

Local apoptotic-like mechanisms underlie complement-mediated synaptic pruning

Balázs A. Györfy^{a,b}, Judit Kun^a, György Török^c, Éva Bulyáki^a, Zsolt Borhegyi^d, Péter Gulyássi^e, Viktor Kis^f, Péter Szocsics^g, András Micsonai^a, János Matkó^h, László Drahos^e, Gábor Juhász^{b,e,i}, Katalin A. Kékesi^{b,j,1}, and József Kardos^{a,1,2}

^aELTE NAP Neuroimmunology Research Group, Department of Biochemistry, Institute of Biology, ELTE Eötvös Loránd University, H-1117 Budapest, Hungary; ^bLaboratory of Proteomics, Institute of Biology, ELTE Eötvös Loránd University, H-1117 Budapest, Hungary; ^cLaboratory of Molecular Cell Biology, Institute of Enzymology, Hungarian Academy of Sciences, H-1117 Budapest, Hungary; ^dDepartment of Biochemistry, Institute of Biology, ELTE Eötvös Loránd University, H-1117 Budapest, Hungary; ^eMTA-TTK NAP B MS Neuroproteomics Group, Research Centre for Natural Sciences, Hungarian Academy of Sciences, H-1117 Budapest, Hungary; ^fDepartment of Anatomy, Cell and Developmental Biology, Institute of Biology, ELTE Eötvös Loránd University, H-1117 Budapest, Hungary; ^gLaboratory of Human Brain Research, Institute of Experimental Medicine, Hungarian Academy of Sciences, H-1083 Budapest, Hungary; ^hDepartment of Immunology, Institute of Biology, ELTE Eötvös Loránd University, H-1117 Budapest, Hungary; ⁱCRU Hungary Ltd., H-2131 Göd, Hungary; and ^jDepartment of Physiology and Neurobiology, Institute of Biology, ELTE Eötvös Loránd University, H-1117 Budapest, Hungary

Edited by Charles F. Stevens, Salk Institute for Biological Studies, La Jolla, CA, and approved May 9, 2018 (received for review December 29, 2017)

C1q, a member of the immune complement cascade, is implicated in the selective pruning of synapses by microglial phagocytosis. C1q-mediated synapse elimination has been shown to occur during brain development, while increased activation and complement-dependent synapse loss is observed in neurodegenerative diseases. However, the molecular mechanisms underlying C1q-controlled synaptic pruning are mostly unknown. This study addresses distortions in the synaptic proteome leading to C1q-tagged synapses. Our data demonstrated the preferential localization of C1q to the presynapse. Proteomic investigation and pathway analysis of C1q-tagged synaptosomes revealed the presence of apoptotic-like processes in C1q-tagged synapses, which was confirmed experimentally with apoptosis markers. Moreover, the induction of synaptic apoptotic-like mechanisms in a model of sensory deprivation-induced synaptic depression led to elevated C1q levels. Our results unveiled that C1q label-based synaptic pruning is triggered by and directly linked to apoptotic-like processes in the synaptic compartment.

synaptic pruning | complement C1q | proteomics | synaptosome sorting | apoptotic-like mechanisms

The complement cascade, as part of the innate immune system, plays a role in the recognition and removal of invading pathogens and stimulating further components of the immune system. It has also been shown that certain complement system molecules are constitutively expressed in diseased and healthy brain (1). Specifically, C1q, the first component of the classical complement pathway, C3, and complement receptor 3 participate in postnatal synaptic development and pruning (2). Schafer et al. (3) reported the role of microglia cells in synapse elimination in the developing murine visual system in a synaptic activity-dependent and complement-mediated manner. Stevens et al. (4) showed the synaptic localization of C1q and its expression in a period of postnatal development of the retina and brain, and they demonstrated that C1q is the pruning-initiator label on certain synapses. Erroneous synapse remodeling was observed in C1q knock-out mice (4). Stephan et al. (5) showed that correlating with cognitive decline, the C1q level dramatically increases during normal aging of mouse and human brain and that C1q is localized in close proximity to synapses. Most recently, participation of C1q-mediated synaptic pruning was described in early-stage synapse loss in animal models of Alzheimer's disease (6) and in frontotemporal dementia (7).

It is well-known that synaptic turnover can occur at high rates even in adulthood (8) and that synaptic C1q, as a molecular “tag,” mediates the selective pruning of unnecessary synapses by the adjacent microglia (6, 7). However, the molecular changes attracting the C1q tag to a synapse are still unknown. In the present work, we addressed the question of what kind of specific synaptic proteome alterations could induce the C1q tagging of a

synapse. We identified the subsynaptic localization of C1q and elaborated a technique of separating C1q-labeled murine synaptosomes by fluorescence-activated cell sorting (FACS). We applied 2D differential gel electrophoresis (2D-DIGE) and mass spectrometry (MS) for quantitative synaptic proteome change discovery. Our results revealed the significance of apoptotic-like mechanisms in the background of synaptic C1q tagging, which was verified experimentally. To our knowledge, this is a unique report on the direct link between synaptic local apoptotic-like processes and C1q-mediated synaptic pruning mechanisms.

Results

Presence of C1q in Synaptic and Subsynaptic Fractions—Preferential Recognition of the Presynapse by C1q. This study was critically dependent on the appropriate purification of synaptosomes from the cerebral cortices of mice. Visual analysis of electron micrographs of the samples demonstrated that ~87% of the particles were synaptosomes (*SI Appendix, Fig. S1 A–C*). We also investigated the enrichment of well-known synaptic protein markers in the cortical synaptosome fraction in comparison with whole cortical tissue homogenate. Our results demonstrated prominent

Significance

Synaptic pruning is dominant in early ontogenesis when a large number of unnecessary synapses are eliminated, and it maintains synaptic plasticity in the mature healthy brain, e.g., in memory processes. Its malfunction is involved in degenerative diseases such as Alzheimer's disease. C1q, a member of the immune complement system, plays a central role in the selective pruning of synapses by microglial phagocytosis. Understanding the molecular aspects of complement-mediated synapse elimination is of high importance for developing effective therapeutic interventions in the future. Our analysis on C1q-tagged synaptosomes revealed that C1q label-based synaptic pruning is linked to local apoptotic-like processes in synapses.

Author contributions: B.A.G., Z.B., J.M., L.D., G.J., K.A.K., and J. Kardos designed research; B.A.G., J. Kun, G.T., P.G., V.K., P.S., L.D., and K.A.K. performed research; B.A.G., J. Kun, G.T., É.B., Z.B., P.G., V.K., A.M., J.M., G.J., K.A.K., and J. Kardos analyzed data; and B.A.G., G.J., K.A.K., and J. Kardos wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

This open access article is distributed under [Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 \(CC BY-NC-ND\)](#).

¹K.A.K. and J. Kardos contributed equally to this work.

²To whom correspondence should be addressed. Email: kardos@elte.hu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1722613115/-DCSupplemental.

Published online May 29, 2018.

enrichment of the postsynaptic marker postsynaptic density protein 95 (Psd95) and a significantly elevated level of the presynaptic synaptophysin (Syn) in the fraction of synaptosomes (SI Appendix, Fig. S1D). Synaptosomes were visible via differential interference contrast (DIC) light microscopy, as they formed large aggregates in PBS medium (SI Appendix, Fig. S1E). Confocal microscopy experiments also confirmed sample purity by labeling the synapse-specific Syn protein (SI Appendix, Fig. S1E).

Another crucial element was the demonstration of the presence of C1q protein in the synaptosome fraction. Our immunoblotting experiments indicated the presence of C1q in the whole cerebral cortex tissue samples. Moreover, C1q was localized to the cerebral cortical synapses of healthy adult mice (SI Appendix, Fig. S1F). In addition, the presence of brain-derived, and particularly synapse-attached C1q in transcardially perfused mice without complement contamination from the blood confirmed our observations on synaptosomes. Individual C1q-tagged synaptosomes have also been observed in sucrose/EDTA/Tris (SET) buffer using confocal microscopy (Fig. 1A). C1q and Syn were clearly colocalized (Fig. 1A), which is in accordance with previous data (5). These results confirmed the presence of a pool of secreted C1q molecules that directly bind to synapses in the adult brain.

C1q has been observed previously in colocalization with and in close vicinity of pre- and postsynaptic molecular markers using different techniques (4, 5). To estimate precisely the distribution of C1q between the two sides of synapses, we fractionated pre- and postsynaptic membranes (PRE and PSD, respectively) from the synaptosomes. Western blot analysis (Fig. 1B) verified the enrichment of the mostly presynapse-derived Syn in the PRE fraction and the postsynapse-marker Psd95 in the PSD fraction (Fig. 1C). Our results revealed that C1q is predominantly presynaptically bound (PSD/PRE C1q levels = 0.26 ± 0.08 , mean \pm SEM; $P = 0.0013$; Fig. 1C). These results suggest that C1q-dependent elimination of synapses originates from the presynaptic compartment in the cerebral cortex.

Sorting of C1q-Tagged Synaptosomes. Analysis (and sorting) of synaptosomes using flow cytometry has been conducted by few groups before (9–11). However, experimental designs varied considerably between the studies (e.g., detection of fluorescence

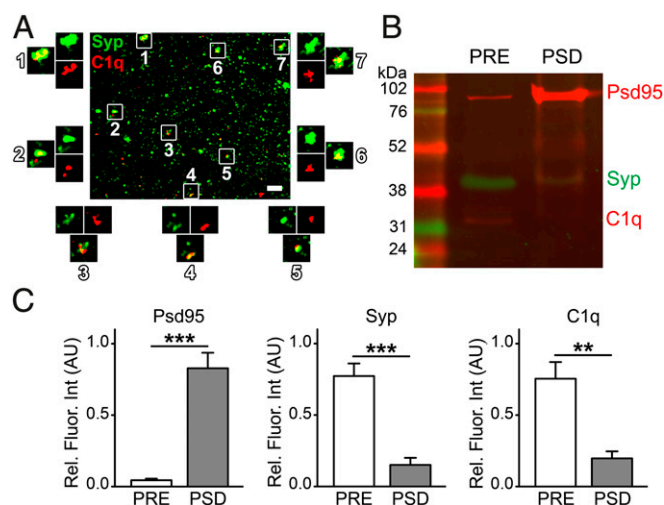


Fig. 1. Presence of C1q in the synaptosome fraction. (A) Confocal image of dispersed Syn-positive synaptosomes in SET buffer showing several points of colocalization with C1q (representative image of four independent experiments). (B) Western blot image and (C) bar graphs showing levels of the pre- and postsynaptic markers Syn and Psd95, respectively, and of C1q in pre- (PRE) and postsynaptic (PSD) membrane fractions ($n = 6$ biologically independent samples). Means \pm SEM are shown. ** $P < 0.01$, and *** $P < 0.001$, two-tailed independent Student's t test. (Scale bar in A, 5 μ m.)

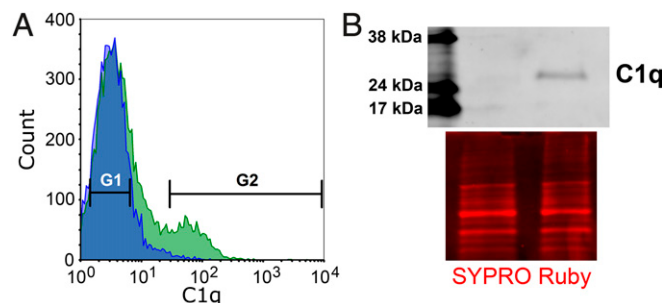


Fig. 2. Sorting of synaptosomes for the synaptic C1q tag. (A) Representative histograms illustrating the fluorescence intensity of C1q-immunolabeled synaptosomes (representative image of at least six independent experiments). The blue histogram in the front represents the negative control sample, labeled solely with the fluorescent dye-conjugated secondary antibody; the green histogram in the back depicts the fluorescence intensity of synaptosomes labeled with anti-C1qA primary antibody as well. G1 and G2 gates show the populations of untagged and C1q-tagged synaptosomes, respectively. (B) Representative Western blot image demonstrating the level of C1q in 6 million sorted untagged and 6 million sorted C1q-tagged synaptosomes complemented with an image of the corresponding total protein staining (SYPRO Ruby) (representative image of four independent experiments).

by antibody-based method or by the presence of transgenically engineered fluorescent protein); thus, we had to optimize the method for our purposes. In contrast to the previous practice, we used SET buffer instead of PBS throughout the immunolabeling of synaptosomes to prevent their aggregation (12) (Fig. 1A). Synaptosome samples were filtered through a 5- μ m pore size membrane to remove large contaminants and aggregated synaptosomes. This step has been successfully used for synaptosome purification previously (13). This protocol enabled the analysis of individual synaptosomes in FACS experiments (SI Appendix, Fig. S2A). Immunolabeled synaptosomes formed a homogeneous population by size and inner complexity in flow cytometry analysis (SI Appendix, Fig. S2B). The diameter of the synaptosomes was in the range of 0.5–1 μ m according to our tests with differently sized flow cytometry calibration beads, which is in accordance with previously published results (9). We excluded the possibility that detection of cellular debris could compromise our observations in flow cytometry experiments because the detected particles could almost be completely labeled with the viability dye calcein-acetoxymethyl (AM) ester (SI Appendix, Fig. S2C). In addition, electron microscopy examinations revealed intact synaptosomes after the sorting procedure (SI Appendix, Fig. S2D). Gating of fluorescently labeled synaptosomes was performed strictly eliminating the false positives based on the histogram of the negative control samples (Fig. 2A). Summarizing our flow cytometry data, $15.60 \pm 3.03\%$ (mean \pm SEM) of the synaptosomes were labeled with anti-C1qA antibody (SI Appendix, Fig. S3). The specificity of the antibody in this experiment was verified in a parallel investigation, as we detected a similar percentage of C1q-positive synaptosomes using an alternative, highly specific, monoclonal anti-C1q antibody (5) (SI Appendix, Fig. S3). In addition, we also assessed the percentage of C1q-positive synaptosomes isolated from the cerebral cortices of newborn mice [at postnatal day 5 (P5)] and observed a lower amount of C1q-positive synaptosomes in comparison with the results obtained using adult mice (SI Appendix, Fig. S3), which is in accordance with the already described notable increase in C1q expression during aging (5). Western blot analysis also verified the reliability of sorting purity via the successful detection of C1q-signal from C1q-tagged synaptosome homogenates and the lack of a detectable signal from the untagged ones (Fig. 2B). In conclusion, immunolabeling of the complement tag of synapses and application of the FACS methodology provided sorted synaptosome samples suitable for characterizing the molecular composition of C1q-tagged synapses.

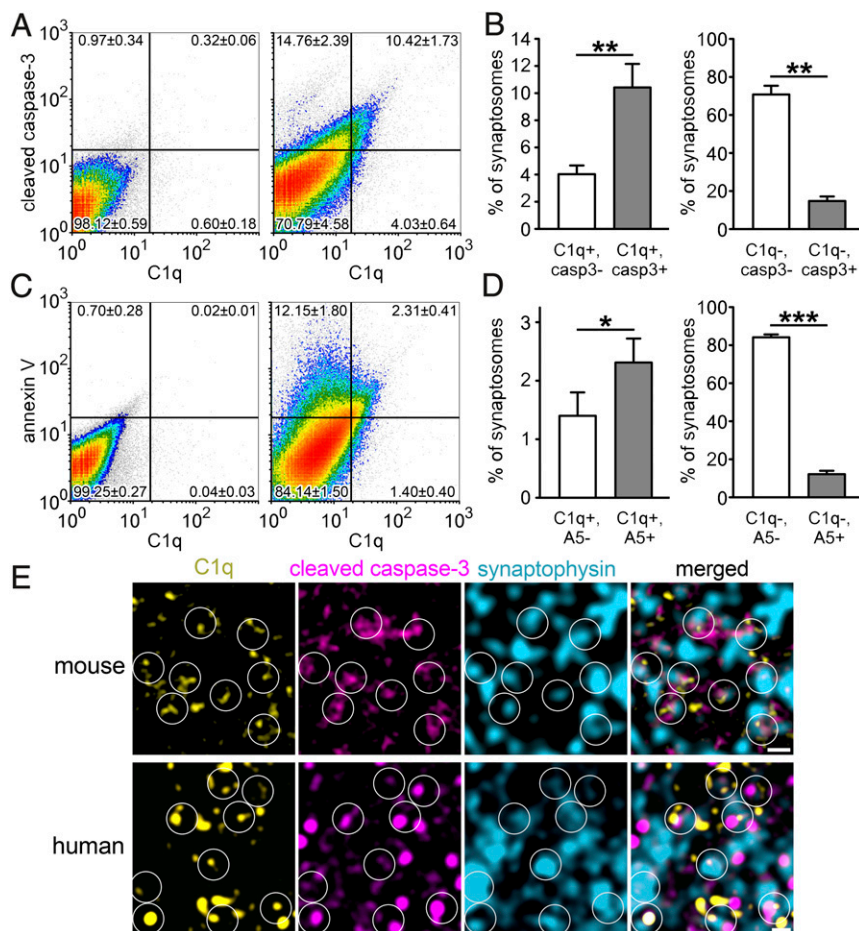


Fig. 4. The role of apoptotic-like mechanisms in the C1q tagging of synapses. (A–D) According to the gating criteria (Left in A and C; negative controls, solely labeled with the secondary antibody), a large proportion of C1q-tagged synaptosomes was also positive for cleaved caspase-3 (casp3) (Right in A and Left in B) and annexin V (A5) (Right in C and Left in D); the untagged synaptosomes were mostly negative for these apoptotic markers (Right in A and Right in B; Right in C and Right in D) as uncovered using flow cytometry. (E) Triple immunostaining of sagittal brain sections of mice (Upper) confirmed the presence of cleaved caspase-3 in C1q-tagged synapses in the molecular layer of the hippocampal dentate gyrus. On stimulated emission depletion (STED)/confocal combined microscopy images, circles indicate examples of colocalization between C1q (yellow) and the presynaptic and apoptotic markers synaptophysin (cyan) and cleaved caspase-3 (magenta), respectively. Moreover, the abundant colocalization of these three proteins was further confirmed on human brain sections (prepared from the temporal cortex) using the HyVolution 2 pseudosuperresolution confocal microscopy technique (Lower). Means \pm SEM are shown; $n = 4$ mice per apoptotic marker. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$, two-tailed Student's t test of paired samples. (Scale bar, 0.5 μ m.)

our translational experiment confirmed the abundant colocalization of C1q, cleaved caspase-3, and Syp in human cerebral cortex (Fig. 4E and *SI Appendix*, Fig. S8). The activation of the molecular machinery, well known in apoptosis, has already been described under synaptic weakening and long-term depression (as excellently reviewed in ref. 24). To test the hypothesis that synaptic downscaling could result in activation of an apoptotic-like molecular process ending up with C1q tagging of synapses involved, we studied a well-established model of synaptic depression in the barrel cortex (25, 26). In this model, whisker removal induces synaptic weakening and degeneration of synaptic boutons and axonal branches in the sensory deprived primary somatosensory barrel cortex (25, 26). Our investigation revealed the elevation of C1q and cleaved caspase-3 levels in homogenates of synaptosomes isolated from affected barrel cortices in comparison with unaffected ones (Fig. 5). Moreover, the level of synaptic vesicle-localized Syp decreased (Fig. 5), which is in accordance with the previously reported presynaptic dysfunction in this model (25). In conclusion, our data strongly suggest that the selective C1q tagging of synapses depends on the occurrence of local apoptotic-like processes.

Discussion

Synaptic pruning is a physiological process necessary in early ontogenesis to shape functional neuronal circuits (27) and to promote synaptic plasticity in the mature central nervous system as it maintains a kinetic balance in synapse turnover (28). However, unbalanced synaptic turnover shifted toward pruning is a hallmark of neurodegenerative diseases, e.g., Alzheimer's disease (29), and schizophrenia as well (30). The classical pathway of the complement system has been repeatedly implicated in the process of synaptic pruning since the pioneering study of

Stevens et al. (4). A detrimental role of the excessive activation of C1q and C3 (6), and other downstream complement components (31, 32) leading to synapse and/or neuronal loss has been identified in neurodegenerative pathology. Despite the significance of complement-mediated synapse elimination in health and disease, knowledge about the molecular changes leading to C1q-tagged synapses is limited.

Our investigations were performed on synaptosomes containing both the pre- and postsynaptic neuronal elements, which can be compartmentalized, and the localization of C1q can be examined (Fig. 1A and *SI Appendix*, Fig. S1). The presence of C1q attached to individual synaptosomes is in accordance with the results of previous studies that revealed detectable C1q levels in the brains of healthy adult rodents and of human subjects as well (5, 6). Separation of the two sides of the synapse unveiled that the C1q tag is dominantly localized to the presynaptic part of the labeled synapse (Fig. 1B and C). Therefore, complement-mediated synaptic pruning might be directly initiated by presynaptic processes. This finding is supported by the fact that microglia engulf specific presynaptic elements (3). Although several interacting partners of C1q are known (33), they were identified in the periphery, not in the central nervous system. Our data suggest that it is worth searching for presynapse-specific interacting partners of C1q in the future.

In this study, we have combined FACS with proteomics techniques to reveal synapse-specific protein alterations in the subset of synapses that are assigned for elimination via the local complement system. The experimental procedure enabled the multiparametric analysis of individual synaptosomes (Fig. 2A and *SI Appendix*, Fig. S2) and the pure separation of C1q-tagged ones (Fig. 2B). Synaptic proteins of altered abundance in the C1q-tagged synapses (Fig. 3A and *SI Appendix*, Fig. S4) were assigned

separation of pre- and postsynaptic membranes was carried out based on the treatment of synaptic junctions with detergents at different pH.

Immunolabeling of Synaptosomes and Assessment of Apoptotic Processes and Viability. Synaptosomes were gently fixed and immunolabeled in SET buffer with anti-C1qA primary and appropriate secondary antibodies. When additional labeling of the intracellular Syp and cleaved caspase-3 proteins was conducted, a permeabilization step was applied using 0.2% Tween-20. Annexin V labeling was performed using annexin V-FITC protein, and for viability tests, calcein-AM labeling was applied.

Fluorescence-Activated Cell Sorting. Flow cytometry was performed on a BD FACSAria III sorter coupled with BD FACSDiva data acquisition and analysis software. The population of synaptosomes was designated based on their FSC and SSC characteristics compared with that of the buffer alone, and further gating of fluorescent and nonfluorescent particles was performed among the members of this population. Fluorescently labeled, C1q-tagged and unlabeled untagged synaptosomes were collected separately and used for the proteomics and validation experiments. To determine the extent of synaptic colocalization of C1q with apoptotic markers, additional flow cytometry experiments were carried out.

Proteomics Experiments. Two-dimensional-DIGE was conducted to compare the proteomes of sorted C1q-tagged and untagged synaptosomes from six mice. Proteins from 2 to 2 million C1q-tagged and untagged synaptosomes were labeled with Cy5, and the pooled sample (2 to 2 million from each

sample) was labeled with Cy3 fluorescent dye. C1q-tagged and untagged synaptosome samples were prepared and run on separate gels together with the same amount of a pooled sample. The fluorescence intensity of the protein spots was detected, and individual spots were identified, matched, and manually verified using the DeCyder 2D Differential Analysis software. For mass spectrometric protein identification, HPLC-MS/MS analysis of the tryptic peptide mixtures was performed using a nanoflow ultra (U)HPLC system coupled to a high-resolution quadrupole time-of-flight mass spectrometer.

Bioinformatics Analysis. Functional classification of the altered proteins was conducted using the GeneOntology (www.geneontology.org) and UniProt (www.uniprot.org) databases as well as with published literature. Common regulator and common target analyses of the differentially regulated proteins were conducted using the Pathway Studio software.

ACKNOWLEDGMENTS. We thank Péter Kovács (CRU Hungary, Ltd.) for generous financial support for the group at ELTE. This work was supported by the National Research, Development, and Innovation Office of Hungary Grants KTIA_NAP_13-2-2015-0003 (to L.D., P.G., and G.J.), KTIA_NAP_13-2-2014-0017 and 2017-1.2.1-NKP-2017-00002 (to B.A.G., J. Kun, É.B., A.M., and J. Kardos, and funding for STED microscopy), K_120391 (to J. Kun, É.B., J. Kardos, and A.M.), FIEK_16-1-2016-0005 (to Z.B., J. Kun, G.J., K.A.K., and J. Kardos), and VEKOP-2.3.3-15-2016-00007 for confocal microscopy. A.M. is supported by the Bolyai János fellowship of the Hungarian Academy of Sciences. Human tissue was provided by the Department of Pathology of the Saint Borbála Hospital, Tatabánya, Hungary.

1. Veerhuis R, Nielsen HM, Tenner AJ (2011) Complement in the brain. *Mol Immunol* 48:1592–1603.
2. Bialas AR, Stevens B (2013) TGF- β signaling regulates neuronal C1q expression and developmental synaptic refinement. *Nat Neurosci* 16:1773–1782.
3. Schafer DP, et al. (2012) Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. *Neuron* 74:691–705.
4. Stevens B, et al. (2007) The classical complement cascade mediates CNS synapse elimination. *Cell* 131:1164–1178.
5. Stephan AH, et al. (2013) A dramatic increase of C1q protein in the CNS during normal aging. *J Neurosci* 33:13460–13474.
6. Hong S, et al. (2016) Complement and microglia mediate early synapse loss in Alzheimer mouse models. *Science* 352:712–716.
7. Lui H, et al. (2016) Progranulin deficiency promotes circuit-specific synaptic pruning by microglia via complement activation. *Cell* 165:921–935.
8. Trachtenberg JT, et al. (2002) Long-term in vivo imaging of experience-dependent synaptic plasticity in adult cortex. *Nature* 420:788–794.
9. Biesemann C, et al. (2014) Proteomic screening of glutamatergic mouse brain synaptosomes isolated by fluorescence activated sorting. *EMBO J* 33:157–170.
10. D'Amelio M, et al. (2011) Caspase-3 triggers early synaptic dysfunction in a mouse model of Alzheimer's disease. *Nat Neurosci* 14:69–76.
11. Gyls KH, Fein JA, Cole GM (2000) Quantitative characterization of crude synaptosomal fraction (P-2) components by flow cytometry. *J Neurosci Res* 61:186–192.
12. Daniel JA, Malladi CS, Kettle E, McCluskey A, Robinson PJ (2012) Analysis of synaptic vesicle endocytosis in synaptosomes by high-content screening. *Nat Protoc* 7:1439–1455.
13. Bajor M, et al. (2012) Synaptic cell adhesion molecule-2 and collapsin response mediator protein-2 are novel members of the matrix metalloproteinase-9 degradome. *J Neurochem* 122:775–788.
14. Roumenina LT, et al. (2006) Interaction of C1q with IgG1, C-reactive protein and pentraxin 3: Mutational studies using recombinant globular head modules of human C1q A, B, and C chains. *Biochemistry* 45:4093–4104.
15. Sia GM, et al. (2007) Interaction of the N-terminal domain of the AMPA receptor GluR4 subunit with the neuronal pentraxin NP1 mediates GluR4 synaptic recruitment. *Neuron* 55:87–102.
16. Pelkey KA, et al. (2015) Pentraxins coordinate excitatory synapse maturation and circuit integration of parvalbumin interneurons. *Neuron* 85:1257–1272.
17. Bjartmar L, et al. (2006) Neuronal pentraxins mediate synaptic refinement in the developing visual system. *J Neurosci* 26:6269–6281.
18. Perry VH, O'Connor V (2008) C1q: The perfect complement for a synaptic feast? *Nat Rev Neurosci* 9:807–811.
19. Kanamori T, et al. (2013) Compartmentalized calcium transients trigger dendrite pruning in Drosophila sensory neurons. *Science* 340:1475–1478.
20. Earnshaw WC, Martins LM, Kaufmann SH (1999) Mammalian caspases: Structure, activation, substrates, and functions during apoptosis. *Annu Rev Biochem* 68:383–424.
21. Shimohama S, Tanino H, Fujimoto S (2001) Differential subcellular localization of caspase family proteins in the adult rat brain. *Neurosci Lett* 315:125–128.
22. van Engeland M, Nieland LJ, Ramaekers FC, Schutte B, Reutelingsperger CP (1998) Annexin V-affinity assay: A review on an apoptosis detection system based on phosphatidylserine exposure. *Cytometry* 31:1–9.
23. Paidassi H, et al. (2008) C1q binds phosphatidylserine and likely acts as a multiligand-bridging molecule in apoptotic cell recognition. *J Immunol* 180:2329–2338.
24. Sheng M, Ertürk A (2013) Long-term depression: A cell biological view. *Philos Trans R Soc Lond B Biol Sci* 369:20130138.
25. Bender KJ, Allen CB, Bender VA, Feldman DE (2006) Synaptic basis for whisker deprivation-induced synaptic depression in rat somatosensory cortex. *J Neurosci* 26:4155–4165.
26. Marik SA, Yamahachi H, McManus JN, Szabo G, Gilbert CD (2010) Axonal dynamics of excitatory and inhibitory neurons in somatosensory cortex. *PLoS Biol* 8:e1000395.
27. Paolicelli RC, et al. (2011) Synaptic pruning by microglia is necessary for normal brain development. *Science* 333:1456–1458.
28. Holtmaat A, Svoboda K (2009) Experience-dependent structural synaptic plasticity in the mammalian brain. *Nat Rev Neurosci* 10:647–658.
29. Selkoe DJ (2002) Alzheimer's disease is a synaptic failure. *Science* 298:789–791.
30. McGlashan TH, Hoffman RE (2000) Schizophrenia as a disorder of developmentally reduced synaptic connectivity. *Arch Gen Psychiatry* 57:637–648.
31. Hernandez MX, Namiranian P, Nguyen E, Fonseca MI, Tenner AJ (2017) C5a increases the injury to primary neurons elicited by fibrillar amyloid beta. *ASN Neuro* 9:1759091416687871.
32. Xiong ZQ, Qian W, Suzuki K, McNamara JO (2003) Formation of complement membrane attack complex in mammalian cerebral cortex evokes seizures and neurodegeneration. *J Neurosci* 23:955–960.
33. Kishore U, Reid KB (2000) C1q: Structure, function, and receptors. *Immunopharmacology* 49:159–170.
34. Stephan AH, Barres BA, Stevens B (2012) The complement system: An unexpected role in synaptic pruning during development and disease. *Annu Rev Neurosci* 35:369–389.
35. Vargas KJ, et al. (2014) Synucleins regulate the kinetics of synaptic vesicle endocytosis. *J Neurosci* 34:9364–9376.
36. Rosahl TW, et al. (1995) Essential functions of synapsins I and II in synaptic vesicle regulation. *Nature* 375:488–493.
37. Tang J, et al. (2006) A complexin/syntaxin 1 switch controls fast synaptic vesicle exocytosis. *Cell* 126:1175–1187.
38. Al Rahim M, Thatipamula S, Hossain MA (2013) Critical role of neuronal pentraxin 1 in mitochondria-mediated hypoxic-ischemic neuronal injury. *Neurobiol Dis* 50:59–68.
39. Ertürk A, Wang Y, Sheng M (2014) Local pruning of dendrites and spines by caspase-3-dependent and proteasome-limited mechanisms. *J Neurosci* 34:1672–1688.
40. Mattson MP, Partin J, Begley JG (1998) Amyloid beta-peptide induces apoptosis-related events in synapses and dendrites. *Brain Res* 807:167–176.
41. Gyls KH, Fein JA, Wiley DJ, Cole GM (2004) Rapid annexin-V labeling in synaptosomes. *Neurochem Int* 44:125–131.
42. Glantz LA, Gilmore JH, Lieberman JA, Jarskog LF (2006) Apoptotic mechanisms and the synaptic pathology of schizophrenia. *Schizophr Res* 81:47–63.
43. Nauta AJ, et al. (2002) Direct binding of C1q to apoptotic cells and cell blebs induces complement activation. *Eur J Immunol* 32:1726–1736.
44. Fraser DA, Pisalyaput K, Tenner AJ (2010) C1q enhances microglial clearance of apoptotic neurons and neuronal blebs, and modulates subsequent inflammatory cytokine production. *J Neurochem* 112:733–743.
45. Hyman BT, Yuan J (2012) Apoptotic and non-apoptotic roles of caspases in neuronal physiology and pathophysiology. *Nat Rev Neurosci* 13:395–406.
46. Li Z, et al. (2010) Caspase-3 activation via mitochondria is required for long-term depression and AMPA receptor internalization. *Cell* 141:859–871.
47. Léveillé F, et al. (2010) Suppression of the intrinsic apoptosis pathway by synaptic activity. *J Neurosci* 30:2623–2635.