging

Neuronal Vesicular Trafficking and Release in Age-Related Cognitive Impairment

Ferenc Deák

Reynolds Oklahoma Center on Aging, Department of Geriatric Medicine, University of Oklahoma Health Sciences Center.

Address correspondence to Ferenc Deák, MD, PhD, Reynolds Oklahoma Center on Aging, Department of Geriatric Medicine, University of Oklahoma HSC, 975 N. E. 10th Street/SLY-BRC 1309-B, Oklahoma City, OK 73104-5419. Email: Ferenc-Deak@ouhsc.edu

Aging is a common major risk factor for many neurological disorders resulting in cognitive impairment and neurodegeneration including Parkinson's and Alzheimer's diseases. Novel results from the fields of molecular neuroscience and aging research provide evidence for a link between decline of various cognitive, executive functions and changes in neuronal mechanisms of intracellular trafficking and regulated vesicle release processes in the aging nervous system. In this Perspective, we review these recent findings and formulate a hypothesis on how cargo delivery to the synapses and the release of neurotrophic factors may be involved in maintaining learning and memory capabilities during healthy aging and present examples on how defects of those disrupt normal cognition. We provide an overview of emerging new concepts and approaches that will significantly advance our understanding of the aging brain and pathophysiology of dementia. This knowledge will be instrumental in defining drug targets and designing novel therapeutic strategies.

Key Words: Brain aging—Synapse—Neurotransmission—Microtubule—Dementia.

Received March 11, 2014; Accepted March 21, 2014

Decision Editor: Rafael de Cabo, PhD

THE nervous system can be viewed as a strategically located organ that collects information about the overall health status of the living organism and harmonizes various physiological functions by distributing signals and could also influence aging processes in different organs. The human brain is probably the most complex organ that the medical sciences have encountered. It contains some 400 miles of vasculature (we refer the reader on vascular aging and brain perfusion related cognitive impairment to excellent articles in this volume (1) and our previous review (2)) and multiple highly specific cell types, the primary four of these include neurons, astrocytes, oligodendrocytes, and microglia. All these cell types are involved in brain aging and their functions deteriorate with age, however, in the current review, we focus on age-related changes only in a specific subset of neuronal functions. In the following sections, we discuss the intracellular trafficking of synaptic and cargo vesicles as an example of emerging directions in cognitive impairment research. We will also summarize new data of the molecular regulation of exocytosis, the pathways responsible for properly delivering synaptic vesicles, neurotrophic factors, and receptors to their cellular targets. We will review both basic science discoveries and case studies regarding recently identified mutations that result in dementia. Our approach is based on the hypothesis that such heritable, familial disorders with specific mutations may serve

as accelerated models of age-dependent disorders and thus provide valuable insights into the pathophysiology of cognitive decline.

POLARIZED STRUCTURE OF NEURONS AND TRAFFICKING PATHWAYS – MICROFILAMENTS AND TAU

Neurons in the human nervous system have a wide variety of shapes and sizes from the few micrometers of interneurons up to the meter long axons of some motor neurons. The highly polarized structure of neurons is established and maintained by intracellular trafficking of organelles along two main types of cytoskeletal structures: actin filaments and microtubules (3). Microtubules are formed via assembly of tubulin heterodimers, a GTP-dependent process. During brain development, mutations of alpha-tubulin encoding the gene, TUBA1A, were described in severe brain malformation cases of lissencephaly (smooth brain) syndrome (4–6). Similar symptoms including cognitive impairment could result from motor protein regulator mutations—for example, LIS1/PFAH1B1 for the dyneins (5)—further highlighting the pivotal role of intracellular trafficking in neuronal cell vitality. Microtubules are vulnerable during aging and in multiple neurological disorders, including Alzheimer's disease (7). Microtubules are stabilized by specific microtubule-binding proteins, of which tau

received the most attention as it forms neurofibrillary tangles in Alzheimer's disease. A critical step in the generation of tangles is the hyperphosphorylation of tau that facilitates tau aggregation. Interestingly, glycogen synthase kinase 3, a major kinase involved in tau hyperphosphorylation, has increased activity in both insulin resistance and insulin-like growth factor-1 (IGF-1) deficiency states (8).

Disorders caused by mutations in the microtubule-binding protein tau are generally referred to as tauopathies. Tauopathies include cortical basal degeneration, primary progressive aphasia, and progressive supranuclear palsy (for more details, see review (9)). Tau mutations were also associated with parkinsonism and frontotemporal dementia (10). Typical symptoms of these disorders are more pronounced compared with those seen in age-related dementia including slow speech and motion, impaired abstract thinking, and progressive loss of working memory. In a mouse model (rTg4510) of human tauopathy expressing the P301L mutant tau protein age-dependent neurofibrillary tangle formation, pronounced neurodegeneration in the CA1 region of the hippocampus and memory impairment were described as early as 4 months (11).

Engine Failure: Motor Proteins in Neurodegeneration

Actin facilitates motility of motor proteins of the myosin family, whereas microtubules serve as tracks for two families of motor proteins, the kinesins and dyneins, which move toward the microtubule plus or minus end, respectively (12,13). Kinesin mutants are indicated in various neuronal developmental and neurodegenerative disorders including Charcot–Marie–Tooth disease (type 2) and lissencephaly (3). Recent experiments in kinesin-1-deficient mice suggested that axonal transport defects can initiate biochemical changes that induce activation of axonal stress kinase pathways leading to abnormal tau hyperphosphorylation. Mice with reduced expression of the kinesin light chain KLC1 protein had not only reduction in axonal transport but the axonal stress was sufficient to induce and accelerate abnormal tau (P301L) aggregation (14).

Recently, interesting mechanistic details have been discovered regarding the pathological consequences of motor protein dysfunction in neurodegenerative disorders of amyotrophic lateral sclerosis, frontotemporal dementia, and Perry syndrome (15). Perry syndrome consists of early-onset parkinsonism, depression, severe weight loss, and hypoventilation (16). Farrer and coworkers (17) found novel mutations of dynactin, a main binding partner of dynein, in families suffering from Perry syndrome. These mutations of the dynactin component encoding the DCTN1 gene diminish microtubule binding and lead to cytoplasmic inclusions in serotonergic and dopaminergic neurons but, surprisingly, spare motor neurons (17,18).

DCTN1 encodes the large subunit of the dynactin complex, also called p150^{glued}, which is essential in many

intracellular transport functions (19). DCTN1 protein interactions are partly mediated by the N-terminal, microtubule binding cytoskeleton-associated protein-glycine-rich (CAP-Gly) domain, the coiled-coiled and the “GKNDG” binding motifs (20). Most recently, the Holzbaur group presented new data about neuron specific isoforms of DCTN1 and its CAP-Gly domain that stabilize microtubules and facilitate retrograde transport (21). They found that low DCTN1 levels induced catastrophic disassembly of microtubules (21). Another DCTN1 mutant of the missense G59S was associated with amyotrophic lateral sclerosis and frontotemporal dementia (15). Although knockout mice heterozygous for DCTN1 had no movement phenotypes, knock-in mice carrying the G59S mutation developed loss of spinal motor neurons and gait abnormalities (22). Aging attenuated dynein–dynactin interaction in primates and was associated with tau accumulation in neurons (23).

Importantly, in a synaptic vesicle distribution study, the Nonet group found that mutation of dynein caused mislocalization of synaptic vesicles in neurons of *Caenorhabditis elegans* (24). Transporting small clear synaptic vesicles to presynaptic boutons or receptor proteins and trophic factors to their mostly dendritic location all depends on microtubule-mediated trafficking. Next, we will focus on the last step of cargo delivery, namely how these vesicles fuse with the plasma membrane in a spatially and temporally coordinated manner. For more information on other roles of intracellular trafficking in such divergent processes as RNA transport to spatially restrict gene expression, autophagy, and mitochondrial transport, we refer the readers to these excellent reviews—Hirokawa and coworkers (12) and Gagnon and Mowry (25).

SYNAPTIC FUNCTION AND COGNITION

It is estimated that on average the human brain consists of some 80–100 billion neurons and almost 100 trillion synapses, special connections between neurons to transfer and process information. Synaptic function and plasticity is viewed as the cornerstone of learning and memory, higher cognitive processes, including executive functions and moral decision making. Overwhelming data support the view that the number of synaptic connections decreases with age. Importantly, even morphologically normal synapses may have disrupted neurotransmission and attenuated synaptic plasticity which leads to impaired learning, memory, and executive performance.

Molecular Mechanism of Neurotransmission Through Regulated Exocytosis of Synaptic Vesicles

Neurotransmission in both excitatory and inhibitory synapses depends on a strictly organized set of events leading to exocytosis of synaptic vesicles executed by the core complex of membrane fusion. This core complex is formed by

three members of the soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) protein family (26) in hippocampal neurons: SNAP-25, syntaxin1, and synaptobrevin/VAMP2 (27–30). This complex is responsible for calcium-dependent, action potential triggered synchronized neurotransmitter release from the presynaptic sites (Figure 1A). The calcium signal is detected by synaptotagmins (31), members of the C2 calcium-binding domain containing protein family primarily residing on synaptic and dense-core vesicles (32), but in special cases (as for that of synaptotagmin-7), also on the plasma membrane (33). It was reported that synaptotagmin-1, -2, and -9 are all capable of serving as calcium sensors for neurotransmitter release (34,35). The diversity of the SNARE proteins associated with neurotransmission is noteworthy as it could explain regional and neuron type differences in the properties of transmitter release including short-term plasticity. Syntaxin1A and syntaxin1B are the products of two separate genes, whereas SNAP-25A and SNAP-25B are splice variants (36,37). Importantly, while hippocampal synaptic vesicles have synaptobrevin2 as the major vesicular SNARE, cortical motor neurons and Purkinje neurons in the cerebellum express synaptobrevin1 (Deak et al., unpublished data). Interestingly, in a recent human prospective study, higher levels of SNARE proteins in postmortem brain samples are associated with lower odds of dementia (odds ratio = 0.36–0.68) and better cognitive function (38). Therefore, elucidation of changes in SNARE proteins with age will be another important area of future research in cognitive decline of the elderly.

Synaptic Plasticity and Long-term Potentiation: The Case of AMPA Receptor Trafficking in the CA1 Pyramidal Neurons

During spatial learning tasks, synaptic responses are potentiated in the CA1 field of the hippocampus. This is the

result of addition of more AMPA (2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl) propanoic acid)-sensitive ionotropic glutamate receptors (AMPA-R) to the postsynaptic site through fusion of AMPA-R-containing vesicles, and receptor diffusion to the postsynaptic density (39). In a series of elegant studies (40,41), the Malenka and Südhof laboratories identified the proteins participating in this step of AMPA receptor exocytosis as SNAP-47, syntaxin3, and synaptobrevin2 (Figure 1B). Munc18 and complexin1 also contribute to the regulation of the membrane fusion process that is critical in long-term potentiation in the CA1 Schaffer collateral synapses (40). At this stage, one can only speculate whether similar molecular mechanisms are involved in the plasticity of inhibitory synaptic strength. Intriguingly, it was suggested that age-related upregulation of inhibitory synaptic transmission in prefrontal cortex may impair working memory and that targeting GABA-B receptors might have therapeutic benefits for age-related deficits in executive function (42).

Secretion of Neurotrophic Factors

Neuronal survival critically depends on a variety of trophic factors including neurotrophic factor-3, brain-derived neurotrophic factor (BDNF), and IGF-1. The Sonntag group and others established that plasma concentrations of IGF-I decrease with age and contribute to the impairment in tissue function that is characteristic of aging (1). Although some IGF-1 comes from the circulation to the nervous system, a significant portion of the peptide is locally synthesized and used in the brain and establishes the level of IGF-1 around neurons. Therefore, the regulation of IGF-1 secretion from brain cells has crucial importance. In extensive studies, Cao and coworkers (43,44) reported that IGF-1 is stored in intracellular vesicles in neurons, and during neuronal activity, it is released via exocytosis. This release is triggered by a marked sudden increase in calcium

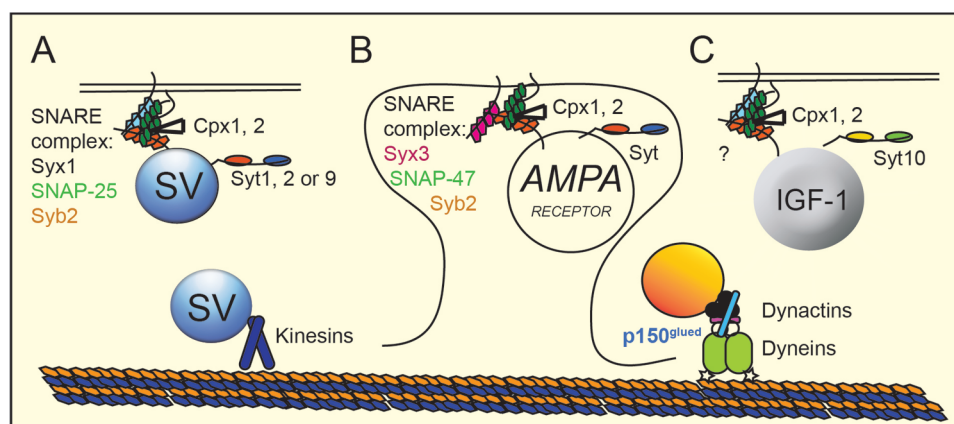


Figure 1. Molecular components of neurotransmitter and neurotrophic factor release and glutamate AMPA receptor membrane insertion. Figure depicts the SNARE protein and synaptotagmin isoforms participating in vesicle exocytosis for three different scenarios: (A) a small glutamatergic synaptic vesicle; (B) glutamate AMPA receptor insertion into the dendrites; and (C) secretion of IGF-1 from large vesicles. AMPA receptor = 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl) propanoic acid-sensitive class of ionotropic glutamate receptors; cpx = complexin; sv = synaptic vesicle; syb = synaptobrevin; syt = synaptotagmin; Syx = syntaxin.

ion concentration that is sensed by synaptotagmin-10 on the surface of the IGF-1 vesicles (Figure 1C). Deletion of synaptotagmin-10 resulted in smaller mitral neurons and an overall decrease in synapse numbers in the olfactory bulb (43). To complete the process, synaptotagmin-10 triggers the SNARE protein complex (for which the exact components have not been identified yet) to release IGF-1, very similarly to neurotransmitter release described above. Restoration or maintenance of adult IGF-1 levels could provide significant benefits in combating cognitive decline in the elderly and justify further studies of both IGF-1 secretion and its neuronal effects (8).

BDNF is associated with complex brain disorders. For instance, decreased BDNF serum levels have been reported in multiple patient groups with major depression (45,46). BDNF increases spine density in apical dendrites of CA1 pyramidal neurons in rat hippocampal slice cultures (47), and BDNF KO mice have impaired synaptic transmission when stimulated at high frequencies (48). This synaptic fatigue is accompanied with lower number of docked synaptic vesicles in hippocampal pyramidal cells.

The molecular mechanism of BDNF secretion is less well characterized than that of IGF-1. Synaptotagmin-4 (syt4) was found to colocalize with BDNF-containing vesicles and its selective deletion increased BDNF release from dendrites by around 25%; overexpression of syt4 decreased BDNF to 70% of control levels (49). Interpretation of the synaptic phenotype of the syt4 KO mice is further complicated by the fact that there are significantly less vesicles in synaptic terminals of cultured syt4 KO hippocampal neurons, with a pronounced decrease in the number of docked vesicles (50). This could arise from the disturbed synaptotagmin–kinesin (kif1A) interaction that is involved in vesicle delivery to the synapse. As BDNF plasma levels decrease with age (51), further studies are required to establish its effect on age-related mood disorders.

The finding that tetanus and clostridium toxins, that cleave synaptobrevin, also blocked the release of neurotrophic factors indicates the involvement of vesicular SNARE synaptobrevins (52) in this process. On the other hand, neurotrophin-induced neurite outgrowth appears to require rac1 activation and intact microtubules, and this process depends on cell membrane incorporation of preformed intracellular “enlargeosomes,” driven by another vesicle-associated SNARE, VAMP4 (53). This is a new area of research and many hypotheses could be developed about its possible roles in adult neurogenesis and in the process of neuronal plasticity to rebuild circuits, for example, after stroke or traumatic brain injury.

CONCLUSIONS AND FUTURE DIRECTIONS

Medicine has achieved a remarkable progress in extending the life of the human population; now the challenge is to find the best way to maximize quality of life for seniors. A key

aspect will be developing preventive strategies and therapies that will be able to maintain cognitive health. Better understanding of aging-induced molecular changes in intracellular trafficking, neurotrophic factor secretion, and neurotransmitter release will be a central research area to achieve that goal. Although many brain disorders are already getting categorized as axonopathies and synaptopathies, clearly, we need more studies on how vital components of neurons are delivered to distal cellular compartments and how synaptic plasticity can encode information accurately for prolonged periods. These future studies should utilize novel experimental tools with unprecedented resolution such as stimulated emission depletion (54,55), total internal reflection fluorescence or single-molecule fluorescence microscopy (56), and live imaging of organelle movements (57) as well as quantifying neurotransmission supporting processes of exocytosis (27,58) and endocytosis (28,39). Taken together, research on neuronal trafficking and synaptic function will be fields to pay close attention to as the ones with potential to bring the urgently needed breakthroughs to research on age-related cognitive impairment.

FUNDING

This work was supported by funding from the Oklahoma Center for the Advancement of Sciences and Technology (OCAST) program grant number of HR13-141. The author would like to express his gratitude for the support of the Donald W. Reynolds Foundation, which funds aging research at the University of Oklahoma Health Sciences Center under its Aging and Quality of Life Program.

ACKNOWLEDGMENTS

We are grateful for the support and comments of Dr. Bill Sonntag during writing this review. We thank Ms. Sarah Green and Ms. Judith Kranz for their kind help with editing this manuscript.

REFERENCES

1. Tucsek Z, Toth P, Tarantini S, et al. Aging exacerbates obesity-induced cerebrovascular rarefaction, neurovascular uncoupling and cognitive decline in mice. *J Gerontol A Biol Sci Med Sci*. 2014.
2. Sonntag WE, Deak F, Ashpole N, et al. Insulin-like growth factor-1 in CNS and cerebrovascular aging. *Front Aging Neurosci*. 2013;5:27. doi:10.3389/fnagi.2013.00027
3. Franker MA, Hoogenraad CC. Microtubule-based transport - basic mechanisms, traffic rules and role in neurological pathogenesis. *J Cell Sci*. 2013;126(Pt 11):2319–2329. doi:10.1242/jcs.115030
4. Kumar RA, Pilz DT, Babatz TD, et al. TUBA1A mutations cause wide spectrum lissencephaly (smooth brain) and suggest that multiple neuronal migration pathways converge on alpha tubulins. *Hum Mol Genet*. 2010;19(14):2817–2827. doi:10.1093/hmg/ddq182
5. Mokánszki A, Körhegyi I, Szabó N, et al. Lissencephaly and band heterotopia: LIS1, TUBA1A, and DCX mutations in Hungary. *J Child Neurol*. 2012;27(12):1534–1540. doi:10.1177/0883073811436326
6. Sohal AP, Montgomery T, Mitra D, Ramesh V. TUBA1A mutation-associated lissencephaly: case report and review of the literature. *Pediatr Neurol*. 2012;46(2):127–131. doi:10.1016/j.pediatrneurol.2011.11.017
7. Stokin GB, Lillo C, Falzone TL, et al. Axonopathy and transport deficits early in the pathogenesis of Alzheimer's disease. *Science*. 2005;307(5713):1282–1288. doi:10.1126/science.1105681
8. Deak F, Sonntag WE. Aging, synaptic dysfunction, and insulin-like growth factor (IGF)-1. *J Gerontol A Biol Sci Med Sci*. 2012;67(6):611–625. doi:10.1093/gerona/gls118

9. Higuchi M, Lee VM, Trojanowski JQ. Tau and axonopathy in neurodegenerative disorders. *Neuromolecular Med.* 2002;2(2):131–150. doi:10.1385/NMM:2:2:131
10. Ittner LM, Fath T, Ke YD, et al. Parkinsonism and impaired axonal transport in a mouse model of frontotemporal dementia. *Proc Natl Acad Sci U S A.* 2008;105(41):15997–16002. PMID:18832465.
11. Ramsden M, Kotilinek L, Forster C, et al. Age-dependent neurofibrillary tangle formation, neuron loss, and memory impairment in a mouse model of human tauopathy (P301L). *J Neurosci.* 2005;25(46):10637–10647. doi:10.1523/JNEUROSCI.3279-05.2005
12. Hirokawa N, Noda Y, Tanaka Y, Niwa S. Kinesin superfamily motor proteins and intracellular transport. *Nat Rev Mol Cell Biol.* 2009;10(10):682–696. doi:nrm2774 [pii]10.1038/nrm2774
13. Vale RD. The molecular motor toolbox for intracellular transport. *Cell.* 2003;112(4):467–480. PMID:12600311.
14. Falzone TL, Gunawardena S, McCleary D, Reis GF, Goldstein LS. Kinesin-1 transport reductions enhance human tau hyperphosphorylation, aggregation and neurodegeneration in animal models of tauopathies. *Hum Mol Genet.* 2010;19(22):4399–4408. doi:10.1093/hmg/ddq363
15. Münch C, Sedlmeier R, Meyer T, et al. Point mutations of the p150 subunit of dynactin (DCTN1) gene in ALS. *Neurology.* 2004;63(4):724–726. PMID:15326253.
16. Wider C, Wszolek ZK. Rapidly progressive familial parkinsonism with central hypoventilation, depression and weight loss (Perry syndrome)—a literature review. *Parkinsonism Relat Disord.* 2008;14(1):1–7. PMID:17870652.
17. Farrer MJ, Hulihan MM, Kachergus JM, et al. DCTN1 mutations in Perry syndrome. *Nat Genet.* 2009;41(2):163–165. doi:10.1038/ng.293
18. Wider C, Dachsel JC, Farrer MJ, Dickson DW, Tsuboi Y, Wszolek ZK. Elucidating the genetics and pathology of Perry syndrome. *J Neurol Sci.* 2010;289:149–154. PMID:19732908.
19. Schliwa M, Woehlke G. Molecular motors. *Nature.* 2003;422(6933):759–765. doi:10.1038/nature01601
20. Weisbrich A, Honnappa S, Jaussi R, et al. Structure–function relationship of CAP-Gly domains. *Nat Struct Mol Biol.* 2007;14(10):959–967. PMID:17828277.
21. Lazarus JE, Moughamian AJ, Tokito MK, Holzbaur EL. Dynactin subunit p150^{Glued} is a neuron-specific anti-catastrophe factor. *PLoS Biol.* 2013;11(7):e1001611. doi:10.1371/journal.pbio.1001611
22. Lai C, Lin X, Chandran J, Shim H, Yang WJ, Cai H. The G59S mutation in p150^{Glued} causes dysfunction of dynactin in mice. *J Neurosci.* 2007;27(51):13982–13990. PMID:18094236
23. Kimura N, Imamura O, Ono F, Terao K. Aging attenuates dynactin-dynein interaction: down-regulation of dynein causes accumulation of endogenous tau and amyloid precursor protein in human neuroblastoma cells. *J Neurosci Res.* 2007;85(13):2909–2916. doi:10.1002/jnr.21408
24. Koushika SP, Schaefer AM, Vincent R, Willis JH, Bowerman B, Nonet ML. Mutations in *Caenorhabditis elegans* cytoplasmic dynein components reveal specificity of neuronal retrograde cargo. *J Neurosci.* 2004;24(16):3907–3916. doi:10.1523/JNEUROSCI.5039-03.2004
25. Gagnon JA, Mowry KL. Molecular motors: directing traffic during RNA localization. *Crit Rev Biochem Mol Biol.* 2011;46(3):229–239. doi:10.3109/10409238.2011.572861
26. Söllner T, Whiteheart SW, Brunner M, et al. SNAP receptors implicated in vesicle targeting and fusion. *Nature.* 1993;362(6418):318–324. PMID:8455717.
27. Schoch S, Deák F, Königstorfer A, et al. SNARE function analyzed in synaptobrevin/VAMP knockout mice. *Science.* 2001;294(5544):1117–1122. PMID:11691998.
28. Deák F, Schoch S, Liu X, Südhof TC, Kavalali ET. Synaptobrevin is essential for fast synaptic-vesicle endocytosis. *Nat Cell Biol.* 2004;6(11):1102–1108. PMID:15475946.
29. Deák F, Shin OH, Kavalali ET, Südhof TC. Structural determinants of synaptobrevin 2 function in synaptic vesicle fusion. *J Neurosci.* 2006;26(25):6668–6676. PMID:16793874.
30. Südhof TC, Rothman JE. Membrane fusion: grappling with SNARE and SM proteins. *Science.* 2009;323(5913):474–477. PMID:19164740.
31. Fernández-Chacón R, Königstorfer A, Gerber SH, et al. Synaptotagmin I functions as a calcium regulator of release probability. *Nature.* 2001;410(6824):41–49. PMID:11242035.
32. Shin OH, Rizo J, Südhof TC. Synaptotagmin function in dense core vesicle exocytosis studied in cracked PC12 cells. *Nat Neurosci.* 2002;5(7):649–656. PMID:12055633.
33. Virmani T, Han W, Liu X, Südhof TC, Kavalali ET. Synaptotagmin 7 splice variants differentially regulate synaptic vesicle recycling. *EMBO J.* 2003;22(20):5347–5357. PMID:14532108.
34. Nagy G, Kim JH, Pang ZP, et al. Different effects on fast exocytosis induced by synaptotagmin 1 and 2 isoforms and abundance but not by phosphorylation. *J Neurosci.* 2006;26(2):632–643. PMID:16407561.
35. Xu J, Mashimo T, Südhof TC. Synaptotagmin-1, -2, and -9: Ca(2+) sensors for fast release that specify distinct presynaptic properties in subsets of neurons. *Neuron.* 2007;54(4):567–581. doi:10.1016/j.neuron.2007.05.004
36. Gerber SH, Rah JC, Min SW, et al. Conformational switch of syntaxin-1 controls synaptic vesicle fusion. *Science.* 2008;321(5895):1507–1510. doi:1163174 [pii]10.1126/science.1163174
37. Sørensen JB, Nagy G, Varoqueaux F, et al. Differential control of the releasable vesicle pools by SNAP-25 splice variants and SNAP-23. *Cell.* 2003;114(1):75–86. PMID:12859899.
38. Honer WG, Barr AM, Sawada K, et al. Cognitive reserve, presynaptic proteins and dementia in the elderly. *Transl Psychiatry.* 2012;2:e114. doi:10.1038/tp.2012.38
39. Czöndör K, Mondin M, Garcia M, et al. Unified quantitative model of AMPA receptor trafficking at synapses. *Proc Natl Acad Sci U S A.* 2012;109(9):3522–3527. doi:10.1073/pnas.1109818109
40. Ahmad M, Polepalli JS, Goswami D, et al. Postsynaptic complexin controls AMPA receptor exocytosis during LTP. *Neuron.* 2012;73(2):260–267. doi:10.1016/j.neuron.2011.11.020
41. Jurado S, Goswami D, Zhang Y, Molina AJ, Südhof TC, Malenka RC. LTP requires a unique postsynaptic SNARE fusion machinery. *Neuron.* 2013;77(3):542–558. doi:10.1016/j.neuron.2012.11.029
42. Bañuelos C, Beas BS, McQuail JA, et al. Prefrontal cortical GABAergic dysfunction contributes to age-related working memory impairment. *J Neurosci.* 2014;34(10):3457–3466. doi:10.1523/JNEUROSCI.5192-13.2014
43. Cao P, Maximov A, Südhof TC. Activity-dependent IGF-1 exocytosis is controlled by the Ca(2+)-sensor synaptotagmin-10. *Cell.* 2011;145(2):300–311. doi:S0092-8674(11)00311-4 [pii]10.1016/j.cell.2011.03.034
44. Cao P, Yang X, Südhof TC. Complexin activates exocytosis of distinct secretory vesicles controlled by different synaptotagmins. *J Neurosci.* 2013;33(4):1714–1727. doi:10.1523/JNEUROSCI.4087-12.2013
45. Nestler EJ, Barrot M, DiLeone RJ, Eisch AJ, Gold SJ, Monteggia LM. Neurobiology of depression. *Neuron.* 2002;34(1):13–25. doi:10.1038/mp.2013.105
46. Molendijk ML, Spinhoven P, Polak M, Bus BA, Penninx BW, Elzinga BM. Serum BDNF concentrations as peripheral manifestations of depression: evidence from a systematic review and meta-analyses on 179 associations (N = 9484). *Mol Psychiatry.* 2013; doi:10.1038/mp.2013.105
47. Tyler WJ, Pozzo-Miller L. Miniature synaptic transmission and BDNF modulate dendritic spine growth and form in rat CA1 neurons. *J Physiol.* 2003;553(Pt 2):497–509. doi:10.1113/jphysiol.2003.052639
48. Pozzo-Miller LD, Gottschalk W, Zhang L, et al. Impairments in high-frequency transmission, synaptic vesicle docking, and synaptic protein distribution in the hippocampus of BDNF knockout mice. *J Neurosci.* 1999;19(12):4972–4983. PMID:10366630.
49. Dean C, Liu H, Dunning FM, Chang PY, Jackson MB, Chapman ER. Synaptotagmin-IV modulates synaptic function and

- long-term potentiation by regulating BDNF release. *Nat Neurosci.* 2009;12(6):767–776. doi:10.1038/nn.2315
50. Arthur CP, Dean C, Pagratis M, Chapman ER, Stowell MH. Loss of synaptotagmin IV results in a reduction in synaptic vesicles and a distortion of the Golgi structure in cultured hippocampal neurons. *Neuroscience.* 2010;167(1):135–142. doi:10.1016/j.neuroscience.2010.01.056
51. Diniz BS, Reynolds CF 3rd, Begley A, et al. Brain-derived neurotrophic factor levels in late-life depression and comorbid mild cognitive impairment: a longitudinal study. *J Psychiatr Res.* 2014;49:96–101. doi:10.1016/j.jpsychires.2013.11.004
52. Wang X, Butowt R, Vasko MR, von Bartheld CS. Mechanisms of the release of anterogradely transported neurotrophin-3 from axon terminals. *J Neurosci.* 2002;22(3):931–945. PMID:11826122.
53. Racchetti G, Lorusso A, Schulte C, et al. Rapid neurite outgrowth in neurosecretory cells and neurons is sustained by the exocytosis of a cytoplasmic organelle, the enlargeosome. *J Cell Sci.* 2010;123(Pt 2):165–170. doi:10.1242/jcs.059634
54. Westphal V, Rizzoli SO, Lauterbach MA, Kamin D, Jahn R, Hell SW. Video-rate far-field optical nanoscopy dissects synaptic vesicle movement. *Science.* 2008;320(5873):246–249. doi:10.1126/science.1154228
55. Dean C, Liu H, Staudt T, et al. Distinct subsets of Syt-IV/BDNF vesicles are sorted to axons versus dendrites and recruited to synapses by activity. *J Neurosci.* 2012;32(16):5398–5413. doi:10.1523/JNEUROSCI.4515-11.2012
56. Tardin C, Cognet L, Bats C, Lounis B, Choquet D. Direct imaging of lateral movements of AMPA receptors inside synapses. *EMBO J.* 2003;22(18):4656–4665. doi:10.1093/emboj/cdg463
57. Mazel T, Biesemann A, Krejczyk M, Nowald J, Müller O, Dehmelt L. Direct observation of microtubule pushing by cortical dynein in living cells. *Mol Biol Cell.* 2014;25(1):95–106. doi:10.1091/mbc.E13-07-0376
58. Sara Y, Virmani T, Deák F, Liu X, Kavalali ET. An isolated pool of vesicles recycles at rest and drives spontaneous neurotransmission. *Neuron.* 2005;45(4):563–573. PMID:15721242.